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Par

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Mechanisms of gustatory perception of dietary lipids: cross-talk with bitter taste and endocannabinoid receptors

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Résumé

Titre : Mécanismes de perception gustative des lipides alimentaires : cross-talk avec les récepteurs du goût amer et des endocannabinoïdes.

Mots clés : Goût du gras, amer, endocannabinoïdes, cellules gustatives, HTC-8, CB_1R

Introduction. L'obésité constitue l'un des principaux problèmes de santé publique en ce début du 21ème siècle. Sa prévalence augmente régulièrement, en particulier chez les enfants. Ce constat n'est pas anodin car l'obésité est généralement associée à diverses pathologies graves (diabète de type 2, hypertension et cancer,…). Ainsi, des investigations sur les mécanismes impliqués dans la perception gustative des lipides alimentaires pourraient éclairer leurs rôles dans l'incidence de l'obésité.

Buts et objectifs. Plusieurs études ont démontré le rôle des endocannabinoïdes et des aliments amers dans l'obésité. Ainsi, nous avons étudié l'interaction (cross-talk) des récepteurs cannabinoïdes et du goût amer avec le goût lipidique. Cette thèse comporte ainsi deux volets : les récepteurs cannabinoïdes (CB_1R), le goût amer et leurs interactions avec les récepteurs lipidiques.

Résultats et discussion. Dans la première partie, nous avons étudié le rôle régulateur de CB_1R. Ainsi, des tests comportementaux sur des souris $CB_1R^{-/-}$ et des souris de type sauvage (WT) ont montré que l'invalidation du gène Cb_1r était associée à une faible préférence pour les solutions contenant de l'huile de colza ou un acide gras à longue chaîne (AGLC) tel que l'acide linoléique (LA). L'administration de rimonabant, un agoniste-inverse de CB_1R, chez la souris a également entraîné une faible préférence pour les acides gras alimentaires. Aucune différence dans l'expression des protéines CD36 et GPR120 n'a été observée dans les cellules des papilles gustatives des souris WT et $CB_1R^{-/-}$. La signalisation calcique via CD36 dans les cellules des papilles gustatives des souris $CB_1R^{-/-}$ diminue de façon significative par rapport à celle observée dans les cellules gustatives des souris WT. Les cellules des papilles gustatives des souris $CB_1R^{-/-}$ présentent également une diminution significative de l'ARNm de Pro-glucagon et de Glp-1r et un faible niveau basal de GLP-1. Nous rapportons que CB_1R est impliqué dans la perception du goût du gras via la signalisation calcique et la sécrétion de GLP-1.

Résultats et discussion. Dans la seconde partie, nous avons d'abord caractérisé le phénotype de cellules fongiformes humaines (HTC-8). En effet, le projet de ma thèse comprend la caractérisation à l'échelle moléculaire des récepteurs amers et lipidiques et leur cross-talk dans ces cellules (collaboration BRAIN, Allemagne). Nous avons démontré que les cellules HTC-8 expriment PLCβ2 et l'α-gustducin à l'échelle des ARNm et des protéines. Elles expriment également TAS2R16 et TAS2R38 et ces mêmes cellules co-expriment CD36 et GPR120. Puis, nous avons étudié la signalisation via ces récepteurs en utilisant l'acide linoléique, un agoniste de CD36 et GPR120, la sinigrin, agoniste de TAS2R16 et TAS2R38, la salicin, agoniste du récepteur TAS2R16 et le phénylthiocarbamide, agoniste du récepteur TAS2R38. De plus, les études du signal calcique ont démontré que la signalisation en aval du goût gras partage une voie commune avec la signalisation en aval du goût amer, mettant en évidence un cross-talk entre ces deux modalités gustatives.

Bien que nous ayons montré le cross-talk entre les modalités gustatives amère et lipidique, il nous reste à étudier ces phénomènes à l'échelle de l'organisme. Ces résultats, d'ores et déjà, montrent que le goût amer et le récepteur cannabinoïde-1 sont liés à la sensibilité au goût du gras et doivent être pris en compte pour la gestion de l'obésité.

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Abstract

Title : Mechanisms of gustatory perception of dietary lipids: cross-talk with bitter taste and endocannabinoid receptors.

Keywords : fat taste, bitter taste, endocannabinoids, taste cells, HTC-8, CB_1R

Introduction. Obesity is one of the major public health problems at the beginning of the 21st century. Its prevalence is increasing steadily, especially among children. This observation is very important because obesity is generally associated with various serious pathologies (type 2 diabetes, hypertension and cancer, etc.). Thus, investigations into the mechanisms involved in the taste perception of dietary lipids could shed light on their roles in the incidence of obesity.

Aims and objectives. Several studies have demonstrated the role of endocannabinoids and bitter foods in obesity. Thus, we studied the cross-talk of cannabinoid receptors and bitter taste with lipid taste. This thesis consists of two components: cannabinoid (CB_1R) and bitter taste receptors and their interactions with lipid receptors.

Results and discussion. In the first part, we studied the regulatory role of CB_1R. The behavioural tests on $CB_1R^{-/-}$ and wild-type (WT) showed that the invalidation of the Cb1r gene was associated with low preference for solutions containing rapeseed oil or a long chain fatty acid (LCFA) such as linoleic acid (LA). Administration of rimonabant, a CB_1R inverse agonist, in mice also resulted in a low preference for dietary fatty acids. No differences in the expression of CD36 and GPR120 proteins were observed in the taste buds cells of WT and $CB_1R^{-/-}$ mice. Calcium signalling via CD36 in the taste bud cells of $CB_1R^{-/-}$ mice decreased significantly compared with those observed in the taste cells of WT mice. The taste bud cells of $CB_1R^{-/-}$ mice also show a significant decrease in Pro-glucagon and Glp-1r mRNA and a low basal level of GLP-1. We report that CB1R is involved in the perception of fat taste via calcium signalling and secretion of GLP-1.

Results and discussion. In the second part, we first characterized the phenotype of human fungiform cells (HTC-8). Indeed, the project of my thesis includes molecular characterization of bitter and lipid receptors and their cross-talk in these cells (collaboration BRAIN, Germany). We have demonstrated that HTC-8 cells express PLCβ2 and α-gustducin at mRNA and protein levels. They also express TAS2R16 and TAS2R38 and the same cells co-express CD36 and GPR120. Then, we studied signalling via these receptors using linoleic acid, a CD36 and GPR120 agonist, sinigrin, TAS2R16 agonist and TAS2R38, salicin, TAS2R16 receptor agonist, and phenylthiocarbamide, TAS2R38 receptor agonist. In addition, calcium signal studies have shown that downstream fatty signalling shares a common path with downstream bitter taste signalling, highlighting a cross-talk between these two taste modalities.

Although we have shown the cross-talk between bitter and lipid taste modalities, we still have to study these phenomena at the level of the organism. These resultsshow that the bitter taste and the cannabinoid-1 receptor are related to the taste sensitivity of fat and must be taken into account for the management of obesity

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Mechanisms of gustatory perception of dietary lipids: cross-talk with bitter taste and endocannabinoid receptors

List of abbreviations

2-AG	2-Arachidonoyl-Glycerol
5-HT	5-hydroxytryptamine - Serotonin
Δ9-THC	Δ9-Tetrahydrocannabinol

A

AEA	Anandamide - N-arachidonoyl ethanolamine
AFC	Alternative force choice
AgRP	Agouti-related peptide
AI	Agranular insular cortex
ARC	Arcuate nucleus

B

BAT	Brown adipose tissue
bPRP	basic proline-rich protein family

C

Ca^{2+}	Calcium ion
$[Ca^{2+}]_i$	Concentration of intracellular calcium
CALHM1	Calcium homeostasis modulator 1
CART	Amphetamine-regulated transcript
Cav1	Caveolin-1
CA-VI	Carbonic anhydrase VI
CB_1R	Cannabinoid receptor-1
CCK	Cholecystokinin
CD36	Cluster of differenciation 36
CNS	Central nervous system
CT	Chorda-tympani
CVP	Circumvallate papillae

D

DMSO	Dimethyl sulfoxide
DRK	Delayed rectifying potassium channels

E

ECS	Endocannabinoid system
ER	Endosplamic reticulum

F

FA	Fatty acid
FFA	Free fatty acid
Fop	Frontal operculum

G

GH	Growth hormone
GI/DI	Granular/dysgranular insular cortex
GL	Glossopharyngeal
GLAST	Glutamate-aspartate transporter
GLP-1	Glucagon-like peptide-1
GPCR	G protein-coupled receptor

GPR120	G protein-coupled receptor 120
GPR40	G protein-coupled receptor 40

H

HDL	High density lipoprotein
HFD	High fat diet
Hipp	Hippocampus

I

IFN	Interferon
IL6	Interleukin 6
IP_3	Inositol trisphosphate

K

$KO/^{-/-}$	Knock-out

L

LA	Linoleic acid
LCFA	Long-chain fatty acid
LDL	Low density lipoprotein
LPS	Lipopolysaccharide

M

MCFA	Medium-chain fatty acid
MSH	Melanocyte-stimulating hormone

N

NAc	Nucleus accumbens
NE	Norepinephrine
NPY	Neuropeptide Y
NST	Nucleus of the solitary tract

O

OBPIIa	Odorant-binding protein IIa
OEA	Oleylethanolamide
OFC	Orbitofrontal cortex
Orai	Calcium release-activated calcium channel protein
OTR	Oxytocin receptor

P

PA	Palmitic acid
PBN	Parabrachial nucleus
PGC	Primary gustatory cortex
P-GCG	Pro-glucagon
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PLCβ2	Phospholipase C β2
POMc	Proopiomelanocortin
PROP	6-n-Propylthiouracil
PTC	Phenylthiocarbamide
PUFA	Polyunsaturated fatty acid
PVN	Paraventricular nucleus
PYY	Neuropeptide Y

S	
siRNA	Small interfering-RNA
SNAP25	Synaptic associated protein, 25kDa
SNP	Single nucleotide polymorphism
SOC	Stored operated calcium channels
SOCE	Stored operated calcium entry
SON	Supraoptic nucleus
Src-PTK	Src protein-tyrosine kinase
SSO	Sulfo-N-succinimidyl oleate
STIM1	Stromal interaction molecule
T	
TAG	Triacylglycerol
TAS1R	Taste receptor type 1
TAS2R	Taste receptor type
TBC	Taste bud cell
TG	Triglyceride
TLR	Toll-like receptor
TNFα	Tumor necrosis factor α
TRC	Taste receptor cells
TRPM5	Transient receptor potential cation channel subfamily M member 5
V	
VEG	Von Ebner's gland
VEGP	Von Ebner's gland protein
VIP	Vasoactive intestinal peptide
VPMPC	Ventral posterior medial nucleus parvocellular subdivision
VTA	Ventral tegmental area
W	
WAT	White adipose tissue

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Modulation of fat preference by bitter agonists

Introduction

Introduction

Due to increasing abundance of food, the Western diet is constituted of almost 45% of fat (i.e. 10% beyond the nutritional recommandations), greatly contributing to the prevalence of obesity. In France, the obesity rate is increased from 8% in 1997 (first survey) to 15% in 2012 according to the last ObEpi report (OMS, 2012), involving serious diseases such as type II diabetes, atherosclerosis and hypertension. The epidemic of obesity is consequently considered as a major public health problem.

The availability of highly palatable foods rich in energy, fat and sugar has been associated with the risk for obesity (Erlanson-Albertsson, 2005). The hedonic property of this type of food is a driving force, promoting its preferential consumption. Mela and Sacchetti (1991) observed that obese subjects had a higher spontaneous preference for dietary lipids than normal subjects (Drewnowski et al., 1985). This suggests the existence of selective detection of these caloric / lipidic foods operating from the oral cavity.

Taste is one of the components that enables orosensory perception of foods and their composition. This plays a crucial role in food choices. Humans have a highly selective taste system that allows them to distinguish between nutritive and potentially toxic foods (Jyotaki et al., 2010). The perception of taste provides with the information on the quality of food ingested by the organism. The signals generated in the oral cavity will transmit the taste information via the central nervous system to different physiological targets like intestine via tongue-brain-intestine loop. The oral cavity, therefore, plays a significant role in the eating behaviour.

The taste buds localized in the lingual papillae relay the information. There are three main types of papillae: fungiform, foliated and circumvallate papillae (Figure 1). The binding of the tastant to its taste receptor located in the papillae induces, via [Ca^{2+}], taste signals transmitted via the cranial nerve muscle IX and VII to the NTS, connected to different areas of the brain involved in food intake and the digestive tract.

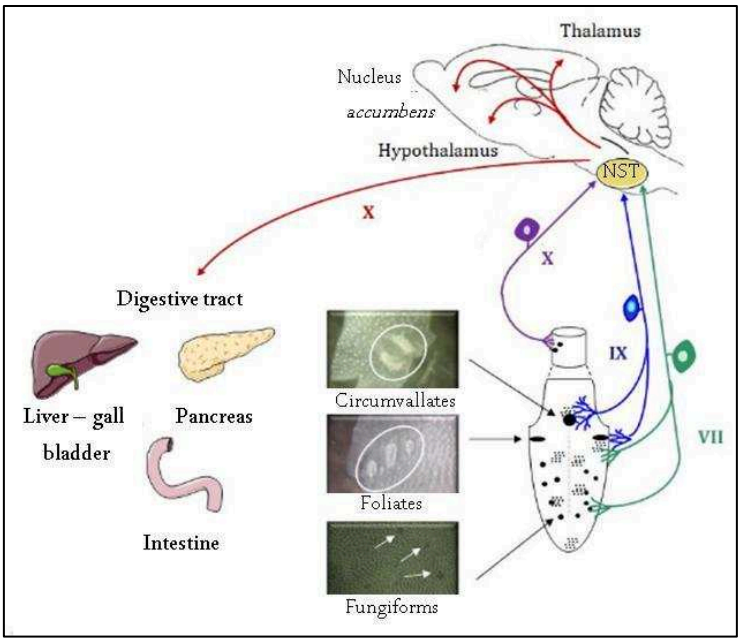

Figure 1. **Nerve pathways involved in taste perception**. At the lingual level, the papillae are innervated by afferent nerve fibers belonging to two pairs of cranial nerves. Thus, the fungiform papillae and the anterior part of the foliated papillae establish synaptic contacts with the chorda-tympani nerve (CT, i.e., pairs of cranial nerves VII). The posteror part of the foliated papillae and the circumvallate papillae are innervated by fibers from the glossopharyngeal lingual branch (i.e., pair of cranial nerves IX). The few papillae present in the upper esophagus are connected to branches of the vagus nerve (nerve X). At the central level, the nucleus of the solitary tract (NST) is the first gustatory relay. The integration of tatse signals from the taste buds is mediated by brain areas (hypothalamus, nucleus accumbens, thalamus) whose role in the resultaion of the eating behavior is well established. The NST sends information back to the digestive tract through efferent vagal nerve fibers. VII tympanic chord, IX glossopharyngeal nerve, NST nucleus of solitary tract. Adapted from Martin et al. (2010).

Orosensory perception of dietary lipids has long been perceived as dependent only on their textural and olfactory properties. However, during the last recent years, the existence of a sixth taste modality, dedicated to the perception of dietary lipids, has been proposed. Indeed, the recent discovery of lipid receptors in rodents and humans suggests that lipids can be detected by the taste pathway (Mattes, 2011; Passilly-Degrace et al., 2009). Hence, CD36 (Cluster of differentiation 36) has been suggested to play the role of a lingual lipid receptor (Laugerette et al., 2005). In addition, it has been proposed, in the first place, that the GPR120 lingual receptor (G protein-coupled receptor 120) also has a role in lipid detection. However, it seems that this is not the case (Ancel et al., 2015). Indeed, GPR120 has been clearly demonstrated to play a "regulatory" role in the control of lipid feeding behaviour (Ozdener et al., 2014). Long-chain fatty acids (LCFA) have been shown to induce a cephalic response and influence the gastric motility of the gastrointestinal system as well as the hormonal cascade of satiety (Little and Feinle-Bisset, 2011). Obese individuals appear to have an attenuated oral and gastrointestinal chemoreception of fatty acids (FA) (Little and Feinle-Bisset, 2011). Oral

detection of fatty acids has two major roles: the cephalic phase activation and the regulation of fat and energy intake (Little and Feinle-Bisset, 2010). In this context, many studies aimed at analyzing lipid perception have been conducted to understand how individuals make high-fat dietary choices in terms of the quality and quantity they consume (Ebba et al., 2012). A large number of studies concluded that oral fat sensitivity is a key factor in food-selection (Keller, 2012; Keller et al., 2012; Melis et al., 2015a; Stewart et al., 2010, 2011a; Tepper and Nurse, 1997) (Figure 2).

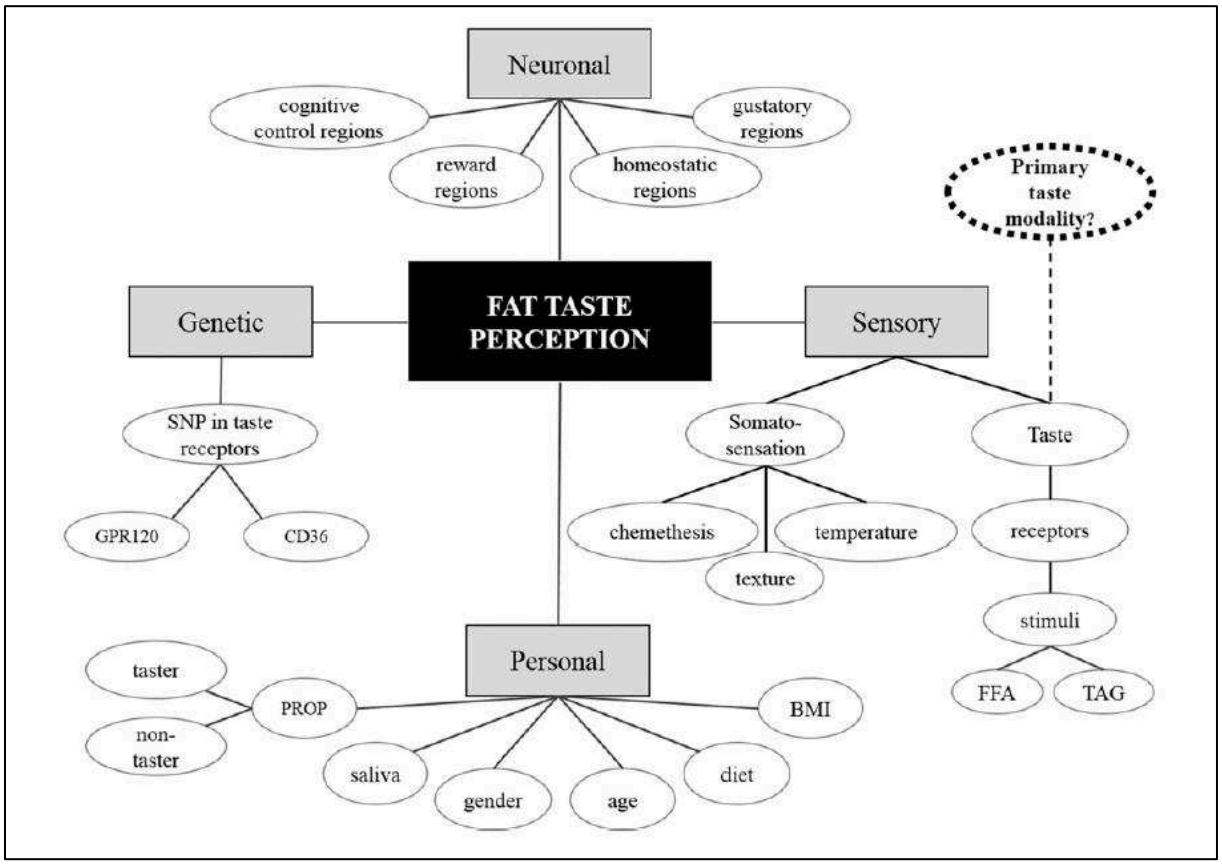

Figure 2. **Sensory, genetic, personal and neuronal factors influencing the perception of fat taste**. BMI body mass index, CD36 cluster of differenciation 36, GPR120 G-protein coupler receptor 120, FFA free fatty acid, PROP 6-n-Propylthiouracil, SNP single nucleotide polymorphism, TAG triacylglycerol. Adapted from Heinze et al. (2015).

A defect in an individual's ability to detect dietary lipids would be linked to overconsumption of fatty foods (Keast et al., 2014).

First Part
State of the Art

Chapter 1
Physiology of the taste system

1. Gustative perception

1.1. General introduction

The primary organ responsible for the sense of taste is the tongue. It contains biological machinery for identifying non-volatile compounds in food and non-food products that are placed in the oral cavity (Keast and Costanzo, 2015).

Humans recognize and distinguish 5 basic taste qualities; sweet, umami, salty, sour and bitter. Basically, sweet detects energy-rich food; umami, the taste of L-glutamate reflecting a food's protein content; salty is responsible for the electrolyte balance; sour prevents ingestion of food that is damaged or not mature enough; finally, bitter warns about potential presence of harmfull compounds (Figure 3) (Chaudhari and Roper, 2010).

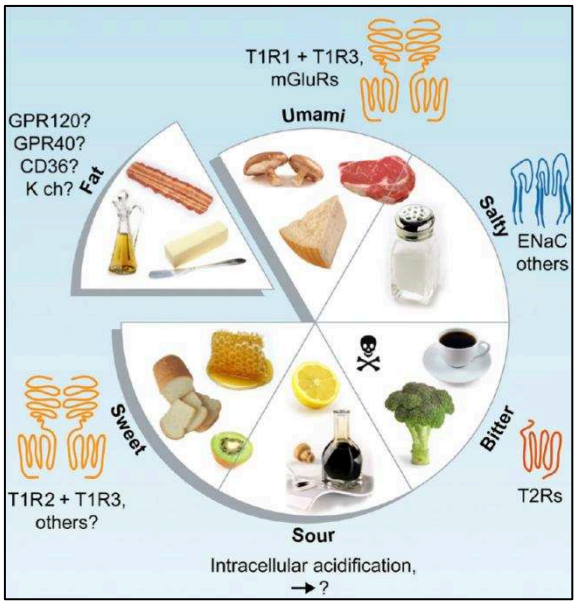

Figure 3. **Taste qualities, taste receptors and natural stimuli**. Human taste is characterized by 5 basic tastes qualities: sweet, umami, salty, sour and bitter. Recently, a sixth taste dedicated to the fat perception has been proposed. Adapted from Chaudhari and Roper (2010).

Taste perception relays the information triggered by ingested food from the oral cavity to the rest of the body. It occurs in the taste buds included in the papillae and mostly located, in the dorsal epithelium of the tongue. There are 3 main types of papillae: fungiform, foliated and circumvallate papillae (Figure 4). Each taste bud contains 30 to 100 taste cells. There are mainly 3 types of cells in the taste buds: type I cells which are qualified by their properties as

glial-like cells; type II cells, corresponding to taste receptor cells, which express phospholipase Cβ2 (PLCβ2) and TRPM5 (Transient receptor potential cation channel subfamily M member 5) channels and type III cells which are pre-synaptic cells (Chaudhari and Roper, 2010). The taste bud cells (TBC) undergo continual turnover with a life span of about 10 days (Perea-Martinez et al., 2013). It has been recently shown that a lingual population of stem cells are involved in the production of mature TBC in the lingual papillae (Takeda et al., 2013). TBC derived from a pool of multipotent progenitor cells that are localized in the basal regions surrounding taste buds (Takeda et al., 2013; Yee et al., 2013). The binding of a sapid molecule to its receptor leads to changes in membrane potential and to an increase in the concentration of free calcium, $[Ca^{2+}]_i$. The depolarization of the taste cell triggers the release of neurotransmitters such as serotonin (5-HT) (El-Yassimi et al., 2008). The taste signals from the oral cavity are then transmitted to cranial nerve VII (the cranial nerve tympanum) and IX (the glossopharyngeal lingual branch) and then to the nucleus of the solitary tract (NST) (Figure 4) (Gaillard et al., 2008). The NST is connected to different areas of the brain involved in food intake, reward, memory and the digestive tract (Besnard et al., 2010).

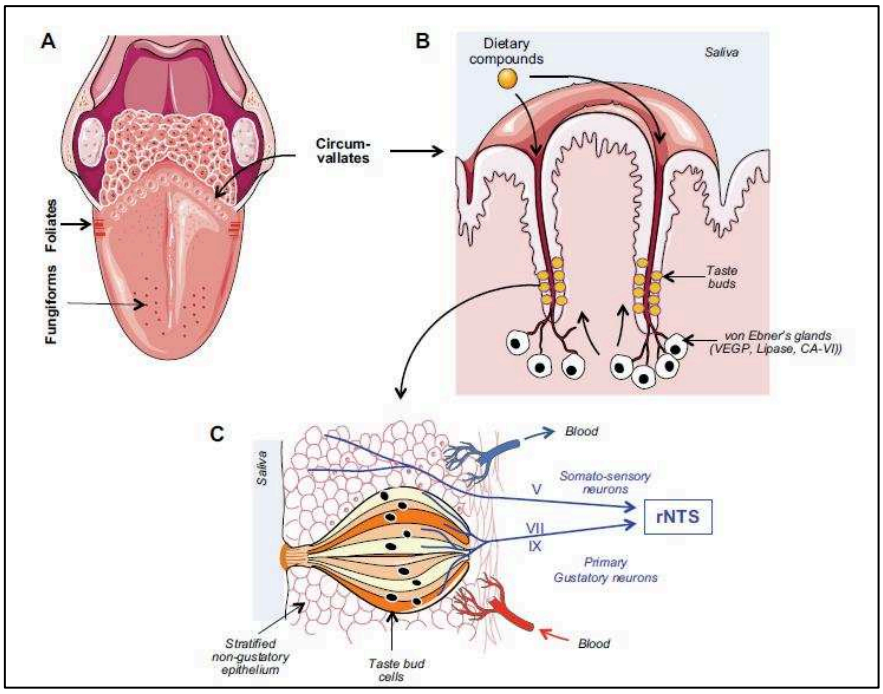

Figure 4. **Peripheral gustatory system.** A. Localization of circumvallate, fungiform and foliate papillae on the human tongue. B. Sagittal section of a circumvallate papilla (CVP) characterized by a dome-shaped structure highlighting the vicinity with the von Ebner glands. C. Schematic representation of a taste bud. CA-VI carbonic anhydrase, rNTS rostral nucleus of the solitary tract, VEGP protein of the Ebner glands, V trigeminal terminations, VII afferent fibers of the nerve of the tympanic chord, IX afferent fibers of the glossopharyngeal nerve. Adapted from Besnard et al. (2016).

1.2. Taste physiology: from detection to perception

The oral detection of tastants by gustatory papillae, as mentioned above, triggers signals that are transmitted by the afferent gustatory fibers from the chorda-tympani (CT) and glossopharyngeal (GL) nerves (cranial nerve VII and IX, respectively) to the nucleus of tractus solitaries (NTS) (Figure 5-1). The NST receives information from the peripheral gustatory detection system, the digestive tract and the brain and is connected to different brain areas. The NST also sends gustatory signals towards the digestive tract via afferent vagal fibers (Figure 5-2). This cephalic phase reflex prepares, by anticipation, the digestive tract to food digestion (Power and Schulkin, 2008). The NTS projects taste signals to the primary gustatory cortex (PGC) constituted of the anterior insula and the frontal operculum (Fop). PCG is involved in identification and evaluation of intensity of tastants. Then, inputs from PGC reach the orbitofrontal cortex (OFC) which is involved in the evaluation of reward value associated with taste (Figure 5-3). The OFC is a sensory platform that receives inputs linked to food palatability such as smell or texture and send important informations with the reward pathway, constituted by the mesolimbic system, about hedonic experience (liking) and incentive salience (wanting) (Berridge, 1996). The mesolimbic system, an integrated pathway including the amygdala and hippocampus are involved in learned memory and the ventral tegmental as well as nucleus accumbens are mainly involved in the motivation of eating behaviour (Besnard, 2016). This system is responsible for taste perception and builds the hedonic value (Figure 5-4).

Post-ingestive regulatory signals provide real-time information about the feeding status to the hypothalamus that constitutes the “metabolic brain”. It is involved in the control of food intake and energy expenditure (Figure 5-5). It has been shown that taste-responsive neurons directly project to the lateral hypothalamus (Li et al., 2013) suggesting that orosensory signals are able to influence homeostatic functions of the hypothalamus and internal signals, in the same way, can modulate the “emotional brain” function (Figure 5-6). Finally, anatomical interconnections link the mesolimbic system and the hypothalamus (Figure 5-7). It emerges that the regulation of appetite is based on a subtle balance between the “emotional brain”, i.e. the hedonic hunger, and the “metabolic brain”.

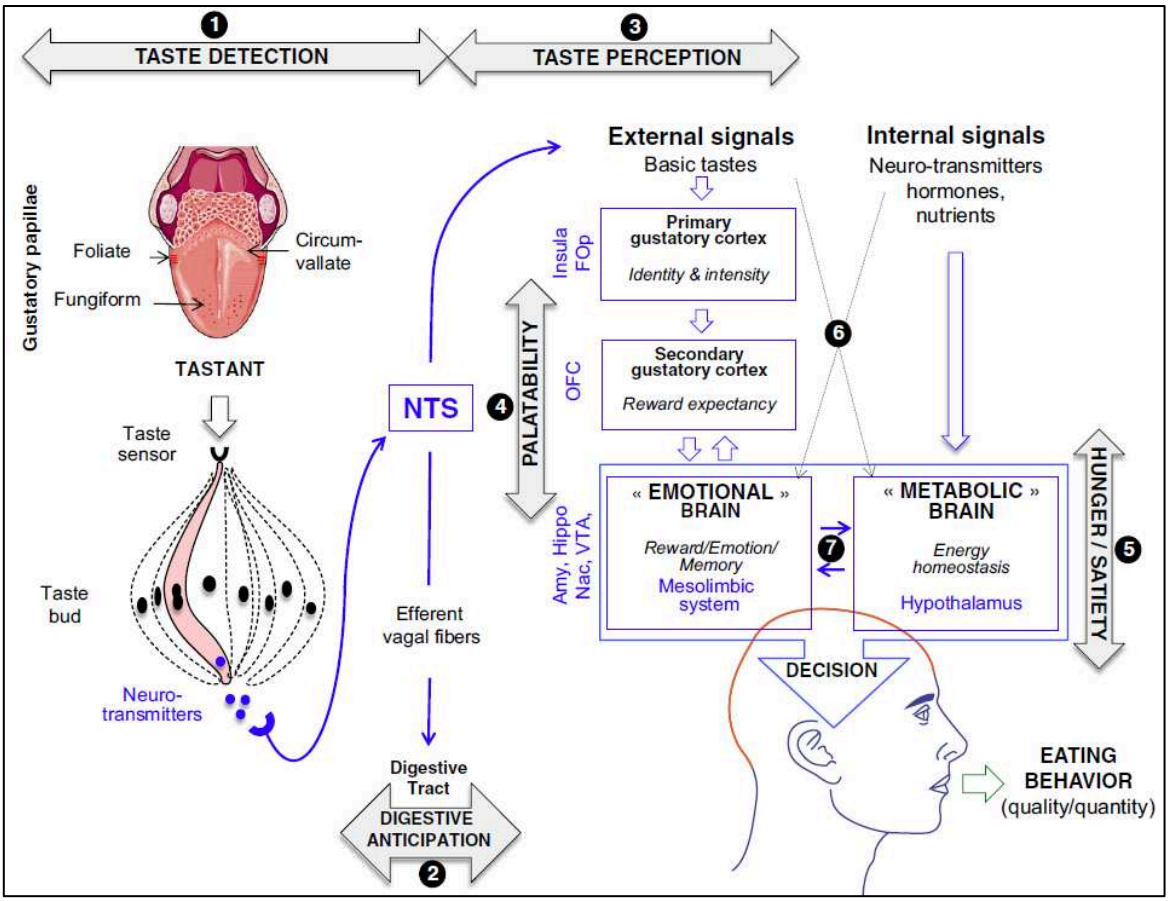

Figure 5. **Taste perception: emotional and metabolic brain involvement.** 1. Oral detection of tastants by gustatory papillae. NTS sends gustatory signals toward the digestive tract allowing a digestive anticipation. 3. Cephalic integration of the taste signals. 4. The mesolimbic system is responsible for taste perception and builds the hedonic value. 5. Post-ingestive regulatory signals provide real-time information about the feeding status. 6. Orosensory signals influence homeostatic functions. 7. Anatomical interconnections link the mesolimbic system and the hypothalamus. NTS, nucleus of tractus solitarius; FOp, frontal operculum; OFC, orbito-frontal cortex. Adapted from Besnard (2016).

2. Orosensory detection of dietary lipids

"Fat" is the term used to refer to triglycerides and fats that are essential components of a traditional food intake in humans. Dietary fatty acid deficiency leads to impaired vision, stunting, skin lesions and reduced learning abilities (Connor et al., 1992). In contrast, overconsumption of fat induces negative health effects such as obesity (Stewart et al., 2011a; Swinburn et al., 2011), diabetes (Ravussin and Smith, 2002) and cancer (Giovannucci et al., 1993; Prentice and Sheppard, 1990).

Fat has been mentioned as a taste modality in 330 years before J-C by Aristotle (Aristotle, 350AD). Recent work has suggested that there is a sixth taste for lipid perception (Khan and Besnard, 2009; Laugerette et al., 2005). To define a flavor as a primary taste, it should meet 4 criteria: a unique class of tastant molecules, a specific reception system, an implication of the

gustatory and physiological pathways. Indeed, the lipid perception system seems to respond to these conditions (Gilbertson and Khan, 2014).

2.1. Fat taste agonists

Dietary fats, i.e., triglyceride, do not appear to be effective stimuli. Indeed, compared to what is observed in intestinal fat sensing, the fat detection in the oral cavity appears to be dependent on the presence of fatty acids (FA). In humans, sensory detection of FA occurs within the millimolar range (0.02–6.4 mM) (Stewart et al., 2010). This detection threshold is consistent with the concentrations of FA naturally present in foods (0.76–3% w/v). It has been asserted that a lipolytic activity in saliva has been shown to be sufficient to produce micromolar amounts of fatty acids within the detectable range (Stewart et al., 2010). Furthermore, lingual lipase appeared to have an influence on fatty acid thresholds as the addition of orlistat (a lipase inhibitor) increased fatty acid thresholds (Pepino et al., 2012).

2.2. Fat taste receptors in taste bud cells

2.2.1. GPR40 and GPR120

G protein-coupled receptors (GPCR) belong to seven-transmembrane domain receptors. Sweet, bitter and umami taste perception is initiated by binding of tastants to specific GPCR. Two GPRCR, GPR120 and GPR40 (G protein-coupled receptor 40), were detected on mouse papillae. GPR120 was co-expressed with TRPM5, a marker of type II taste receptor cells (TRC) (Cartoni et al., 2010) whereas GPR40 was mainly found in type I cells which are not true TRC (Cartoni et al., 2010). Nevertheless, Galindo et al. (2012) failed to detected GPR40, at protein and mRNA levels, in human papillae and Hirasawa et al. (2008) also failed to detect GPR40 in rat gustatory papillae.

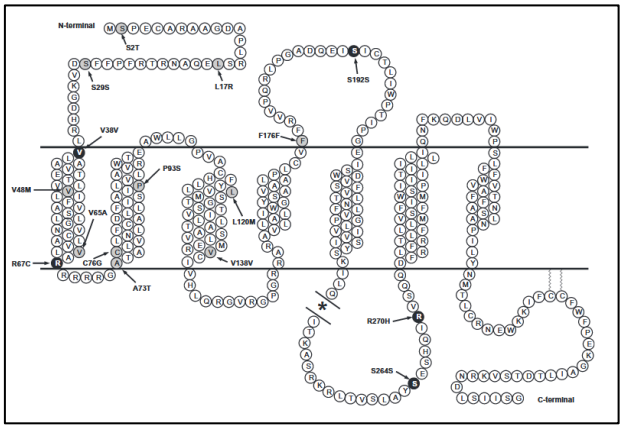

Figure 6. **Predictive structure of human GPR120**. Arrows indicate polymorphisms. Adapted from Mo et al. (2013).

GPR120, also known as FFAR4 (free fatty acid receptor 4) has been identified as an orphan receptor in 2003 (Fredriksson et al., 2003) (Figure 6). GPR120 has been disorphaned in 2005. Indeed, GPR120 is activated by MCFA (Medium-chain fatty acid) and LCFA (Hirasawa et al., 2005). GPR120 is widely expressed in tissues such as brain, adipose tissue, heart and especially in lungs and gut (Hirasawa et al., 2005). GPR120 has been shown to mediate anti-inflammatory effects (Oh et al., 2010). Over the last few years, GPR120 has raised interest as it has been identified as a potential target for metabolic disorders such as type 2 diabetes mellitus or obesity (Mo et al., 2013; Ulven and Christiansen, 2015).

GPR120 was initially reported to be a plausible candidate for LCFA detection by taste buds because GPR120$^{-/-}$ mice were unable to properly detect an oily source during a behavioural test (Cartoni et al., 2010). Nevertheless, this finding has not been reproduced (Ancel et al., 2015; Sclafani et al., 2013). Moreover, healthy volunteers subjected to two-alternative force choice (AFC) tests were unable to detect an oral sensation consecutive to a pharmacological activation of GPR120 (Godinot et al., 2013). These inconsistencies strongly suggest that GPR120 is not a major player in oral fat detection. In contrast, an implication of GPR120 in the LCFA mediated release of the incretin hormone glucagon-like peptide-1 (GLP-1) by mouse TBC is likely, as previously demonstrated in enteroendocrine L-cells (Hirasawa et al., 2005). Indeed GPR120 and GLP-1 are found to be co-localized in a subset of mouse TBC (Martin et al., 2012). Moreover, LCFA elicit GLP-1 release by freshly isolated mouse circumvallate papillae (Martin et al., 2012). Ozdener et al. (2014) clearly demonstrated that GPR120 plays an alternative role in lingual detection of fatty acids. In fact, in the fasting situation, GPR120 is downregulated and in a fed-situation, it is upregulated in the raft fraction of mouse TBC.

2.2.2. CD36

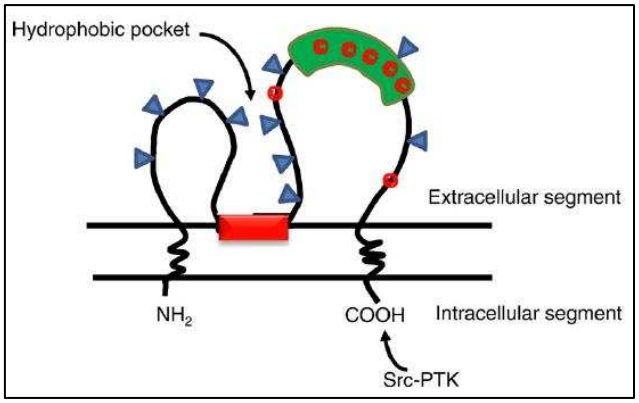

Figure 7. **Structural features of CD36**. It is noteworthy the hair-pin loop structure of the extracellular segment, suitable for the LCFA binding. Solid triangles show potential glycosylation sites. The red-coloured box shows a potential membrane-embedded domain. The green-coloured box on the outer membrane segment shows a proline rich region with the location of cysteine residues indicated within red circles. Adapted from Khan and Besnard (2009).

The Cluster of Differenciation 36 (CD36) formerly known as FAT for fatty acid translocase is a multifunctional glycoprotein. CD36 has been first identified 50 years ago (Silverstein and Febbraio, 2000) in mammary globules. The protein has been very well conserved over 300 million years (Ozbek et al., 2010). The first ligand has been described in 1993. Indeed, CD36 has been identified as a macrophage receptor for oxidized low density lipoprotein (Endemann et al., 1993).

CD36 is expressed in various tissues such as the heart, the kidney and the gut and is involved in various functions such as immunity, inflammation, angiogenesis, thrombosis or atherogenesis. CD36 has also been found to be a lipido-sensor in numerous organs including the brain, the lingual and the gut epithelium suggesting a functional continuity between these tissues (Martin et al., 2011a).

CD36 is an integral membrane ditopic glycoprotein with a large extracellular hydrophobic hair-spin structure between two short cytoplasmic tails (Figure 7) (Abumrad et al., 1993; Greenwalt et al., 1992). It belongs to scavenger receptor family and expresses the binding sites for a number of ligands such as fatty acids, thrombospondin-1, oxidized low-density lipoproteins (LDL), growth hormone (GH)-releasing peptides (Silverstein and Febbraio). CD36 exhibits high affinity (in the order of nanomolar) for fatty acids (Baillie et al., 1996) (Figure 8). CD36 appeared to be highly expressed on the apical region of mice circumvallate papillae. The foliate and fungiform papillae also express this glycoprotein but to a lesser

extent. In human beings, CD36 has been detected in human foliate and circumvallate papillae (Simons et al., 2011).

Family type	CD36	GPR120 (FFAR4)
FA binding specificity	LCFA ≥16C	MCFA-LCFA ≥ 14C
LCFA affinity	nM	µM
KO mouse models	~~CD36~~ = GPR120 → LCFA detection : No	~~GPR120~~ = CD36 → LCFA detection : Yes
Lipid-mediated regulation in TBC	Yes	No
Attraction for fat	Yes	No

Figure 8. **Comparison of the main characteristics of CD36 and GPR120 in the mouse.** FA, fatty acid; KO, knockout; LCFA, long-chain fatty acid; MCFA, medium-chain fatty acid; TBC, taste bud cells. Adapted from Besnard et al. (2016).

In mice, the CD36 deletion completely suppressed spontaneous preference for lipid solutions (Laugerette et al., 2005). This effect on feeding behaviour is lipid-specific since sweet preference and bitter aversion were not affected in these transgenic mice. It is remarkable that CD36 failed to influence post-oral fat conditioning in mice. Indeed, Sclafani and Ackroff (2012) and Sclafani et al. (2007) reported that CD36 deletion decreased fat preference and consumption but also enhanced the ability of mice to compensate for the calories provided by their optional fat intake in fat conditioned experiments. They also proposed that CD36 might be involved in fat "detection" when GPR120 whereas take part in the regulation of post-ingestive lipid behaviour.

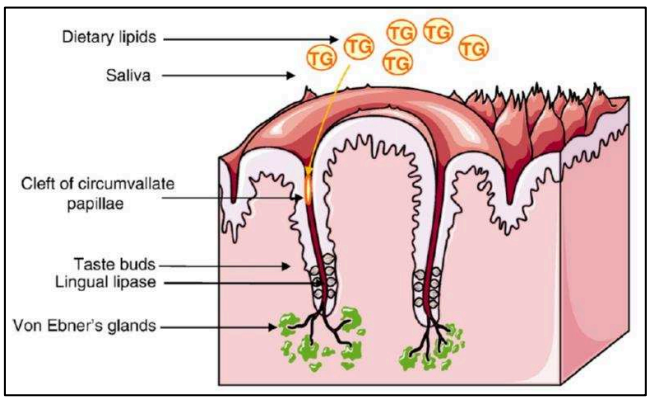

Figure 9. **Localization of lingual von Ebner's gland.** Sagittal section of a circumvallate papilla (CVP) with its typical dome-shaped structure and its anatomical relationship with the von Ebner glands. Adapted from Khan and Besnard (2009).

The local release of lingual lipase by the von Ebner's gland in the clefts of circumvallate papillae (Kawai and Fushiki, 2003) exposes the CD36 directly to a microclimate potentially rich in long-chain fatty acids (LCFA) (Figure 9). This organization allows the degradation of triglyceride into fatty acids (FA) that promote the fat detection in the oral cavity.

2.2.3. Others candidates

Other orphans GPCR have been suggested to bind fatty acids. GPR41and GPR43 are receptors for the short chain (C2:0–C6:0) saturated fatty acids (Galindo et al., 2012; Miyamoto et al., 2016; Ulven, 2012) whereas GPR84 appears to be predominately activated by medium chain (C8:0–C14:0) fatty acids (Venkataraman and Kuo, 2005; Wang et al., 2006). Besides, GPR41 and GPR84 have been shown to be abundantly expressed by posterior, probably circumvallate, papillae in rat (Gilbertson et al., 2010) whereas absent on fungiform papillae. As for GPR43, the receptor was present on both mouse fungiform and posterior papillae. There are currently no functional data on GPR41/43 in the taste system, preliminary data suggest that mouse TBC respond to medium chain fatty acids like myristic acid and lauric acid that are ligands of GPR84. However, the role of these TBC-expressed fatty acid-activated GPCR in fat taste perception remains to be elucidates in detail.

2.3. Signalling transduction mediated by LCFA in taste bud cells

During the eating period, LCFA bind to CD36, followed by the communication with GPR120. This binding triggers intracellular signal transduction involving successively the Src-PTK, phospholipase C (PLC) activation and inositol trisphosphate (IP_3) production which induces Ca^{2+} release by the endoplasmic reticulum (ER) (Figure 10-1). Ca^{2+} activates stromal interaction molecule (STIM1) reinforcing the $[Ca^{2+}]_i$ accumulation by opening Orai1 and Orai1/3 channels (Figure 10-2). The rise in $[Ca^{2+}]_i$ induces a TBC depolarization via TRPM5 channels (Figure 10-3). LCFA also block the delayed rectifying potassium channels (DRK) channels via CD36 translocation or directly in order to maintain the cell activation (Figure 10-4). The Ca^{2+} rise produces cell depolarization leading to ATP efflux triggering serotonin (5-HT) release by type III cells via the calcium homeostasis modulator 1 (CALHM1) channels (Figure 10-5). Indeed, Subramaniam et al. (2016) showed that LA-evoked Ca^{2+} signalling and ERK1/2 phosphorylation were decreased in CALHM1-deficient TBC. Moreover, $Erk1^{-/-}$ and $Calhm1^{-/-}$ mice exhibit reduced preferences for oily solutions. These results suggested that ERK1/2-MAPK cascade and voltage-gated CALHM1 Ca^{2+} channels are involved in oro-

gustatory detection of dietary lipids (Subramaniam et al., 2016). ATP leads to the activation of primary gustatory neurons, type II and type III cells (Figure 10-6). Then, 5-HT mediates the modulation of the taste signal (Figure 10-7). It is noteworthy that FFA could also convey the taste signal to the NST via the non gustatory epithelium and the trigeminal endings (Figure 10-8).

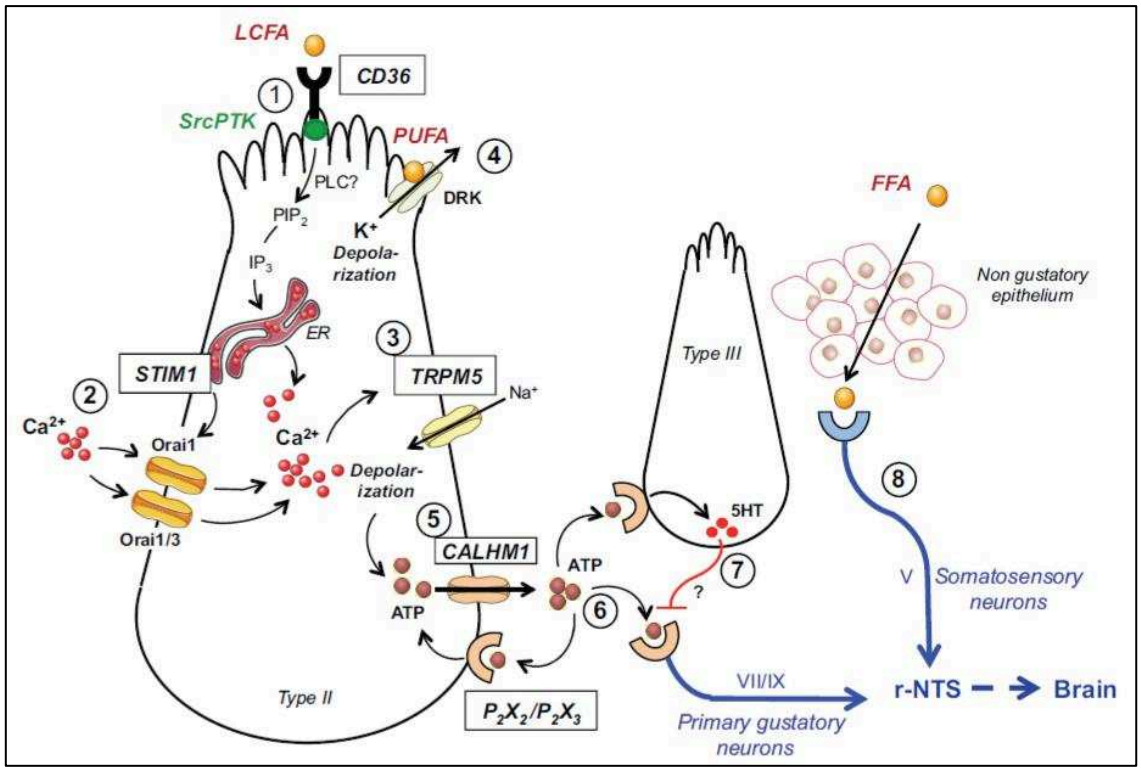

Figure 10. **Signalisation induced by LCFA (working model).** 1. LCFA binds to CD36 and activates the cascade of PLCβ-IP3 thus releasing Ca^{2+} from the ER pool. 2. The depletion of the ER Ca^{2+} pool induces the aggregation of STIM1 toward SOC channels like Orai1 and Orai1/3. 3. The Ca^{2+} increase also activates the TRPM5 channels responsible for the cell depolarization. 4. The activation of the DRK channels is triggered by the Na^{+} influx. 5. Ca^{2+} rise produces cell depolarization leading to ATP efflux. 6. ATP leads to the activation of primary gustatory neurons, type II and type III cells and to 5-HT liberation from Type III cells. 7. 5-HT mediates the modulation of the taste signal. 8. FFA could also convey the taste signal to the NST via the non gustatory epithelium and the trigeminal endings Obligatory genes for the "fatty" taste transduction are shown framed. FFA, free fatty acids; LCFA, long-chain fatty acids; PUFA, polyunsatured fatty acids; 5-HT, serotonin; r-NTS, rostral nucleus of solitary tract; V,trigeminal nerve; VII, chorda tympani nerve; IX, glosso-pharyngeal nerve. Adapted from Besnard et al. (2016).

After cell depolarisation, neurotransmitters such as 5-HT are secreted toward the afferent gustatory nerve fibres (specifically VIIth and IXth cranial nerves) (Passilly-Degrace et al., 2014), which relay the signal to the nucleus of solitary tract (NST), and then to the brain stem and digestive tract (Besnard et al., 2016a; El-Yassimi et al., 2008; Gilbertson and Khan, 2014; Passilly-Degrace et al., 2014) (Figure 10).

2.3.1. CD36/GPR120 cooperation hypotheses

CD36 and GPR120 are co-expressed in mouse and human TBC (Martin et al., 2012; Ozdener et al., 2014) and both are able to bind and to be activated by LCFA (Baillie et al., 1996; Hirasawa et al., 2005). They are two putative gustarory lipid receptors however, a functional redundancy seems unlikely.

2.3.1.1. Direct cooperation hypothesis

This hypothesis suggests that the simultaneous presence of CD36 and GPR120 in TBC is absolutely required to properly detect the presence of LCFA in saliva. It was postulated that LCFA binding to CD36 should activate GPR120 to initiate the signal transduction cascade leading to transmitter release and, hence, fat detection (Gilbertson and Khan, 2014; Gilbertson et al., 2010). This prioritization is based on the fact that CD36 displays a greater binding affinity for LCFA than GPR120 and is known to act as a cofactor facilitating recognition of derived lipids by Toll-like receptors in the mouse (Hoebe et al., 2005). However, this scenario is challenged by behavioural studies performed using knockout mouse models Indeed, detection and preferential consumption of lipid sources are not affected by a targeted GPR120 gene deletion (Ancel et al., 2015; Sclafani et al., 2007), likely because CD36 gene expression in circumvallate papillae (CVP) is maintained in this mouse model (Ancel et al., 2015). Therefore, a role for GPR120 as a primary lipid receptor responsible for the oral fat detection appears to be largely speculative (Besnard et al., 2016).

2.3.1.2. Indirect cooperation hypothesis

An indirect cooperation between CD36 and GPR120 is suggested by several observations. Indeed, hormones and LCFA are known to regulate the membrane localization of CD36. In the digestive tract, the presence of LCFA in the intestinal lumen also induced a rapid disappearance of CD36 from the apical side of enterocytes, leading to a partial degradation by the ubiquitin-proteasome pathway (Tran et al., 2011). This ligand-mediated negative feedback likely constitutes a physiological desensitization mechanism that progressively abolishes the LCFA-mediated CD36 signalling in enterocytes during the postprandial period (Tran et al., 2011). A similar dynamic regulation of CD36 seems to exist in TBC. Indeed, lipid deposition onto the lingual epithelium is enough to induce a dramatic decrease in the CD36 protein levels in mouse CVP (Martin et al., 2011b). In line with this observation, CD36 in CVP displays a diurnal variation, with a progressive decrease during the active feeding period (i.e., dark

period). It is tempting to speculate that this downregulation of CD36 might lead to a progressive decrease in the motivation to eat fatty foods during a meal as lingual CD36 is significantly involved in the avidity for lipids (Laugerette et al., 2005; Martin et al., 2011b; Ozdener et al., 2014; Sclafani et al., 2007). Conversely to CD36, GPR120 levels in the mouse CVP remains globally stable throughout the day and appear to be insensitive to the lipid content of the diet (Martin et al., 2011b).

It is also known that GPR120 is broadly expressed in the distal small intestine and colon, where it is coexpressed with GLP-1 in the enteroendocrine L cells (Hirasawa et al., 2005). During the prandial period, stimulation of GPR120 by LCFA promotes the secretion of intestinal GLP-1, increasing circulating insulin (Hirasawa et al., 2005). GLP-1 is known to modulate sweet and umami taste sensitivities in the mouse (Martin et al., 2009; Shin et al., 2008). Behavioural experiments, conducted using GLP-1R$^{-/-}$ mice, have shown a reduced capacity to detect low concentrations of rapeseed oil, demonstrating for the first time that GLP-1 also modulates the fat taste sensitivity (Martin et al., 2012). The molecular mechanism by which this control takes place is not yet fully established. However, the dynamic downregulation of CD36 in CVP, normally triggered by LCFA (Martin et al., 2011b), was lacking in GPL1-R$^{-/-}$ mice (Martin et al., 2012). This finding provides the first evidence that GRP120 might be able to modulate the CD36-mediated detection of oral lipid, via GLP-1 secretion.

Thus, Besnard et al. (2016) proposed the following “indirect cooperation” scenario (Figure 11). At the beginning of a feeding period, LCFA binding to CD36 into lipid rafts (Pohl et al., 2005) results in the formation of a multimolecular complex of transmembrane proteins, including adaptors (Heit et al., 2013), members of Src protein-tyrosine kinase (Src-PTK) , and caveolin-1 (Cav1) (Fig 11-1). This multimolecular platform should be able both to trigger a downstream signal and to limit its duration by the partial degradation of the ligand-receptor complex. Indeed, because Src kinases activate PLC (Makranz et al., 2004), this event might result in a signalling cascade responsible for a rapid rise in Ca^{2+} levels, initiating neurotransmitter release and, in turn, transfering a lipid signal to the brain (Figure 11-2).

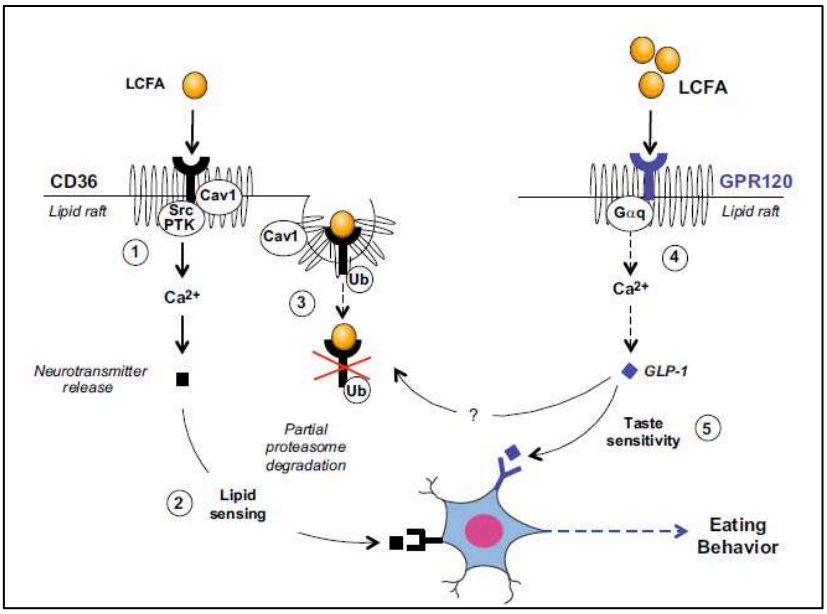

Figure 11. **Respective role of CD36 and GPR120 in taste bud cells: indirect cooperation hypothesis**. 1. LCFA binding to CD36 into lipid rafts results in the formation of a multimolecular complex of transmembrane proteins, including adaptors, members of Src protein-tyrosine kinase (Src-PTK) , and caveolin-1 (Cav1). 2. This multimolecular platform should be able both to trigger a downstream signal and to limit its duration by the partial degradation of the ligand-receptor complex. 3. Ubiquitination of CD36 induces a gradual decrease in the attraction for fat during the meal. 4. When the oral LCFA levels become sufficient to allow the binding and activation of GPR120 in lipid raft triggers the GLP-1 release by TBC. 5. GLP-1 acts on its cognate receptor located in gustatory nerve endings to modulate taste sensitivity. LCFA, long-chain fatty acid; Src PTK, Src protein tyrosine kinases; Cav1, caveolin 1; Ub, ubiquitination; Gαq, protein G; GLP-1, glucagon like peptide-1. Adapted from Besnard et al. (2016).

Ubiquitination of CD36, by inducing its progressive removal from the plasma membrane (Su and Abumrad, 2009), might allow its subsequent partial degradation by the proteasome pathway (Tran et al., 2011), inducing a gradual decrease in the attraction for fat during the meal (Martin et al., 2011b) (Figure 11-3). The molecular mechanism by which this negative feedback takes place in TBC remains to be elucidated. One potential route of internalization of CD36 is the caveolae-dependent pathway used by toll-like receptors (TLR) (Shuto et al., 2005). Studies showing that Caveolin-1 (Cav1) gene disruption is associated with altered subcellular localization and function of CD36 (Ring et al., 2006) are consistent with this hypothesis. When the oral LCFA levels become sufficient to allow the binding and activation of GPR120 in lipid raft (Ozdener et al., 2014) triggers the GLP-1 release by TBC (Martin et al., 2012; Ozdener et al., 2014) (Figure 11-4). GLP-1 acts on its cognate receptor located in gustatory nerve endings (Shin et al., 2008) to modulate taste sensitivity (i.e., fat, sweet, and umami tastes; Figure 11-5). The use of Glp-1r$^{-/-}$ mice has revealed an involvement of GLP-1 in the dynamic regulation of CD36 by LCFA (Martin et al., 2012). The mechanism by which this regulation takes place is presently unknown (Figure 11).

It seems that chemical information combines with textural signals to form the complete sensory perception of fat (Liu et al., 2016).

2.4. Gustatory and reward brain circuits

Peterschmitt et al. (2018) recently studied the implication of gustatory and reward brain circuits following the lingual application of LCFA (LA). They found that LA induced c-Fos expression in the NST, the parabrachial nucleus (PBN) and the ventroposterior medialis parvocellularis (VPMPC) of the thalamus, that are regions known to be activated by gustatory signals. LA also induced c-Fos expression in central amygdala and ventral tegmental area (VTA) involved in food reward. Finally, they observed that LA induced high expression of genes involved in memory consolidation in the arcuate nucleus (Arc) and hippocampus (Hipp). They consequently proposed the following model: LA deposition induces the activation of the gustatory pathway. The gustatory signal is conveyed to the NST that transmits the information to the PBN. Next, the gustatory part of the thalamus (VPMPC) is activated and the signal reaches the gustatory insular cortical areas (AI,GI/DI) with modulatory influences of the central amygaloid nucleus (CeA) and the posterior part of the lateral hypothalamus, the parasubthalamic nucleus/calbindin nucleus (PSTN/CbN) (Figure 12). Thus, they demonstrated that oral lipid taste perception triggers both gustatory and reward pathways.

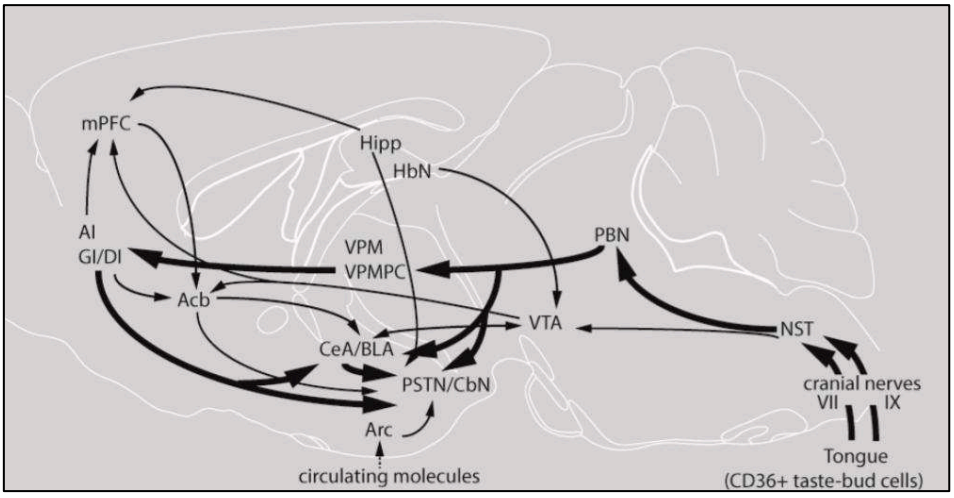

Figure 12. **Schematic representation of the gustatory pathway activation involving metabolic, reward and learning, and memory processes.** Acb, accumbens nucleus; AI, agranular insular cortex; Arc, arcuate nucleus; BLA, basolateral amygdaloid nucleus; CeA, central amygdaloid nucleus; GI/DI, granular/dysgranular insular cortex; Hbn, habenula; Hipp, hippocampus; mPFC, medial prefrontal cortex; NST, nucleus of the solitary tract; PBN, parabrachial nucleus; PSTN/CbN, parasubthalamic nucleus/calbindin nucleus; VPM, ventral posteromedial thalamic nucleus; VPMPC, ventroposterior medialis parvocellularis; VTA, ventral tegmental area. Adapted from Peterschmitt et al. (2018).

3. Orosensory detection of bitter compounds

3.1. Bitter taste agonists

The ability to taste bitterness is linked to a critical function for survival allowing animals to discriminate safe from potentially harmful foods. Thank to its innate negative hedonic value (Steiner et al., 2001), bitterness perception prevents feeding, and differences in bitter taste sensitivity influence human dietary behaviour. The bitter taste perception is controlled by the taste 2 receptor (TAS2R) gene family. There are 25 human TAS2R genes (Drayna, 2005) with many associated polymorphisms and a wide range of individual differences in bitter taste sensitivity (Chandrashekar et al., 2000). This explains in part why thousands of chemically dissimilar agonists can elicit a single taste quality. The most common role is played by TAS2R16 and TAS2R38.

3.1.1. TAS2R16

TAS2R16 is a bitter taste receptor gene located on chromosome 7, has traditionally been associated with the detection of toxic β–D-glucopyranosides, such as salicin from willow bark (Bufe et al., 2002; Greene et al., 2011; Soranzo et al., 2005). Several studies have also reported that bitter taste receptors are expressed in cell types that are not directly involved in oral sensory perception. Indeed, nucleotide polymorphisms in or near the coding exon of TAS2R16 have been identified as risk factors for alcohol and nicotine dependence (Hayes et al., 2011; Hinrichs et al., 2006; Mangold et al., 2008) in African Americans. These polymorphisms have also been correlated with differences in lifespan and senescence among individuals in an isolated European population from southern Italy (Campa et al., 2012). Thus, bitter taste receptors participate in a variety of biological processes, including metabolism and immune response.

3.1.2. TAS2R38

The TAS2R38 gene is one of the most widely studied. TAS2R38 encodes a seven transmembrane G protein-coupled receptor (Drayna, 2005). It binds to thiourea group contained in synthetic compounds such as phenylthiocarbamide (PTC) and 6- n-propylthiouracil (PROP) (Kim and Drayna, 2005).

Fox (1932) serendipitously discovered that phenylthiocarbamide (PTC) concentration that tasted extremely bitter for some people were virtually tasteless for others. Indeed, sensitivity to PTC, a compound containing an -N-C=S group, is used as a phenotypic marker for oral sensations (Tepper, 2008).

3.2. Signal transduction mediated by bitter in taste bud cells

The generation of bitter taste starts when a bitter compound enters the oral cavity. The ligand binds to a TAS2R receptor expressed in the apical membrane of receptor cells in taste buds. It triggers a cascade of signalling events, involving α-gustducin, PLCβ2 and inositol trisphosphate (IP_3) activation (Zhang et al., 2003b), leading to Ca^{2+} influx and ultimately to the release of neurotransmitter. The neurotransmitters activate an afferent nerve fiber that transmits the signal via the cranial nerves VII and IX to the brain (Figure 13). The taste signals transit through the brain and provide input to circuits involed in various functions, such as physiological reflexes, discriminative perception, and affective processing. It illustrates the complexity of the mechanisms intervening between the application of the bitter stimulus and the generation of the behavioural response.

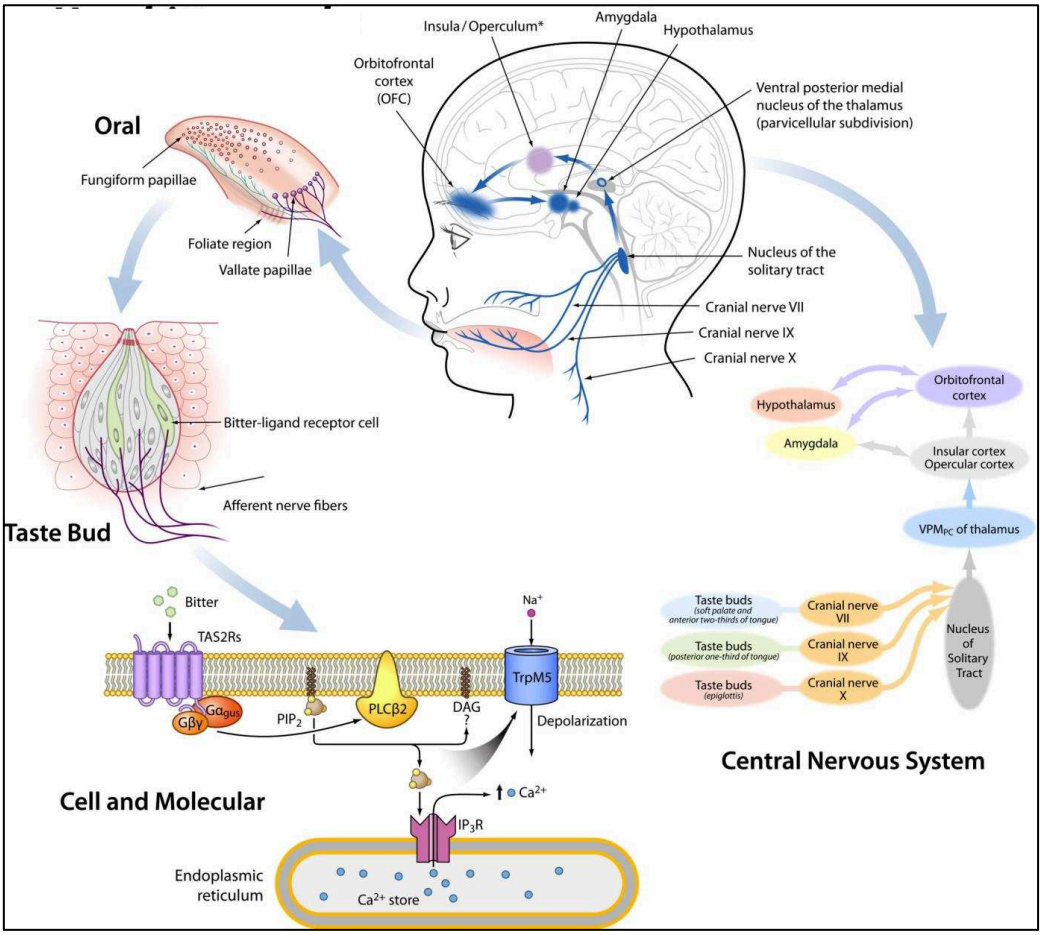

Figure 13. **Mechanism occuring between the application of the bitter tastant and the generation of the behavioral response.** Ligand binds to a T2R G-protein coupled receptor expressed in the apical membrane in taste buds triggereing a cascade of signaling events, leading to the release of neurotransmitters. This mechanism activates afferent nerve fibers that transmit the signal via the cranial nerve to the brain. Papillae are distributed in distinct fields in the oral, pharyngeal and laryngeal epithelia with each field innervated by a different cranial nerve branch. VPMPC, ventral posterior medial nucleus, parvocellular subdivision. Adapted from Mennella et al. (2013).

Chapter 2
Taste system and its regulatory pathways

1. Factors involved in impaired oro-sensory perception and obesity

1.1. Alteration of the salivary composition

The flow of saliva is increased during a meal and it is utilized to form a moisturized bolus of foodstuff thus soaking the masticated food in salivary enzymes to increase its flavor perception, initiate the digestion, and promote swallowing (Zolotukhin, 2013). Saliva plays a significant role in the orosensory perception of dietary components. Indeed, during a meal, the major part of saliva is produced by the salivary glands and only a few is produced by minor salivary glands such as the von Ebner's glands (VEG; Figure 14). VEG secrete directly into the clefts of papillae, known to house the great majority of taste buds (Figure 14). A preduodenal lipase (LIPPF) has been shown to be synthesized and secreted by VEG in rodents (Hamosh and Scow, 1973), its anatomical location highlights an involvement in promoting the enzymatic release of FFA from dietary triglycerides (TG) directly in the vicinity of taste buds, thus facilitating their detection by the TBC (Figure 14).

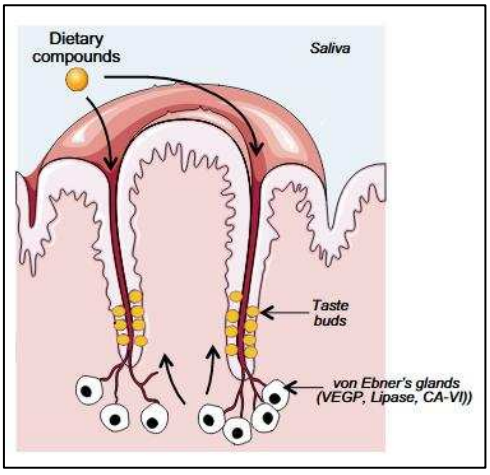

Figure 14. **Location of von Ebner's glands in the circumvallate papillae.** Sagittal section of a circumvallate papillae (CVP) highlighting the vicinity of taste buds with the von Ebner's glands in the clefts of the CVP. Adapted from Besnard et al. (2016).

LIPPF expression is lacking in human VEG (Spielman et al., 1993), nevertheless a similar mechanism involving a lipase activity leading to a partial TG breakdown of FFA is likely to occur in human saliva (Neyraud et al., 2012; Pepino et al., 2012; Stewart et al., 2010; Voigt et al., 2014). Indeed, a recent study revealed the presence of alternative lipase isoforms (LIPK, LIPM, LIPN) that are also known to induce TG cleavage (Voigt et al., 2014). The interindividual variation in oral fat detection could be caused by variability in the expression

of these lipases (Chevrot et al., 2013; Mattes, 2009; Tucker and Mattes, 2013). Indeed, a positive relationship between lipolysis efficacy and intensity of perceived fattiness was recently reported in healthy humans (Neyraud et al., 2012). VEG has also been shown to produce and secrete proteins such as VEG protein (VEGP) belonging to the lipocalin superfamily (Kock et al., 1994; Schmale et al., 1990) (Figure 14). This lipophilic ligand carrier protein is known to bind a remarkable array of small hydrophobic molecules, including LCFA, or bitter compounds. VEGP could also be involved in TBC protection against the detergent effects of FFA (Bläker et al., 1993). VEG also produce carbonic anhydrase VI (CA-VI) involved in the regulation of the acid-base balance in different tissues (Leinonen et al., 2001; Sly and Hu, 1995). CA-VI generates a neutral or slightly alkaline microclimate in the immediate proximity of TBC thought to play a role in taste bud responsiveness by controlling proton-gated K channels involved in TBC depolarization (Leinonen et al., 2001), and by limiting TBC apoptosis (Henkin et al., 1999a).

Obesity has been implicated in impaired salivary secretion. Lasisi et al. (2018) showed that the re-establishement of normal diet in high fat-diet-induced obesity rats reversed the altered salivary composition.

Although it is well established that saliva plays a significant role in taste receptor activation by orally applied chemical compounds, its involvement has not been extensively studied. Variations of general taste sensitivity as been associated with differences in the expression of salivary proteins, such as carbonic anhydrase 6 (Feeney and Hayes, 2014). Also, oral sensitivity to PROP is related to expression of specific salivary proteins (Glendinning, 1992; Mennella and Beauchamp, 2008). Perception of PROP bitterness is also governed by chemical composition of saliva (Melis et al., 2013a). Mennella et al. (2013) highlighted that proline-rich proteins found in saliva can bind with bitter-tasting tannins found in some foods, increasing their acceptability (Glendinning, 1992). Moreover, proline-rich proteins arise from gene clusters that are interleafed with bitter receptor genes, highlighting a common regulatory mechanism and function (Cabras et al., 2012). Besides, greater PROP responsiveness has been also shown to be related to high salivary levels of Ps-1 and II-2, two specific proteins that belong to the basic proline-rich protein family (bPRP) (Cabras et al., 2012; Melis et al., 2013a).

Melis et al. (2015) explained that the formation of this PROP•ArgH+hydrogen-bonded adduct could enhance bitterness intensity by increasing the solubility of PROP in saliva and its availability to receptor sites. Thus, L-Arginin could act as a ‘carrier’ of various bitter

molecules in saliva and enhance PROP taste responsiveness by facilitating the availability of PROP molecule at receptor site.

1.2. Alteration in the oral microbiota

The oral microbiota is comprised of about 700 bacterial species (Paster et al., 2001). The bacteria of the oral cavity can be altered in pathological conditions such as oral cancer and dental caries. DiBaise et al. (2008) first demonstrated a direct association between gut microbiota and obesity. Germ-free C57Bl6 mice infected by fecal samples from WT mice displayed 60% more fat than their germ-free counterparts. The bacteria responsible from this effect belonged to the Firmicutes phylum. The author proposed that subjects predisposed to obesity may have Firmicutes gut levels that promote more efficient extraction and/or storage of energy from the diet. 1 gram of bacteria (10^{11} bacteria) is swallowed daily with the saliva (Socransky and Haffajee, 2005). It is likely that salivary microbiota would affect gastointestine microbiota. Furthermore, it has been shown that periodontitis has been correlated with overweight conditions (Socransky and Haffajee, 2005; Wood et al., 2003). Goodson et al. (2009) showed that 98.4% of the overweight women could be identified by the presence of Selenomonas noxia bacterium (belonging to the Firmicutes phylum) at a level greater than 1.05% of the total salivary bacteria. The authors also proposed different mechanism by which oral bacteria could affect body weight (Figure 15).

First, the oral bacteria may contribute to increased metabolic efficiency. Indeed, by this mechanism, even a small excess in calorie consumption might result in weight gain. For example, an increase by 5% (100 calories/day) would add approximately 10 pounds of fat per year. A second hypothesis is that oral bacteria could increase weight gain by increasing appetite. By stimulating host appetite, the bacteria get more to eat. However, no data is available to corroborate this assumption. A third hypothesis is that oral bacteria redirect energy metabolism by facilitating insulin resistance through increasing levels of tumor necrosis factor α (TNFα) or reducing levels of adiponectin leading to increased fat storage.

The composition of salivary bacteria seems to change in overweight subjects. It seems likely that S. noxia could serve as biological indicators of change in oral microbial ecology and of a developing overweight condition. Of even greater interest is the possibility that oral bacteria may participate in the pathology that leads to obesity (Goodson et al., 2009).

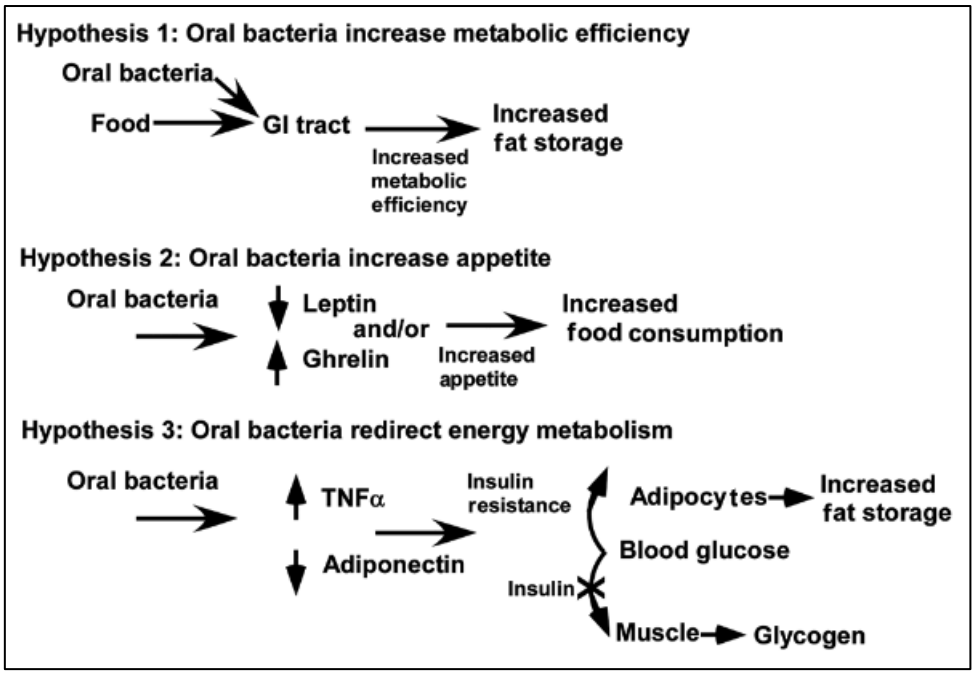

Figure 15. **Three possible hypotheses by which oral bacteria could affect body weight and contribute to obesity**. Hypothesis 1, the oral bacteria may contribute to increased metabolic efficiency. Hypothesis 2, the oral bacteria increase appetite. Hypothesis 3, the oral bacteria redirect energy metabolism. Adapted from Goodson et al. (2009).

1.3. Inflammatory status

It has been shown that overweight or obese people were related to an increase in inflammatory dental and periodontal diseases with an altered health profile and plasma inflammatory mediators (Thanakun et al., 2017). Wang et al. (2009) suggested that TLR and interferon (IFN) pathways function collaboratively in recognizing pathogens and mediating inflammatory responses in taste tissue. This process, however, may interfere with normal taste transduction and taste bud cell turnover and contributes to the development of taste disorders.

Acute lipopolysaccharide (LPS)-induced inflammation appeared to inhibit proliferation from taste progenitor cells, to decrease the number of newly born cells entering taste buds and to shorten the average lifespan of mature TBC (Cohn et al., 2010). It has been shown that a state of low-grade chronic inflammation plays a crucial role in obesity-related metabolic disorders (Hotamisligil, 2006; Shoelson et al., 2006). Indeed, the increased amount of visceral adipose tissue is linked to pro-inflammatory cytokine productions, such as tumor necrosis factor α (TNFα) and interleukin 6 (IL6). This production is up-regulated in obese subjects and is likely to act on receptors in tast buds, such as cell death cascades (Feng et al., 2014, 2015). TNFα is known to be a key mediator of obesity-related pathologies. Ideed, $TNF\alpha^{-/-}$ mice exhibit higher insulin sensitivity, lower free-fatty acid levels, triglycerides and leptin levels (Uysal et al.,

1997; Ventre et al., 1997). It has been shown that, in HFD-fed mice, expression of TNFα in taste tissue was significantly higher compared to lean controls (Kaufman et al., 2018) suggesting that prolonged obesity is linked to inflammatory response in taste epithelium. Moreover, direct injection of TNFα into the tongues of lean mice triggered to an increase in apoptosis in taste buds. Obese $TNF\alpha^{-/-}$ mice did not show a reduction in taste bud abundance or in taste progenitor cells demonstrated in obese WT mice. Finally, mice adiposity-specific deletion of Sel1L, an essential adaptor protein in the ER-associated degradation also know as a universal quality-control sytem, did not show an increase in TNFα expression and taste bud machinery and marker of self-renewal were unaffected (Kaufman et al., 2018).

It has been reported a correlation between taste bud density and perceived taste intensity (Miller and Reedy, 1990). Also, human taste function has been shown to decline with weight gain in college-age males (Noel et al., 2017). Kaufman et al. (2018) showed that obese mice displayed significant reduction in tast bud abundance but with no difference in the size and in the balance between Type I, II and III cells compared to lean mice. These findings highlight that taste dysfunction that occurs in obese subjects may originate from a fundamental change in gustatory morphology arising from a reduction in taste bud abundance.

Kaufman et al. (2018) suggested that their data imply that the metabolic effects of obesity trigger a decline in taste and not merely the oral exposure to fat and that gross adiposity stemming from chronic exposure to a HFD is associated with a low-grade inflammatory response, causing a disruption in the homeostatic mechanisms of taste bud maintenance and renewal. Inhibition of proinflammatory cascades, whether by genetic deletion of TNFα or by resistance to adipogenesis, is sufficient to avert the systematic loss of taste buds due to an obesogenic diet.

1.4. The “obese tongue” phenotype?

The overconsumption of lipid-rich food in obese subjects could be caused by an impairment in fat taste sensitivity linked to an altered gustatory papillae microbial and salivary environment. This may constitute the “obese tongue” phenotype as evoked by Besnard et al. (2018). Indeed, as obesity is associated with a dysbiosis in the gut microbiota (Nicholson et al., 2012), a similary phenomene could occur at the oral level. Moreover, the bacterial composition in saliva from overweight subjects appeared to be different from lean subjects (Goodson et al., 2009) as mention in the 1.2. section. The composition of the oral microbiota is highly sensitive to environmental condition changes such as variation in the salivary flux

and composition (Avila et al., 2009) and obesity is likely to be associated with modifications in salivary parameters (saliva or lipase activity) that are involved in oral fat detection (Vors et al., 2015). It appeared that the salivary composition was limited between normal weighted and obese subjects revealing no association between BMI and oral ecology. However, the salivary composition from non taster subjects, i.e. subjects that experience the sense of taste with far lower intensity than taster subjects, were characterized by a greater bacterial variety including members of families belonging to Gram-negative species linked to pro-inflammatory LPS molecules. Conversaly, salivary from taster subjects displayed increased abundance of the Lactobacillaceae family that exhibits anti-inflammatory functions. Thus, resident CVP microbiota in non taster subjects is prone to local inflammation that could impact the sensitivity of fat taste perception. The salivary composition also highlighted differences between taster and non-taster subjects (Figure 16). Besnard et al. (2018) proposed the following working hypothesis: taster subjects are characterized by an oral microbiota prone to decrease local inflammation contrary to non-taster subjects (Figure 16-1). Furtermore, taster subjects exhibit a salivary lysozyme activity associated with a lower bacteria diversity (Figure 16-2) and with fat emulsion destabilization that facilitates the release of LA that will activate lipid sensors in TBC (Figure 16-3). Finally, the total antioxidant capacity of saliva in taster subjects is more important than in non-taster subjects facilitating the lipid sensing by protecting LA and the gustatory epithelium against, respectively, lipoperoxidation and tissue damage (Figure 16-4). Thus, non-taster subjects are characterized by an oral microbiota prone to increase local inflammation (Figure 16-5), a sustained salivary flux that might prevent access of LA to the TBC (Figure 16-6) and by a high carbonic anhydrase-VI activity, nevertheless, its modality of action remains elusive (Figure 16-7).

Indeed, taster subjects were characterized by higher lysozyme levels which are linked to antimicrobial activity (Jollès and Jollès, 1984), correlated with a weaker bacterial diversity. Moreover, the lysozyme-mediated destabilization of the negatively charged fatty acid has been proposed to increase fatty sensation (Feron and Poette, 2013; Poette et al., 2014). Indeed, CD36 binds ionized LCFA with an affinity in the nanomolar range (Baillie et al., 1996). Besnard et al. (2018) proposed that the higher total antioxidant capacity (Greenberg et al., 1999) of saliva in taster subjects might protect unsaturated FFA (e.g. LA) and taste receptors against the lipoper-oxidation and the tissue damage, respectively (Mounayar et al., 2013), facilitating activation of the fatty acid signalling pathway in taste bud cells. Conversely in the non taster group, salivary flow rate and CA-VI activity were positively correlated to higher

LA detection threshold values (i.e. lower fatty taste sensitivity). A high salivary flow, diminishing by dilution the LA access to taste receptor cells (i.e. CD36), might render these subjects poorly sensitive to lipids. The CA-IV activity was identified as a marker of the oral sensitivity to lipids (Mounayar et al., 2014) and plays a role in the salivary buffer capacity (Peres et al., 2010), the growth and the development of taste buds (Henkin et al., 1999b) and bitter taste sensitivity (Padiglia et al., 2010). Taken altogether, these findings suggest that specific microbial and salivary environments surrounding CVP are involved in the fatty taste sensitivity and suggest the existence of an "obese tongue" phenotype (Besnard et al., 2018).

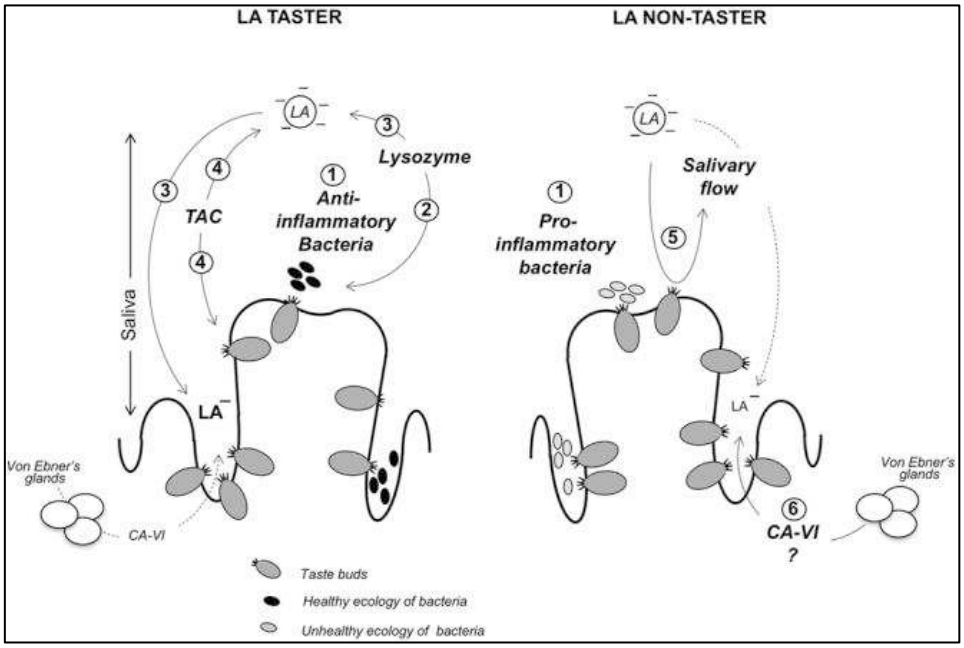

Figure 16. **Involvement of the micro-environment surrounding the gustatory circumvallate papillae (CVP) in tasters (T) and non-tasters (NT) and their putative consequence on the fatty taste sensitivity (working model)**. 1. Tasters are characterized by a host-associated microbiota prone to decrease local inflammation whereas non-tasters are characterized by a microbiota prone to increase local inflammation. 2. In the salivary, the lysozyme activity is associated with a lower bacteria diversity 3. It induces destabilization of the fat emulsion facilitating the release of linoleic acid (LA). 4. The total antioxidant capacity of saliva facilitates the lipid sensing by protecting LCFA and gustatory epithelium against lipoperoxidation and tissue damage. In non-tasters, 5. the salivary flow might decrease the LA access to taste receptors and 6. The involvement of a high carbonic anhydrase-VI activity remains elusive. CA-VI, carbonic anhydrase-VI; TAC, total antioxidant capacity. Adapted from Besnard et al. (2018).

2. Detection of dietary lipids and bitterness by the olfactory system

The olfactory sensation is triggered by the detection of odor ligands by olfactory receptors. The human genome encodes more than 400 olfactory receptors, thus it is hypothesized that a combinatorial code occurs in which one olfactory receptor can be activated by a set of odorant ligands and one odorant ligand can activate a combination of olfactory receptors (Malnic et

al., 1999). The ligand-biding cavity associated with olfactory receptors response cover a large spectrum. Orthonasal activation refers to sniffing through the nose which leads to sensory cells activation in the olfactory epithelium. This is the pathway used to sense odours in the environment. While, retronasal stiulation occurs during food ingestion when volatile molecules released from the food in the mouth are pumped from the back of the oral cavity up through the nasopharynx to the olfactory epithelium (Figure 17).

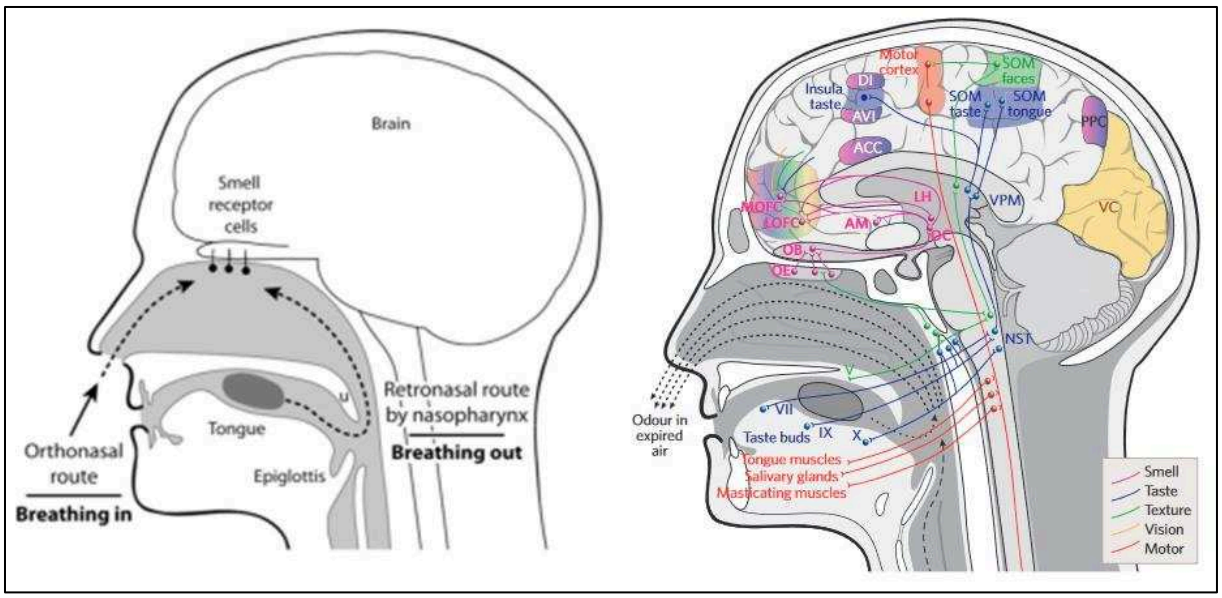

Figure 17. **Orthonasal and retronasal smell mechanism in humans**. (on the left) Orthonasal and retronasal smell humans. (on the right) Brain systems involved in smell perception during retronasal olfaction (breathing out), with food in the oral cavity Air flows indicated by dashed and dotted lines; dotted lines indicate air carrying odour molecules. ACC, accumbens; AM, amygda la; AVI, anterior ventral insular cortex; DI, dorsal insular cortex; LH, lateral hypothalamus; LOFC, lateral orbitofrontal cortex; MOFC, medial or bitofrontal cortex; NST, nucleus of the solitary tract; OB, olfactory bulb; OC, olfactory cortex; OE, olfactory epithelium; PPC, posterior parietal cortex; SOM, somatosensory cortex; V, VII, IX, X, cranial nerves; VC, primary visual cortex; VPM, ventral posteromedial thalamic nucleus. Adapted from Shepherd (2006, 2011).

Odorants, like fats and many bitter compounds, are hydrophobic and rely on protein binding to effectively travel hydrophobic mediums, such as nasal mucus or saliva, to reach their receptors (Briand et al., 2002). It has been shown that retronasal odors can enhance taste intensity (Melis et al., 2015b).

2.1. Fat taste receptors

Olfaction plays a role in the preference for fatty foods and triglycerides (Ramirez, 1993). Humans are also able to detect fatty content, fatty acids, through their sense of smell alone (Bolton and Halpern, 2010) even in combination with other food scents (Boesveldt and

Lundström, 2014). The system of detection by olfactory sensory neurons remains unknown. One study has shown that up to 8% of mature olfactory neurons express CD36 (Oberland et al., 2015). CD36 has been shown to be expressed by olfactory receptor cells in mouse and plays a role in olfaction (Lee et al., 2015).

2.2. Bitter taste receptors

It has also been shown that PROP and oleic acid induced $[Ca^{2+}]_i$ responses in cultured human olfactory cells respond (Tomassini Barbarossa et al., 2017). The bitter receptors expressed in the nose and upper airways contribute to innate defense by detecting invading pathogens and toxins (Lee et al., 2014) (Figure 18).

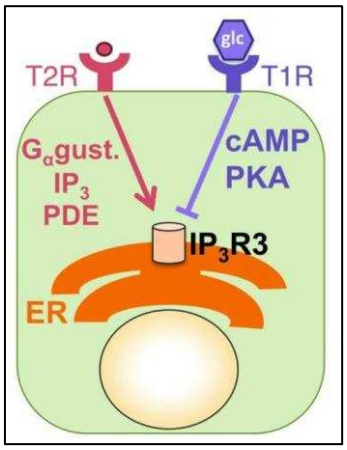

Figure 18. **Proposed mechanism for T2R and T1R signalling in sinonasal chemosensory cells**. The binding of a bitter agonist to its receptor induces a T2R signaling Gα gust dependent and activates IP_3 production which induces Ca^{2+} release by the ER. The binding of a sweet agonist with its receptor triggers the T1R signaling that likely activates an alternative G protein that acts through cAMP/PKA and may inhibit IP_3R-mediated calcium signaling. Adapted from Lee et al. (2014).

These findings suggest an olfactory role in ingestive behaviour. Besides, interesting findings have been shown investigating the odorant-binding protein 2a (OBPIIa) gene that is expressed in the olfactory cleft with high affinity for LCFA. Barbarossa et al. showed that a common single nucleotide polymorphism in the OBPIIa gene were involved in bitterness perception of fat (Tomassini Barbarossa et al., 2017). They found that the subjects homozygous for rs2590498 A-allele or heterozygous perceived bitterness in milkshakes containing oleic acid but not when noseclips were included.

Subjects homozygous for the rs2590498 A-allele also perceived PROP as more bitter than subjects homozygous for the G-allele in every TAS2R38 groups, i.e., AVI/AVI, PAV/AVI,

PAV/PAV. These findings suggest a role of the olfactory system in the modulation of taste sensitivity that might have physiological implications.

Flavour perception is one of the most complexes of human behaviour. It involves almost all the senses. The sense of smell, among others, is involved through odour images generated in the olfactory pathways. The perceptual systems are closely linked to learning, memory, emotion so that the distributed neural mechanisms contribute to food preference and food craving (Shepherd, 2006).

3. Existence of a taste system in the digestive tract

3.1. Digestive tract

It has been established that an infusion of a lipid emulsion in the small intestine decreases food intake in animals and humans (Greenberg et al., 1999; Matzinger et al., 2000). The understanding of this underlying mechanism has led to the gut-brain axis paradigm. LCFA induce intestinal lipid sensors activation which induces the release of appetite regulating peptides that transit via the vagal afferent fiber and/or blood flow. It eventually modulates the activity of hypothalamic areas corresponding to the "metabolic brain" known to be involved in food intake regulation and energy expenditure (Blouet and Schwartz, 2010) (Figure 19).

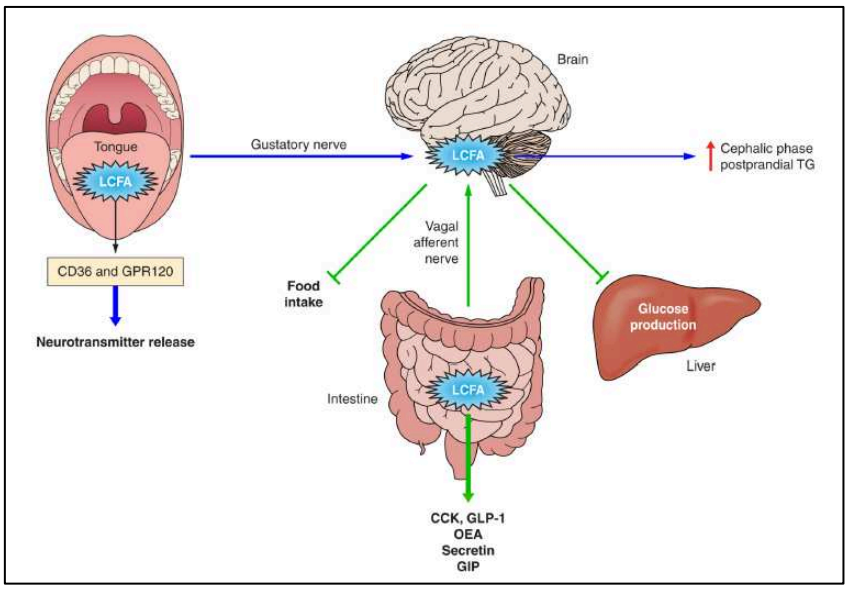

Figure 19. **Role of the gut in lipid homeostasis.** During digestion, LCFA are released from dietary triglyceride. In the oral cavity, fat taste receptors (CD36, GPR120), on the surface of TBC located on the apical surface of the tongue, trigger fat taste perception through the secretion of neurotransmitters, signal transmission to the brain and the induction of the early cephalic phase of digestion (blue arrows). In the intestinal tract, LCFA have satiety effects (green arrows) mediated by the release of anorexigenic peptides and lipid messenger (GLP-1, CCK, OEA). Lipid sensing in the gut mediated by the increase in long-chain faty acyl-coenzyme A inhibits glucose production by the liver through an intestine-brain-liver axis. Adapted from Abumrad and Davidson (2012).

CD36 has been shown to be mainly expressed in the proximal party of the small intestine in rodents (Poirier et al., 1996) and humans (Lobo et al., 2001) and in the apical side of entero-endocrine cells involved in secretin and cholecystokinin (CCK) secretion (Sundaresan et al., 2013) (Figure 19). Furthermore, acute intra-duodenal infusion of sulfo-N-succinimidyl oleate (SSO), a CD36 inhibitor (Kuda et al., 2013), leads to anorectic response that is abolished in $CD36^{-/-}$ mice. Then, CD36 is involed in enterocyte production of oleylethanolamide (OEA), an anorectic lipid messenger synthesized from dietary oleix acid (Schwartz, 2011), that is lacking in $CD36^{-/-}$ mice (Schwartz et al., 2008). Besides, OEA is also involved in the release of GLP-1 by the entero-endocrine L cells (Lauffer et al., 2009).

The perception of LCFA by lingual CD36 induces the generation of taste signals that are conveyed to the "emotional brain" (Figure 20-1). A cephalic reflex loop triggers the digestive anticipation via CD36 (Figure 20-2). The binding of LCFA to intestinal CD36 leads to the release of satiety related hormones such as secretin and CCK by the entero-endocrine cells (Figure 20-3) to the production of the endocannabinoid lipid messenger OEA by enterocytes (Figure 20-4). OEA, then, promotes GLP-1 release, known to decrease the food intake (Figure 20-5). In the same time, chylomicrons (CM) and the satiety peptide apoprotein AIV (ApoAIV) are synthesized and secreted by the enterocytes (Figure 20-6). Finally, postprandial hyperinsulinemia alters the intestinal CD36 mediated lipid sensing (Figure 20-7).

Taken together, these findings support the implication of CD36 as an intestinal lipid sensor in food regulation (Figure 20).

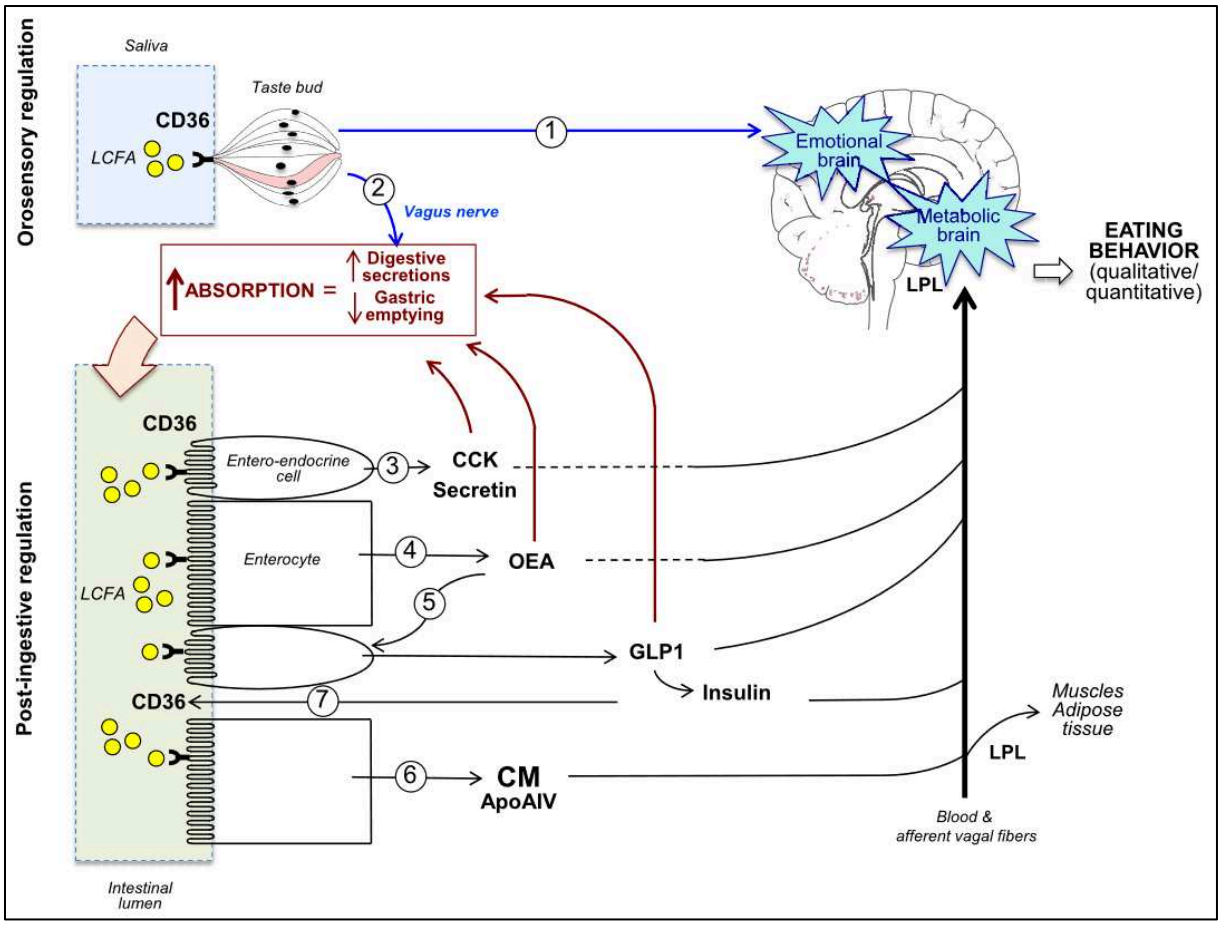

Figure 20. **Working hypothesis of the role de CD36/SR-B2 expressed along the tongue-gut axis on eating behaviour**. 1. Fat taste perception by the "emotional brain". 2. Cephalic reflex loop responsible for the digestive anticipation via CD36. 3. Release of satiety hormones secretin and cholecystokinin (CCK) by entero-endocrine cells. 4. Production of the endocannabinoid anorectic lipid messenger oleoylethanolamide (OEA) by enterocytes. 5. Production of the anorexigenic peptide, glucagon-like peptid-2 (GLP-1) by OEA. 6. Synthesis and secretion of intestinal TG-rich lipoprotein such as chylomicrons (CM) and the satiety peptid apoprotein AIV (ApoAIV) by enterocytes. LCFA, long-chain fatty acids; LPL, lipoprotein lipase. Adapted from Niot and Besnard (2017).

It is noteworthy that CD36 is also involved in the synthesis and secretion of large chylomicrons, lipoproteins responsible for lipids transport, by enterocytes during the post-prandial period (Niot et al., 2009). Indeed, $CD36^{-/-}$ mice exhibit a reduction of chylomicrons size leading to postprandial hyperglycemia (Drover et al., 2005; Masuda et al., 2009).Intestinal CD36 contribute to regulate the eating behaviour. As suggested in the taste bud cell, CD36 rapidly diseappeared from the brush border membrane of enterocytes in the presence of LCFA in the intestinal lumen suggesting a role of CD36 in the early stage of fat absorption (Tran et al., 2011).

Regarding the bitter taste and TAS2R, taste receptors have also been identified in a variety of nongustatory tissues, such as the gut, where they have been proposed to play a role in nutrient and toxin sensing (Mennella et al., 2013).

Thus, taste not only helps decide whether a food is noxious or palatable but also prepares the gastrointestinal tract for post-ingestive metabolic events.

3.2. The tongue-gut axis

The relation of the tongue-gut axis in relation to appetite control is to be considered.
There is a functional continuum along the oro-intestinal axis allowing the brain to analyze and regulate in real time the ingestion, digestion, absorption and metabolic fate of dietary lipids. This system requires the presence of sensors capable of detecting the presence of lipids at the lingual, intestinal and cerebral level. We have shown that CD36 plays an essential role in this system. At the lingual level, CD36 is clearly involved in taste perception of dietary lipids (Kuda et al., 2013; Laugerette et al., 2005). At the intestinal level, CD36 allows the detection of lipids during the postprandial period and adapts their absorption to make it optimal (Niot et al., 2009). It also transmits this information (presence or absence of lipids at the intestinal level) to the brain thus participating in the establishment of satiety. Finally, at the central level, CD36 is found in certain hypothalamic neurons known to be involved in the control of food intake.
The orosensory detection of fat food induces cephalic reflex triggering early digestive secretion such as exocrine pancreatic secretion and biliary secretion (Figure 21-1). The “emotional brain” perceives the taste signals and instantly evaluates the hedonic value of food (Figure 21-2). A bidirectional commucation between the “emotional” and the “metabolic” brain leads to the regulation of the eating behaviour towards food choice (Figure 21-3 and 4). Then, food arrival in the small intestine leads to postingestive detection of energetic nutrients by the taste receptors located on enteroendocrine cells (Figure 21-5). This detection triggers the release of neurotransmitters and hormones that modulate the eating behaviour via enteric vagal afferents (Figure 21-6). The cascade of events triggered by the detection of energetic nutrients in the small intestine induces the production of metabolic signals that allow a homeostatic regulation of the “metabolic brain” (Figure 21-7).

There appears to be a coordinated response of fatty acids throughout the digestive tract (Figure 21). In this way, the insensitive individuals orally, are also in the gastrointestinal tract and thus overconsumption fatty foods and energy (Keast and Costanzo, 2015).

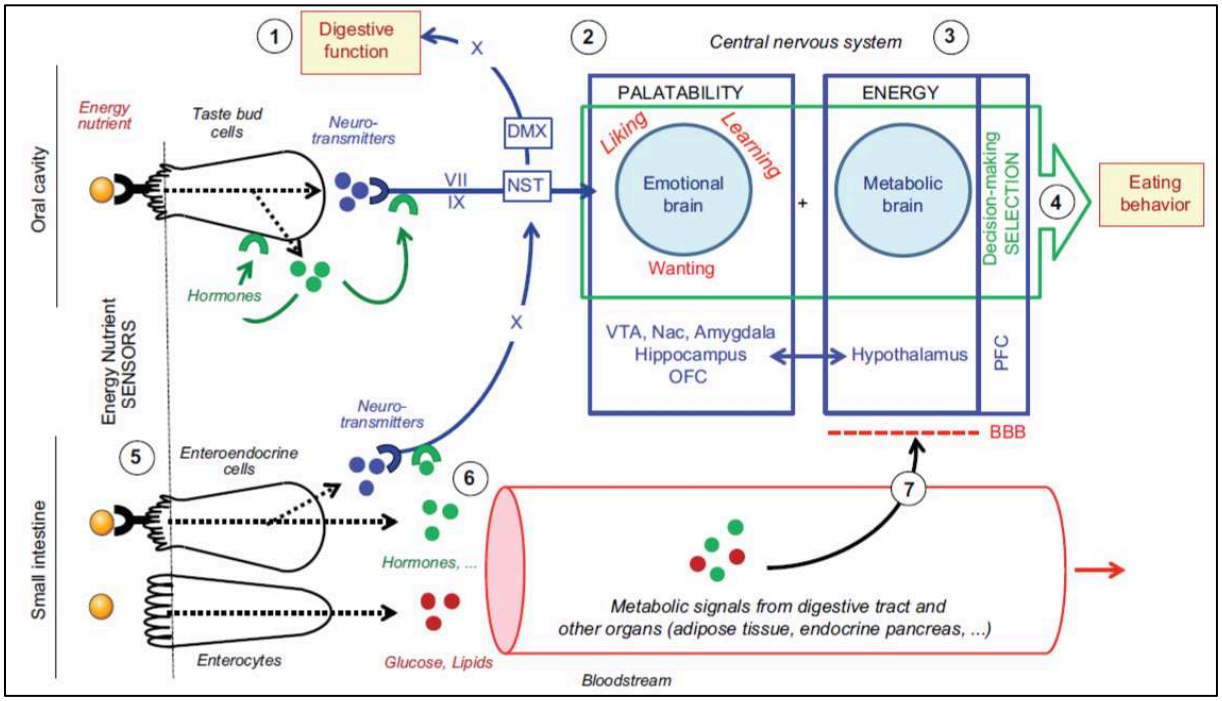

Figure 21. **Physiological consequences on the digestive function and eating behaviour of oral and post-oral detection of energy nutrients.** 1. Taste-induced cephalic reflex leading to early digestive secretion preparing the body for food arrival. 2. Activation of the "emotional" brain responsible for an instantaneous evaluation of hedonic value of foods. 3 and 4. Dialogue between the "emotional" and "metabolic" brain leading to the decision making at the basis of eating behavior, including food choice. 5. Postingestive detection of energy nutrients by receptors, including taste receptors, expressed by enteroendocrine cells. 6. Modulation of eating behavior by the enteroendocrine-derived neurotransmitters and hormones via receptors located in the enteric vagal afferents. 7. Homeostatic regulation of the "metabolic brain" by metabolic signals. BBB, blood-brain barrier; DMX, dorsal motor nucleus of vagus nerve; Nac, nucleus accumbens; NST, nucleus of solitary tract; OFC, orbito-frontal cortex; PFC, prefrontal cortex; VTA, ventral tegmental area; VII, chorda tympani nerve; IX, glosso-pharyngeal nerve; X, vagus nerve. Adapted from Besnard et al. (2016).

4. Endocrine hormones

4.1. Oxytocin

Oxytocin is a neuropeptide composed of 9 amino acids produced in the hypothalamus. It has been discovered in 1906 and named "oxytocin", "swift birth" in Greek, because of it involvement in the contraction of the uterus of pregnant cats (Dale, 1906). Oxytocin is widly spread as it can be found across almost the entire animal kingdom (Donaldson and Young, 2008).

The physiological function of oxytocin was originally known for promoting delivery and milk ejection in mammalian females. However, its involvement in new physiological functions such as change in paternal and maternal behaviour toward children (Naber et al., 2010), increasing capacity to understand other people feelings (Domes et al., 2007), improvement of social communication (Burkett et al., 2016) and also in psychiatric diseases (Feldman et al., 2016). Another important issue involving oxytocin is its implication in food intake regulation.

4.1.1. Physiological role of oxytocin in feeding behaviour regulation

Oxytocin is synthesized in the paraventricular nucleus (PVN) and by the supraoptic nucleus (SON). The magnocellular oxytocin neurons in the PVN and SON project to the posterior pituitary gland and secrete oxytocin into the periphery circulation system (Bargmann and Scharrer, 1951) and into the central nervous system (CNS) (Maejima et al., 2014). Albeit the oxytocin is secreted into the peripheral circuitry and in the CNS, the effect of oxytocin on food intake is generally considered to be regulated mainly by the oxytocin secreted within the CNS (Altirriba et al., 2015).

Arletti et al. (1989) first reported the anorexigenic effect of oxytocin in rat. Afterward, the effect was also shown in mice (Maejima et al., 2011), monkeys (Blevins et al., 2015) and humans (Lawson et al., 2015; Thienel et al., 2016). However, the body weight (BW) reduction observed with oxytocin treatment would not be only due to food intake reduction but also to increase in lipolysis in adipose tissue and decrease in fat mass (Altirriba et al., 2014; Blevins et al., 2015; Gajdosechova et al., 2014; Maejima et al., 2017; Yi et al., 2015).

Oxytocin neurons are activated following food consumption or ingestion (Hume et al., 2017; Johnstone et al., 2006). Thus, oxytocin plays an important role in the end of food intake. Indeed, hypothalamic Oxytocin mRNA expression is reduced with fasting and recovers with refeeding (Kublaoui et al., 2008). Several studies explored the involvement of oxytocin in food intake. In this way, suppression of oxytocin exocytosis or genetic reduction of oxytocin increased food intake (Kublaoui et al., 2008; Zhang et al., 2011); suppression of oxytocin receptor or ablation of neurons expression oxytocin receptors increased body weight gain (Wu et al., 2012) and, in the NTS, triggered hyperphagia (Baskin et al., 2010; Ong et al., 2017). Finally, oxytocin receptor activation in the arcuate nucleus (ARC) leads to satiety (Fenselau et al., 2017).

Furthermore, the oxytocin secretion shows a circadian rhythmic pattern. Indeed, circulation oxytocin levels is higher during the light phase corresponding to the suppression of feeding in rodents (Zhang and Cai, 2011). Moreover, oxytocin antagonist ICV injection increased food intake more effectively in the light phase than in the dark phase, the feeding phase (Zhang and Cai, 2011). Oxytocin seems to be involved in suppressing food intake during non-feeding phase. However, some reports on oxytocin receptor-deficient mice showed no difference in food intake (Takayanagi et al., 2008; Wu et al., 2012). But it can also reveal the existence of compensatory mechanisms.

All together, these findings suggest that oxytocin receptor signalling seems to be involved in energy expenditure and feeding regulations. However, the precise mechanism involved is not completely elucidated.

4.1.2. Oxytocin anorexigenic neural pathway

The oxytocin is synthesized in the PVN and SON of the hypothalamus (Bargmann and Scharrer, 1951). The PVN receives projections from the ARC (Bouret et al., 2004). Two opposite types of neurons regulate feeding behaviour and are found in the ARC: neurons containing proopiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART); and neurons containing neuropeptide-Y/agouti-related peptide (NPY/AgRP) (Schwartz et al., 2000). POMPC is the precursor of α-melanocyte-stimulating hormone (α-MSH), a strong anorexigenic peptide whereas NPY is a strong orexigenic peptide. Due to the fact that the tight junctions of the blood brain barrier are looser in this brain area, the ARC receives the peripheral conditions through factors in the blood stream such as leptin and glucose (Langlet et al., 2013). The ARC constitutes, thereby, the first-order neurons (Schwartz et al., 2000). The peripheral hormonal information received is then conveyed to the second-order neurons that include the PVN. The communication from the first-order to the second-order neurons triggers orexigenic or anorexigenic behaviour (Figure 22).

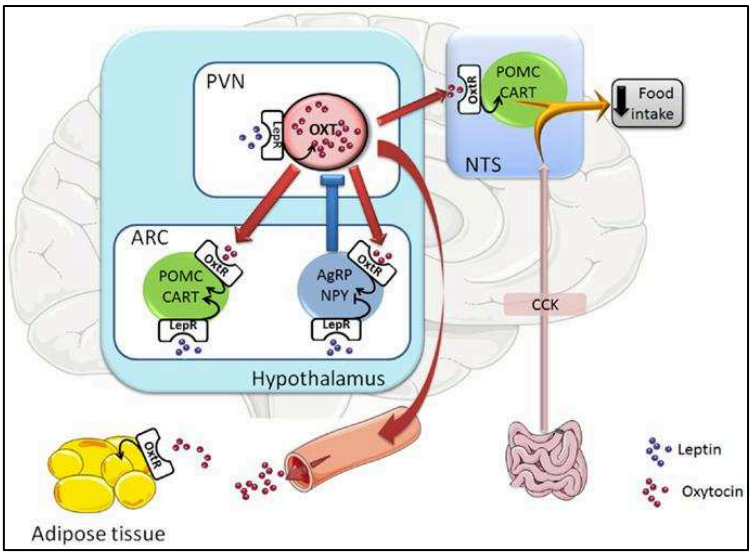

Figure 22. **Representative scheme of the oxytocin neuronal circuits controlling food intake.** Arrow-headed lines and bar-headed lines indicate activation and inhibition, respectively. Oxytocin neurons and innervations are drawn in red, POMC/CART neurons in green, AgRP/NPY neurons in blue, leptin protein in purple circles, and oxytocin protein in red circles. Oxt, oxytocin ; POMC, pro-opiomelanocortin ; CART, cocaine- and amphetamine-regulatedtranscript ; AgRP, agouti-related protein;NPY, neuropeptideY ; CCK, cholecystokinin ; LepR, leptinreceptor ; OxtR, oxytocin receptor ; ARC, arcuatenucleus ; PVN, paraventricular nucleus ; NTS, nucleus of the solitary tract. Adapted from Altirriba et al. (2015).

4.1.3. Oxytocin anorexigenic neuronal pathway and peripheral inputs

The NST is connected to different brain areas associated with food intake, rewarding, memory, and processes integrating visceral signals (Besnard et al., 2016). It responds to meal-related signals from the vagal afferent (Schwartz, 2006). The vagal afferent senses gut-secreted peptides such as CCK or GLP-1 and conveys the information to the NST that receives abundant projections from the PVN oxytocin neurons (Affleck et al., 2012). It has been shown that CCK intraperitoneal injection leads to c-fos expressions in the PVN where oxytocin neurons are selectively activated (Katoh et al., 2014). Gut hormone secreted postprandially participate to suppress food intake via the vagal afferent NTS-PVN oxytocin neurons.

4.1.4. Oxytocin and reward-related brain regions

Projections from the tegmental area (VTA) to the nucleus accumbens (NAc) play a crucial role in reward-related feeding behaviour (Adamantidis et al., 2011). Dopamine neurons are largely distributed in the VTA. It has been shown that neural pathway from VTA dopamine neurons to NAc is related to drug addiction (Volkow and Morales, 2015) and eating a palatable food intake activates dopamine neurons in the VTA and NAc (Valdivia et al., 2014).

Several studies showed that oxytocin treatment decreased the fructose-sweetened beverages consumption in monkeys (Blevins et al., 2015), intra-VTA oxytocin injection reduced sucrose intake in rats (Mullis et al., 2013) and oxytocin curtailed reward-driven food intake such as highly palatable chocolate cookie consumption in humans (Ott et al., 2013). Xiao et al. (2017) showed that PVN oxytocin neurons project to the VTA dopamine neurons and activate dopamine neurons, suggesting oxytocin is involved in the balance of dopamine neurons. Finally, oxytocin administration to NAc decreases food and palatable sucrose intake (Herisson et al., 2016) (Figure 23).

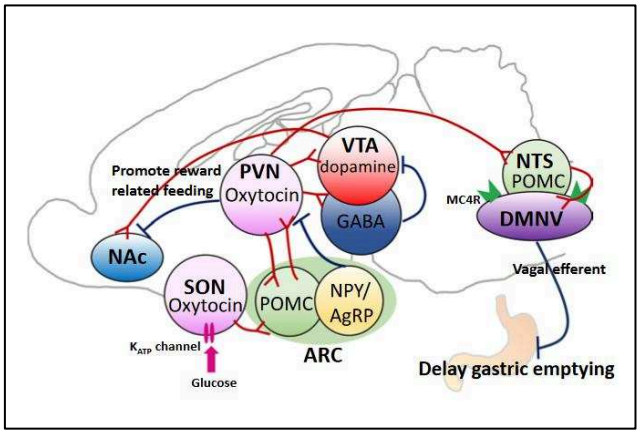

Figure 23. **Proposed model for oxytocin-related neural pathways that regulate food intake.** Oxytocin neurons in the PVN and SON activate POMC neurons in the ARC. Oxytocin neurons in the PVN are subsequently activated by the POMC derived α-MSH. In the SON, oxytocin neurons express K_{ATP} channel and are glucose sensors that activate POMC neurons in the ARC. NPY/AgRP neurons project to the PVN oxytocin neurons and suppress their neural activity. PVN oxytocin neurons project to the POMC neurons in the NTS. Melanocortin receptors are expressed in the DMNV constituting the first nucleus of the vagal afferent. The DMNV regulates the gastrointestinal system and delays gastric emptying. In reward-related feeding, oxytocin neurons in the PVN activate VTA neurons. VTA dopamine neurons project to the NAc and promote reward-related feeding. PVN oxytocin suppresses these neurons and decreases reward-related feeding. The red arrows indicate the stimulatory projections and the blue arrows indicate the inhibitory projections. Adapted from Maejima et al. (2018).

Taken together these findings exhibit the oxytocin as a promising neuropeptide for the prevention and the treatment of obesity.

Spetter et al. (2018) concluded that during the preprandial state, oxytocin stimulates dopaminergic reward-processing circuits. Whereas during the prandial state, oxytocin curbs the food intake by enhancing the activity of brain regions link to exert cognitive control, while increasing the activity of regions involved in the process of food reward value.

4.1.5. Fields of applications of oxytocin

Oxytocin is involved in psychological research areas such as autism (Aoki et al., 2014) or schizophrenia (Bradley and Woolley, 2017) in humans. Regarding obesity treatment, oxytocin showed encouraging effects. Indeed, oxytocin infusions using mini-pumps for two weeks in HFD-induced obese mice decreased significantly the body weight (BW) in only two days. Moreover, the decrease in food intake observed lasted seven days after the treatment. The effect on BW was dose dependent and maintained throughout the oxytocin treatment period (Maejima et al., 2017). This effect was also observed in human (Thienel et al., 2016). Besides, oxytocin seems to induce increased energy expenditure with decreased respiratory quotient in mice during the light phase of the treatment period (Maejima et al., 2011). The reduction of BW induced by oxytocin could also be due to diet-induced thermogenesis (Ong et al., 2017). Oxytocin treatment also improved factors linked to the metabolic disorder: decreased lipid-droplets in the hepatocyte and decreased size of adipocytes in the mesenteric fat (Maejima et al., 2011), decreased abdominal and subcutaneous fat without reducing muscle mass (Maejima et al., 2017), increased lipolysis and fatty acid β-oxidation (Deblon et al., 2011; Eckertova et al., 2011). It also noteworthy to highlight the fact that chronic treatment with oxytocin seems also to be effective in decreasing BW of leptin-resistnt obese mice (Altirriba et al., 2014; Iwasaki et al., 2015) and rats (Balazova et al., 2016) suggesting that oxytocin is independent from leptin sensitivity. It is of paramount importance as human obesity is characterized by a leptin resistance, the ability of oxytocin to be effective despite leptin-resistance provides it an important benefice as an anti-obesity agent.

As stated above, oxytocin effects are more potent in severe obesity. Maejima et al. (2018) hypothesized that this effect may be due to the increment of oxytocin receptor expression in the brain and adipose tissue. The authors also reported that severely obesity is generally linked with insulin and leptin resistance associated with increases in plasma leptin and insulin levels that promotes insulin and leptin resistances and, by the way, diabetes and obesity. Conversely, in obese state, plasma oxytocin levels are decreased and oxytocin receptors are increased without oxytocin resistance establishment. These statements are to be considered since, as an anti-obesity agent, oxytocin may decrease fat mass and therefore induce reduction of plasma leptin levels and consequently improve leptin resistance (Figure 24). In addition, oxytocin, known to protect insulin-secreting β-cells from chronic high-glucose conditions in humans (Elabd et al., 2014) may improve insulin secretion (Maejima et al., 2015) and, therefore, improve glucose tolerance.

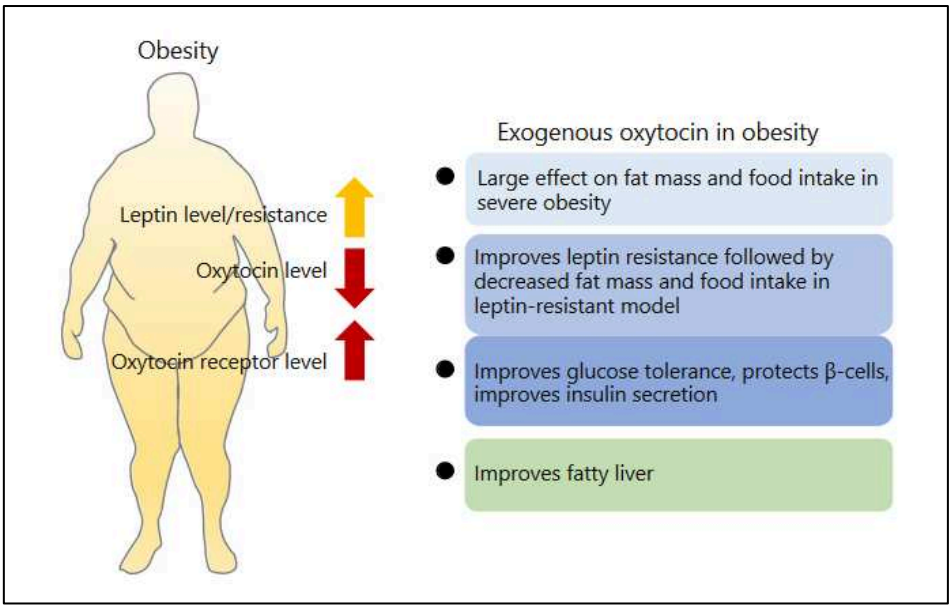

Figure 24. **The potential of exogenous oxytocin as an anti-obesity treatment.** Obesity is characterized by increased plasma leptin levels, leptin resistance, decreased plasma oxytocin levels and increased oxytocin receptor expression in adipose tissue and the CNS. Exogenous oxytocin has been shown to decrease fat mass and food intake and also to improve leptin resistance in obese animals and humans and in leptin-resistant animal models. Furthermore, oxytocin has been shown to protect insulin-secreting pancreatic β-cells in obese conditions, promote insulin secretion and improve fatty liver. Oxytocin emerges as a potential anti-obesity drug. Adapted from Maejima et al. (2018).

Considering the number of advantages presented by oxytocin in an obesity treatment context, it appeared to be an effective anti-obesity drug (Figure 25). Moreover, the anorexigenic effect of oxytocin are also found with nasal treatment that is noninvasive and a recent study showed that oral oxytocin supplementation could be also considered (Higashida et al., 2017).

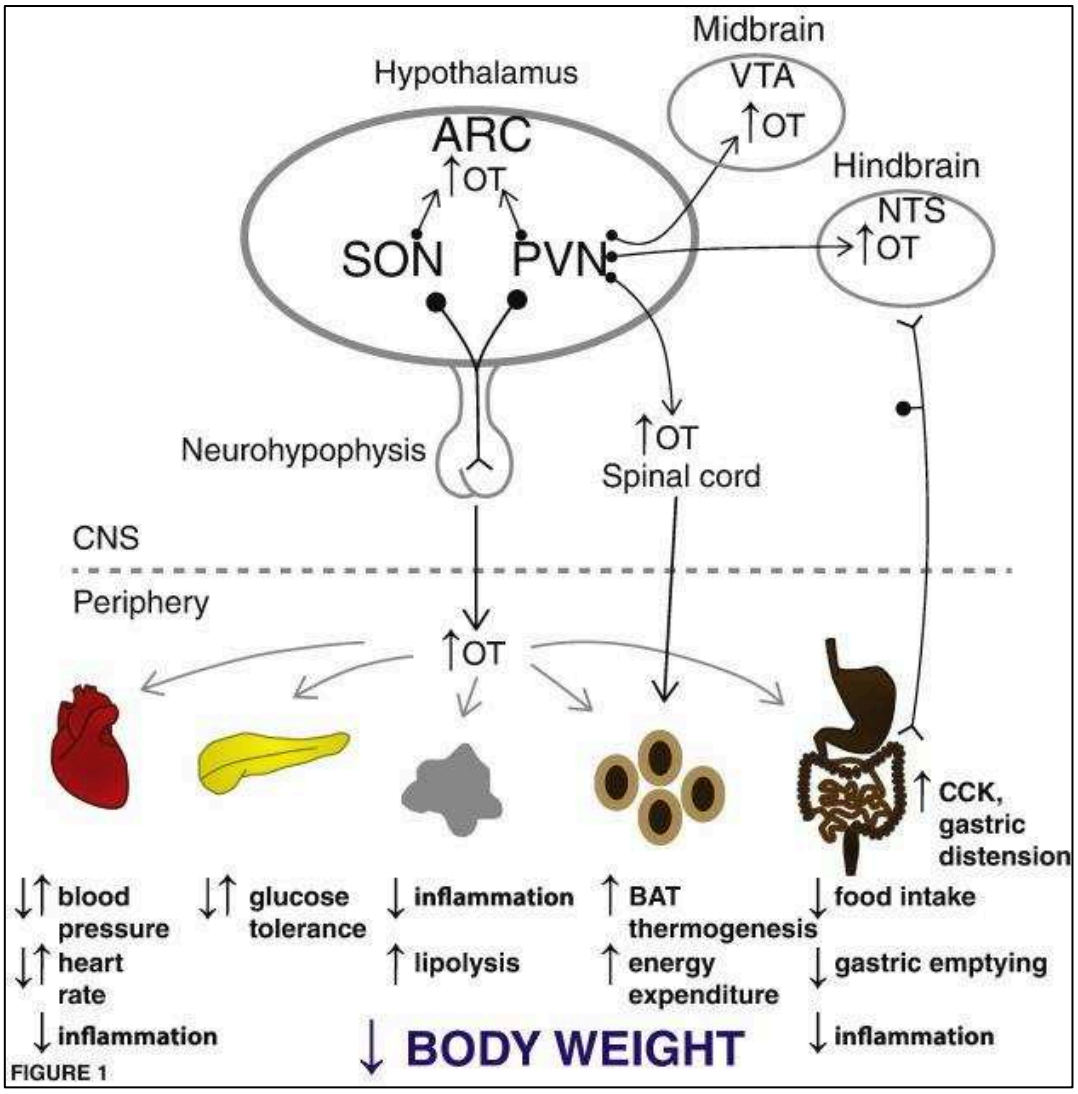

Figure 25. **Schematic representation oxytocin involvement on energy homeostasis.** OT release within the CNS, spinal cord, and from the neurohypophysis into the circulation (shown in black arrows) may impact metabolic processes (shown in gray) that result in the reduction of body weight. Dotted arrow represents implicated pathways from sympathetic preganglionic neurons in the spinal cord to WAT. Reported opposing effects denoted by arrows pointed in opposite directions to one another. ARC, arcuate nucleus; CCK, cholecystokinin; BAT, brown adipose tissue; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus; SON, supraoptic nucleus; and VTA, ventral tegmental area. Adapted from Blevins and Baskin (2015).

4.2.7. Oxytocin in the peripheral taste system

In addition to act within central circuits, oxytocin seems to also influence the peripheral taste system. Indeed, mouse taste buds have been shown to express oxytocin receptor (OTR) (Sinclair et al., 2010). Besides, oxytocin, in the nanomolar range, elicits Ca^{2+} responses in taste bud cells (Sinclair et al., 2010). OTR appeared to be expressed only by Glial –like (Type I) taste cells. It means that, if confirmed, oxytocin would modulate taste signals via a cell-to-cell communication between Glial-like cells and Receptor cells. Besides, it highlights an

analogy with the central nervous system, where OTR is also co-expressed with glutamate-aspartate transporter (GLAST) located in glial cells (Yoshida et al., 2009).

It is likely oxytocin modulates centrally but also peripherally the feeding behaviour.

4.2. Other endocrine hormones

4.2.1. NPY

Hormones appeared to have an important role in the modulation of taste sensitivities. Neuropeptide Y (NPY) is an orexigenic peptide in the central nervous system (Beck et al., 2002). NPY is found in the neurons projecting from the arcuate nucleus (ARC) to the paraventricular nucleus (PVN). Intracerebroventricular injection of NPY induced an increase in food intake (Beck et al., 2002) whereas destruction of NPY neurons by a neurotoxic agent triggers a decrease in food intake (Stricker-Krongrad and Beck, 2004). NPY has been shown to reduce the delay between food intakes (Sindelar et al., 2005) and to increase eat motivation (Jewett et al., 1995). Interestingly, NPY is involved in the consumption of palatable food as demonstrated by the fact that the endocannabinoid receptor antagonist Rimonabant prevented the release of NPY (Gamber et al., 2005).

4.2.2. Cholecystokinin

Cholecystokinin (CCK) functions as a satiey signal (Ritter, 2004). CCK is secreted from enteroendocrine cells in presence of lipids and proteins (Sayegh and Ritter, 2003). CCK binds to its receptor, CCK1 or CCKA receptor, that is found on vagal afferents innervating the intestinal mucosa (Moran et al., 1997). The vagal afferent fibers project to the nucleus of the solitary tract and are involved in feeding termination. CCK has been shown to inhibit food intake (Lieverse et al., 1994) and the use of a CCK receptor antagonist increased food intake (Reidelberger and O'Rourke, 1989).

During the prandial phase, fatty acids reach the small intestine triggering a slowdown of the gastric emptying and suppressing appetite through the release of the hormones CCK, glucagon-like peptide-1 (GLP-1) and peptide Y (PYY), and inhibition of ghrelin release (Feinle et al., 2003). An high-fat diet decreases the response to dietary fat in the GI tract (Park et al., 2007; Stewart et al., 2011b).

4.2.3. Ghrelin

Ghrelin has been discovered in 1999. It is produced by closed-type enteroendocrine cells in the oxyntic glands of the gastric fundus (Date et al., 2000; Kojima et al., 1999), by some small intestinal enteroendocrine cells, pancreatic-islet cells, and neurons in various brain areas, including the arcuate nucleus of the hypothalamus (Arc) (Date et al., 2000; Kojima and Kangawa, 2005; Müller et al., 2015; Verhulst and Depoortere, 2012). The ghrelin receptor was described in 1996 as the growth hormone-secretagogue receptor-1A (GHSR1A) (Howard et al., 1996). It is widely expressed peripherally and centrally (Kojima and Kangawa, 2005; Müller et al., 2015; Verhulst and Depoortere, 2012). Plasma ghrelin levels increase progressively before meal onset and fall precipitously afterwards (Gibbons et al., 2013; Kirchner et al., 2012; Spiegel et al., 2011). Ghrelin concentrations at meal onset correlated with meal size in healthy-weight and overweight men and women (Gibbons et al., 2013).
Ghrelin acts in the brain to stimulate eating, in the stomach to stimulate gastric emptying, and on the pancreatic-cells to inhibit insulin secretion. Ghrelin secretion is stimulated mainly by neural controls. Feedback from small-intestinal nutrient sensing, mediated in part by CCK and PYY(3–36) cells, inhibits ghrelin secretion during and after meals.

In obese subjects, a diminished postprandial suppression was observed compared with lean individuals (le Roux et al., 2005). PYY infusion has also been shown to reduce the orexigenic hormone ghrelin levels (Batterham et al., 2003). Ghrelin, the only orexigenic hormone, secreted by the fundus cells of the stomach, has been a target for potential vaccination. Anti-ghrelin vaccine has been shown to decrease food intake, decrease hypothalamic orexigenic signals, and increase energy expenditure in rodents and pigs (Altabas and Zjačić-Rotkvić, 2015; Bohórquez and Liddle, 2015). However, human studies have been disappointing with no weight loss shown in clinical trials (Altabas and Zjačić-Rotkvić, 2015; Colon-Gonzalez et al., 2013) indeed, it seems that IgG anti-ghrelin autoantibodies are able to protect ghrelin from degradation, suggesting that an autoimmune response may be involved in ghrelin's orexigenic effects (Takagi et al., 2013).

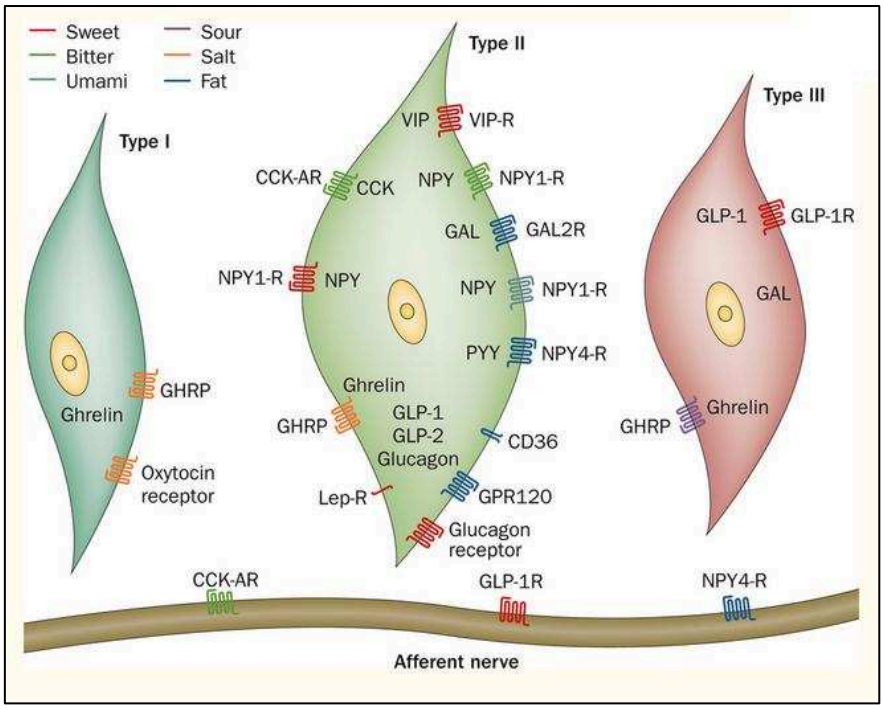

Figure 26. **Expression of hormones and their receptors in the three subtypes of taste bud cells**. The specific taste perceptions are represented as sweet, bitter, umami, sour, salt and fat. CCK, cholecystokinin; CCK-AR, cholecystokinin-A receptor; CD36, platelet glycoprotein 4; GAL, galanin; GAL2-R, galanin receptor type 2; GHRP, ghrelin receptor; GLP-1, glucagon-like peptide 1; GLP-1R, glucagon-like peptide 1 receptor; GLP-2, glucagon-like peptide 2; GPR120, free fatty acid receptor 4; Lep-R, leptin receptor; NPY, neuropeptide Y; NPY1-R, neuropeptide Y receptor type 1; NPY4-R, neuropeptide Y receptor type 4; PYY, peptide tyrosine tyrosine; VIP, vasoactive intestinal peptide; VIP-R, vasoactive intestinal peptide receptor. Adapted from Calvo and Egan (2015).

Numerous gastro-intestinal hormones, known to regulate both the digestive tract (i.e. motility, secretions) and the appetite, such as GLP-1, CKK, neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) and ghrelin are produced by the gustatory papillae, as well as their respective receptors (Figure 26).

4.2.4. GLP-1

The multifunctional hormone GLP-1 (Baggio and Drucker, 2007) is expressed on subsets of Type II and Type III taste cells, known to support the gustatory function, whereas its receptor is expressed on afferent gustatory nerves (Shin et al., 2008). LCFA lead to the release of GLP-1 in freshly isolated mouse CVP (Martin et al., 2012). As demonstrated in the entero-endocrine cells (Hirasawa et al., 2005), this event seems to be dependent of the activation of lingual GPR120 by LCFA (Martin et al., 2012). The fact that sweet and umami tastes are modulated by GLP-1 (Martin et al., 2009; Shin et al., 2008) and sweet taste sensitivity is enhanced by LCFA via the GLP- 1 signalling pathway (Martin et al., 2012) raised the

question of a possible role of GLP-1 on "fat taste" sensitivity. To address this issue, behavioural experiments were conducted using GLP-1R$^{-/-}$ mice. Interestingly, these mice displayed a lower sensitivity to oily solution (Martin et al., 2012). This effect is likely related to the disappearance, in GLP-1R$^{-/-}$ mice, of the down-regulation of CD36 protein levels normally triggered by LCFA in TRC (Martin et al., 2012). This finding provides the first evidence that GLP-1 regulates the signalling cascade controlling "fat taste" sensitivity, via CD36 which is expressed in taste buds. This regulatory loop (i.e. LCFA/GPR120/GLP-1/CD36/sensitivity to lipids), which occurs locally, might be reinforced by GLP-1 produced elsewhere.

Given the expression of the taste receptors throughout the alimentary canal, a mechanism may play an important role in this association.

Indeed, LCFA combine with the fat receptors present on the taste bud cells and on the enteroendocrine cells of the intestine and releases satiety hormones (CCK, GLP-1 and PYY). This mecanism allow the transmission of the satiety information to the NST.

Liu et al. (2016) proposed the following working hypothesis: LCFA are released by lingual lipase from dietary lipids present in food. LCFA binds to the fat receptors, CD36 and GPR120, inducing a rise in intracellular Ca^{2+}. Consequently, a release of neurotransmitters such as 5-HT and NA and gastro-intestinal hormones such as CCK, GLP-1 and neuropeptide Y (NPY) is triggered. In return, hormones are able to regulate the functions of the fat taste receptors (Figure 27-1). The taste bud cell afferent nerve fibres VII and IX transmit the information to the NST (Figure 27-2). The integration of the signals by the NST induces the reflex to early digestive secretion. Indeed, pancreatic exocrine, gastric lipase and other hormones are secreted to hydrolyse the ingested LCFA. This mechanism allows the inhibition of ghrelin, the food intake stimulating hormone (Figure 27-3). LCFA, in turn, bind to fat taste receptors in the enteroendocrine cells triggering the increase of $[Ca^{2+}]_i$ and also induce the release of satiety hormones such as CCK, GLP-1 and PYY (Figure 27-4). The nerve fiber X (vagus nerve) conveys the satiety information to the NST (Figure 27-5). Obese subjects appeared to express less receptors in the TBC and in intestinal cells than lean subjects. This could compromised fat sensing system by attenuating the oral signals conveyed to the brain and thus delays the satiety response inducing excess food intake.

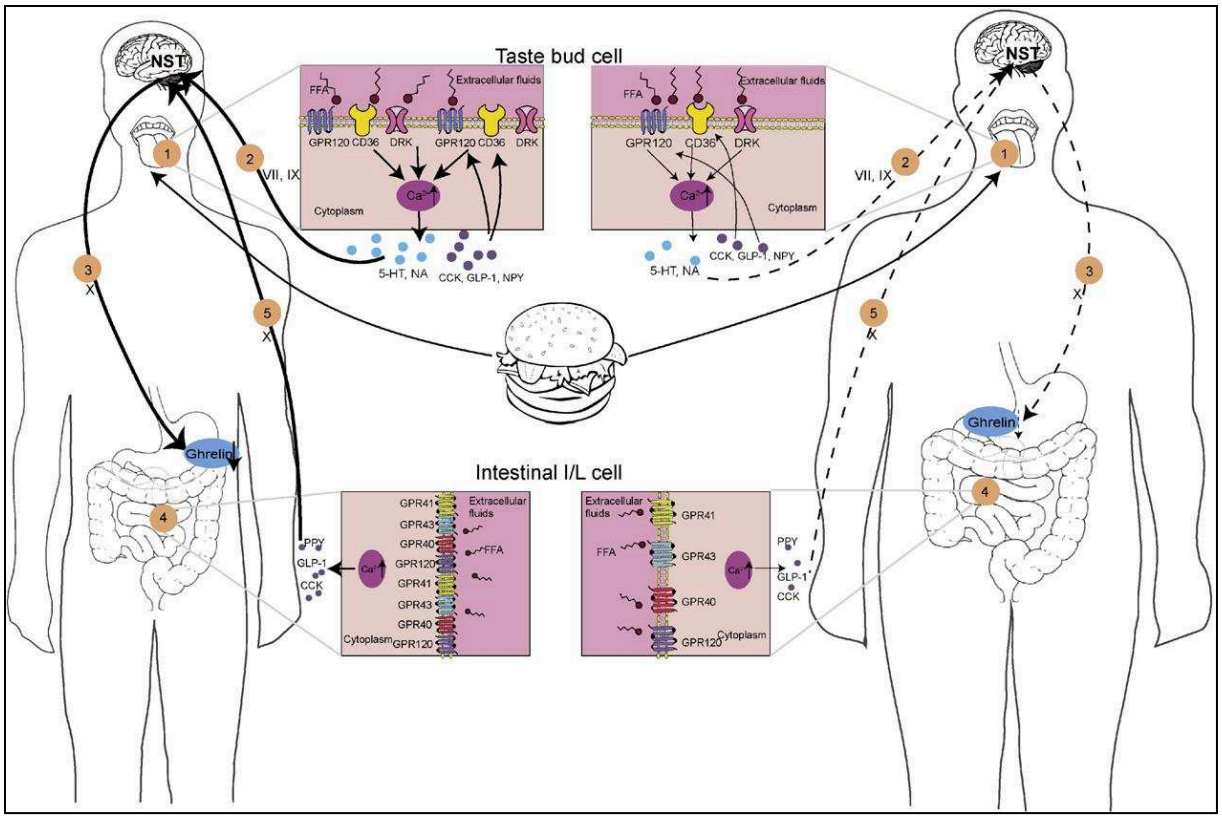

Figure 27. **Proposed mechanisms of the systemic energy regulation via fat taste receptors.** 1. In the oral cavity, LCFA receptors (CD36, GPR120) present on the apical surface of taste bud cells in the tongue contribute to fat taste perception which associates with secretion of neurotransmitters, signal transmission to brain centers, and induction of the early cephalic phase of digestion. 2. The afferent nerve fibres VII and IX transmit the taste information to the nucleus of solitary tract (NST) of the brain. 3. The NST integrates the signals and induces the reflex to early digestive secretion. As a response, gastric lipase, pancreatic exocrine and other hormones are secreted and further hydrolyse the ingested fats. Thus, the food intake stimulating hormone-ghrelin is inhibited. 4. The LCFA combine with the fat receptors GPR120, GPR41 and GPR43 on the enteroendocrine cells of the intestine and releases satiety hormones (CCK, GLP-1 and PYY). The signal transduction involves the elevation of Ca^{2+}. 5. The vagus nerve (nerve fiber X) conveys the satiety information to the NST. The dash lines represent the compromised responses in the obese individuals. Adapted from Liu et al. (2016).

Chapter 3
Modulation of fat taste preference

1. Modulation of fat preference by endocannabinoids

1.1. The endocannabinoid system

The endocannabinoid system (ECS) comprises endogenous agonists, the endocannabinoids and receptors responsible for its actions: cannabinoid-1 receptor (CB_1R) and cannabinoid-2 receptor CB_2R, as well as enzymatic system that catalysis its biosynthesis and degradation (DiPatrizio et al., 2013).

The establishment of this system follows the discovery of Δ-9-tetrahydrocannabinol (Δ^9THC) by Raphael Mechoulam in 1964 (Gaoni and Mechoulam, 1964). Δ^9THC is the most active compound produced by the plant Cannabis sativa. Thus, the cannabinoid receptors have been identified: CB_1R, a G protein-coupled membrane receptor (GPCR). The binding of Δ^9THC to CB_1R inhibits adenylyl cyclase and modulates the activity of Ca^{2+} and K^+ channels in neurons (Matsuda et al., 1990). CB_1R is widely expressed in the central and peripheral nervous system mainly in limbic structures (hypothalamus) and nucleus accumbens (Moldrich and Wenger, 2000). They have also been detected in taste buds (Yoshida et al., 2010). CB_2R, the second cannabinoid receptor subtype, meanwhile, is primarily expressed in the cells of the immune system.

N-arachidonoyl ethanolamine (anandamide, AEA) (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Sugiura et al., 1995) have been identified as the two major endogenous endocannabinoids acting on CB_1 and CB_2 receptors. They are derivatives of arachidonic acid, a ω6-polyunsaturated fatty acid. Anandamide and 2-AG are rapidly hydrolyzed by the fatty acid amide hydrolase and the mooacylglycerol lipase, respectively, into inactive compounds at cannabinoid receptors (Cravatt et al., 1996; Dinh et al., 2002).

The cannabinoid receptors, the endocannabinoids and the enzymes catalyzing their biosynthesis and degradation constitute the endocannabinoid system (Figure 28).

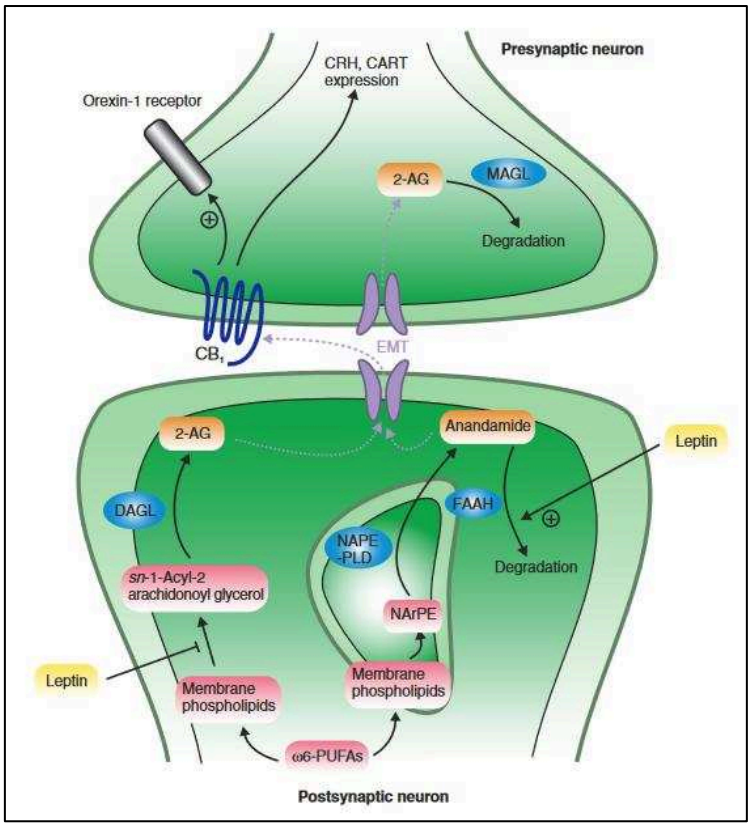

Figure 28. **The endocannabinoid system in neurons: CB_1 signalling affects the expression of orexigenic and anoretic mediators in the hypothalamus.** DAGL: sn-1 selective diacylglycerol lipase; EMT: putative endocannabinoid membrane transporters; FAAH: fatty acid amide hydrolase; MAGL: monoacylglycerol lipase; NArPE: N-arachidonoyl-phosphatidyl-ethanolamine; NAPE-PLD: N-acyl-phosphatidylethanolamine–selective phospholipase D; CRH: corticotropin-releasing hormone; CART: cocaine-amphetamine–regulated transcript. Blunt-ended line indicates inhibition. Adapted from Di Marzo and Matias (2005).

1.2. Involvement of brain endocannabinoids in the regulation of food intake

It has been known since the 1970s that cannabis increases appetite and food consumption, particularly of palatable foods, in humans (Abel, 1975). Several studies have looked at endocannabinoids. As a result, exogenous administration of cannabinoids (anandamide) induces hyperphagia and preference for palatable substances (Williams and Kirkham, 1999). Endocannabinoids are thus orexigenic mediators. In addition, the use of CB_1 receptor antagonists, such as rimonabant (SR-14716A), the first selective CB_1R antagonist (Rinaldi-Carmona et al., 1994), reduces food intake (Arnone et al., 1997). CB_1 receptors therefore seem to be involved in food intake. It appeared that the endocannabinoid system tonically reinforces the motivation to find and consume food with high caloric value. It is likely that this reinforcement is mediated by the mesolimbic pathways involved in reward mechanisms. Besides, it seems that the ECS is activated "on demand" in the hypothalamus after short-term

food deprivation and then transiently regulates the levels and actions of other orexigenic and anorexigenic mediators to induce appetite (Di Marzo and Matias, 2005). It may exist a dual action in mesolimbic and hypothalamic regions. Indeed, endocannabinoid levels vary in the hypothalamus and the limbic forebrain during the phases of feeding behaviour in rats. The levels are high during food deprivation and low during food consumption (Kirkham et al., 2002). Moreover, the changes in endocannabinoid levels in the hypothalamus seemed to be inversely correlated with the leptin blood levels. Leptin appeared to decrease endocannabinoid levels in the hypothalamus (Di Marzo et al., 2001).

The ECS appeared to modulate food intake by regulating the expression of hypothalamic mediators. Indeed, CB_1R have been shown to colocalize with corticotrophin-releasing hormone (CRH) in the paraventricular nucleus (PVN), with melanin-concentrating hormone in the lateral hypothalamus and also with pre-pro-orexin in the ventromedial hypothalamus (Cota et al., 2003). Furthermore, the CB_1R-activation sensitizes orexin-1 receptors which is likely to enhance appetite-inducing action of orexins (Hilairet et al., 2003). Conversely, CB_1R inhibits anorectic actions occurring downstream of melanocortin-4 receptors (Verty et al., 2004). Finally, in the mesolimbic system, endocannabinoids are believed to enhance appetite by increasing dopamine release in the nucleus accumbens shell or by synergizing with opioides. Di Marzo and Matias (2005) suggested that endocannabinoids maintain directed behaviours elicited by a stimulus instead of reinforcing the ability of a stimulus to elicit approach behaviour.

1.3. Peripheral control of food intake and endocannabinoids

Another important target of endocannabinoids is the peripheral target, such as the digestive tract. Indeed, the ECS controls the food intake via the vagus nerve that connects the gastrointestinal tract and the medulla and brainstem involved in satiety. For example, it has been shown, in rats, that food deprivation increases anandamide levels in the duodenum. In the same way, food deprivation increases CB_1R expression in CCK-1 receptor-expressing neurons of the nodose ganglion projecting to the duodenum. Feeding and CCK contribution re-establish low levels of CB_1R in these neurons (Burdyga et al., 2004; Gómez et al., 2002). Di Marzo and Matias suggested that inhibition of ECS activity could mediate induction of satiety by CCK. They also suggested that fasting overcomes satiety by elevating small intestine endocannabinoid levels and by releasing vagal CB_1R from CCK inhibition (Di Marzo and Matias, 2005). In other words, the desinhibition of the ECS in the vagus nerve leads to the lifting of satiety.

Given the many targets associated to food regulation and the possible benefits linked to the ECS activation or inhibition, rimonabant have been used in numerous studies. Rimonabant decreased food intake in ob/ob and db/db mice (Di Marzo et al., 2001) and also in Zucker rats (Vickers et al., 2003). Rimonabant treatment induced a significant decrease in fat mass, an improvement of metabolic parameters such as a decrease in insulin plasma levels, leptin levels, cholesterol and an increase in HDL/LDL ratio (Ravinet Trillou et al., 2003). Surprisingly, $CB_1R^{-/-}$ mice consume as much high-fat food as wild-type mice but do not become obese and do not show insensitivity to insulin or leptin (Ravinet Trillou et al., 2004). It suggests that CB_1R antagonists induce body weight and fat mass loss via their anorexigenic action and act on lipogenesis and fat accumulation by counteracting the peripheral endocannabinoid effects.

CB_1R have been shown to be expressed in white adipocytes. The activation of CB_1R triggers activation of the lipoprotein lipase (Cota et al., 2003) and the CB_1R blockade leads to up-regulation of adiponectin, involved in reducing the expression of enzymes contributing to lipogenesis (Poirier et al., 2005).

CB_1R induced de novo fatty acid biosynthesis in the liver and hepatocytes by increasing, especially, the fatty acid synthase (Osei-Hyiaman et al., 2005). Moreover, it has been sown that CB_1R agonist enhances the expression of the fatty acid synthase in the hypothalamus too (Osei-Hyiaman et al., 2005). Taken together, this findings support that endocannabinoids regulate energy homeostasis centrally, via the hypothalamus, and peripherally (Figure 29).

	The local endocannabinoid system is:	Endocannabinoid activation leads to:
Hypothalamus	Stimulated by fasting	Enhancement of orexin action
	Stimulated by ghrelin[a]	Downregulation of CRH
	Inhibited by leptin	Inhibition of MC4R action
		Increased appetite following food deprivation
Mesolimbic system	Stimulated by palatable (high fat) food	Enhanced dopaminergic signaling in NAc
		Synergism with the opioid system
		Translation of motivation to eat into action
Brainstem	Stimulated by fasting	Effects on nodose ganglion and NTS neurons
	Inhibited by CCK	Inhibition of satiety and emesis
Gastrointestinal tract (duodenum)	Stimulated by fasting	Stimulation of TRPV1/CB_1 neurons in the vagus nerve
		Inhibition of satiety
White adipose tissue	Hyperactivated by fat diet[a]	Downregulation of adiponectin[a]
		Increased lipogenesis

Figure 29. **Endocannabinoid system involvement**. CCK, cholecystokinin; CRH, corticotrophin releasing hormone; MC4R, melanocortin receptor type 4; NTS, nucleus tractus solitarus; TRPV1, transient receptor potential canilloid 1 channel for capsaicin; Nac, nucleus accumbens. Adapted from Di Marzo and Matias (2005).

Endocannabinoid antagonists were proposed for the treatment of obesity (Berry and Mechoulam, 2002). Three one-year studies were carried out in human using oral dose of rimonabant: Rimonabant in Obesity (RIO)-North America, RIO-Lipids and RIO-Europe trials. After one year treatment, the weight loss observed was between 5 and 10%, many parameters were improved such as blood triglyceride levels, high density lipoprotein (HDL) cholesterol levels, fasting insulin levels and adiponectin levels. Nevertheless, the treatment induced side effects, i.e., nausea, diarrhea, dizziness, depression, anxiety and tolerance.

Thus, the ECS is activated transiently after fasting and/or exposure to palatable foods and triggers appetite by reducing satiety and eventually stimulating lipogenesis and decreasing energy expenditure (Figure 29). It raised the hypothesis of a hyperactive ECS as a factor contributing to obesity and related disorders.

These results also led to the hypothesis that the endogenous ECS plays a role in the regulation of appetite and food intake. As reported above, the ECS would also be involved in the reward process that drives the motivation and hedonic value of foods (Kirkham, 2003). Indeed, CB_1 receptors are expressed in the nucleus accumbens and the hypothalamus, regions belonging to the reward circuit. The endocannabinoid system is therefore a key regulator of palatable food intake (DiPatrizio and Piomelli, 2012). Indeed, endocannabinoids induce changes in eating

behaviour via the sensation of palatability of food components (Yoshida et al., 2010). Thus, the endocannabinoid system could be a critical component of the positive feedback mechanism required to maintain fat intake (DiPatrizio et al., 2013).

CB_1R has recently been shown to be expressed in taste bud cells (Yoshida et al., 2010). Subsequently, it was observed that the administration of cannabinoids had no effect on the food intake of $CB_1R^{-/-}$ mice with respect to palatable foods (Yoshida et al., 2010). However, preference of oily solutions in $CB_1R^{-/-}$ mice has never been investigated and could bring new insights in the treatment of obesity.

2. Modulation of fat preference by bitter agonists

2.1. Detection threshold and fat sensitivity

The threshold of detection is the lowest level at which a stimulus is perceived (Keast and Roper, 2007; Webb et al., 2015). It is the concentration from which a subject can detect the presence of a compound other than water in the solution but cannot identify the quality. A high detection limit induces low sensitivity or a compromised taste function (Liu et al., 2016). The increase in the concentration of the stimulus makes it possible to reach the recognition threshold. This is the minimum level that characterizes the taste of the compound, i.e., sucrose is sweet. Beyond and for high concentrations, the supra-threshold is reached, the flavor is generally unpleasant and it appears that other sensory systems are involved, i.e., smell and chemesthesia (Keast and Costanzo, 2015). Oral sensitivity to fatty acids refers to an individual's ability to detect fatty acids in a complex matrix when tasting a solution. The ability to detect fatty acids differs between individuals and this divergence is the result of oral peripheral mechanisms responsible for chemoreception (Chalé-Rush et al., 2007). The measurement of the taste of fat in humans is complex (Figure 30). Some researchers believe that fatty acids do not elicit a full sensory taste quality of fat such as sweet or bitter, but rather define a threshold of detection (Newman and Keast, 2013; Stewart et al., 2010) but this remains controversial (Running et al., 2015).

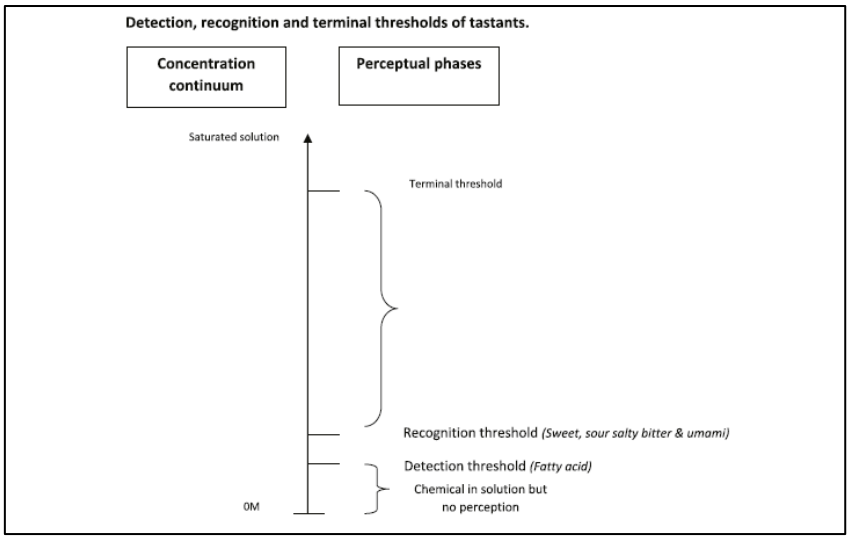

Figure 30. **Relationship between chemical concentration, detection threshold and recognition threshold.** The left-hand side represents chemical concentration from 0 M solution to a saturated solution. The right-hand side represents the perceptual relationship to increasing concentration and where fatty acid detection is placed in comparison to the five basic tastes. Adapted from Keast & Costanzo (2015).

CD36 and GPR120 genetic polymorphism is associated with low LCFA detection in some obese subjects. In the same way, the body-mass index (BMI) has been correlated with decreased detection sensitivity (high thresholds) of LCFA in obese subjects (Stewart et al., 2011c). However, this assumption is still under debate as the absence of a correlation between body weight and fatty taste threshold in humans (Tucker et al., 2017), in contrast to what was observed in rodents. Thus BMI appeared to be a poor predictor of the fatty taste sensitivity.

Nevertheless, detection thresholds for oleic acid were greater in overweight or obese than in lean subjects (Stewart et al., 2011b). Stewart and Keast (2012) also studied the effects of a high-fat and low-fat diet on LCFA-taste sensitivity in lean and overweight/obese subjects. Thay showed that fat contents of the diet modulated taste sensitivity to oleic acid among lean subjects. Indeed, oleic acid sensitivity was increased following a 4-week period of fat restriction and attenuated following the high-fat diet (Stewart and Keast, 2012). The failure of the high-fat diet to alter fatty acid taste thresholds among obese subjects suggests that these individuals were “adapted” to high-fat exposure.

Gilbertson and Khan (2014) suggested that excessive dietary fat attenuates nutrient oro-gustatory sensing, which could be associated with changes in diet and weight status. It is relevant as, in another study, subjects were categorized, on the basis of LCFA oral-detection thresholds, into two categories: hyposensitive or hypersensitive, and hyposensitive subjects consumed significantly more energy, fat, saturated fat, fatty foods, had greater BMI and were

less perceptive of small changes in the fat content of meal compared to hypersensitive subjects (Stewart et al., 2011a).

2.2. Polymorphism

2.2.1. CD36 single nucleotide polymorphism

Interestingly, CD36 AA-genotype of rs1761667 has been associated with a highly functional CD36 and thus a high detection thresholds for fatty acids in different populations (Melis et al., 2017; Mrizak et al., 2015; Pepino et al., 2012; Sayed et al., 2015). Furthermore, CD36 polymorphism has been proposed to be closely related to disorders linked with excess of body lipids (Love-Gregory and Abumrad, 2011). In addition, this genotype is also linked to de novo lipogenesis markers in obese subjects (Melis et al., 2017).

In humans, the common single-nucleotide polymorphism rs1761667 has been shown to reduce the gene expression of CD36 (Love-Gregory et al., 2011). It is also linked to a deep attenuation of the sensitivity to oral fat (Pepino et al., 2012). Thus, several pieces of evidence have been reported concerning the effect of the polymorphism rs1761667 on CD36 protein expression levels, which could explain variations in orosensory perception of fats leading to body weight gain. Subjects with the CD36 polymorphism rs1761667 are likely to be prone to become obese.

In our team, a study has been recently conducted on obese Tunisian women and showed that the participants with A-allele of rs1761667 polymorphism exhibited decreased oral sensitivity (high thresholds) to oleic acid (Mrizak et al., 2015). In another study conducted on young Algerian children age seven to eight, we have observed higher A-allele frequency of rs1761667 polymorphism in obese children compared to leans (Sayed et al., 2015). As expected, the obese young children exhibited higher detection threshold for oleic acid than lean participants (Sayed et al., 2015).

2.2.2 TAS2R38 single nucleotide polymorphism

The TAS2R38 gene presents three functional single nucleotide polymorphisms (SNPs) in gene nucleotide positions 145 (rs713598 C/G), 785 (rs1726866 C/T) and 886 (rs10246939 G/A) encoding for amino acid substitutions at position 49 (alanine/proline, A49P), 262 (valine/alanine, V262A) and 296 (isoleucie/valine, I296V) (Drayna, 2005; Kim et al., 2003). The SNP result in two major haplotypes PAV and AVI, Proline-Alanine-Valine and Alanine-Valine-Isoleucine rescpectively, and three rare (AAI, PVI and AAV) involved in bitter taste

perception of phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP). It has been reported that PTC tastes extremely bitter to people and not bitter to authors (Fox, 1932). A subset of the population, classified as “non-tasters,” cannot detect the presence of the compounds propylthiouracil (PROP) and phenylthiocarbamide (PTC) at moderate concentrations that all others, referred to as “tasters,” find exceptionally bitter.

Recently, the PROP taster status has been link to the genetic ability to taste the bitterness of PROP and is used as an index of chemosensory perception (Drayna, 2005; Kim et al., 2003; Tepper, 2008). Bartoshuk and al. first described the existence of groups, equivalent to 25% of individuals, defined as “supertasters” who perceive intense bitterness from PROP and PTC (Bartoshuk et al., 1994).

PAV represents the taster allele while AVI stands for the non-taster allele. Thus, subjects carrying the dominant diplotype (PAV/PAV or PAV/AVI) detect higher bitternerss intensity towards PROP whereas individuals carrying the homozygous recessive diplotype (AVI/AVI) detect less or not bitterness (Garneau et al., 2014). Interestingly, the TAS2R38 phenotype seems to influence dietary habits. Indeed, it has been reported that individuals carrying AVI/AVI diplotype have higher vegetable consumption belonging to brassica vegetable compared to individuals carrying PAV/PAV and PAV/AVI diplotypes (Duffy et al., 2004; Sacerdote et al., 2007). Moreover, taster individuals reported less liking for vegetables and, thus, consumed fewer vegetables (Bell and Tepper, 2006; Drewnowski and Gomez-Carneros, 2000).

Various factors such as experiences, income and cultural food habits may also influence food preferences and consumption (Mennella et al., 2005). By the way, salt taste has been shown to inhibit bitterness in food and enhances flavour (Keast et al., 2003). Besides, Burd et al. (2013) showed that two children groups exhibiting a non-taster status were likely to develop obesity, the non-tasters from unhealthy food environment had higher BMI and developed more incidence of obesity than the non-tasters from healthy food environment.

Lipchock et al. (2013) provided the first experimental evidence that mRNA expression amounts of the PAV allele of the TAS2R38 taste receptor gene correlate with sensory perception of bitterness and with dietary habits in humans.

Taken together, PTC and PROP may affect food preferences and susceptibility to certain diseases. Polymorphisms in TAS2R38 can alter the perception characteristics of food and thus impact ingestive behaviour and nutrient intake.

2.3. Link between CD36/TAS2R38

The taste modalities might interact with each other and also with other senses and these interactions might be critical in obesity (Khan et al., 2018a). A relationship between fat and bitter taste has been recently proposed. Indeed, some reports have suggested there exists a higher preference for dietary fat in PROP non-taster than that in PROP-taster participants (Karmous et al., 2018a; Tepper and Nurse, 1997; Tepper et al., 2008).

Moreover, Melis et al. (2015) showed there is an association between orosensory perception of oleic acid, rs1761667 single nucleotide polymorphism (SNP) in the CD36 gene and PROP tasting status. These investiagtions further observed that bitter non-taster subjects also presented a reduced oral fat perception, inducing high fat food consumption and, consequently, high risk for obesity (Keller, 2012). Our team showed that CD36 rs1761667 genotype and TAS2R38 rs1726866 and rs10246939 genotypes were associated with higher PROP detection thresholds and higher BMI in obese subjects than normal weight participants. These observations revealed the existence of an association between PROP detection threshold and CD36 and TAS2R38 genotypes (Khan et al., 2018a).

Recently, bitterness perceived in milkshakes containing very dilute concentrations of oleic acid and that bitter perception was dramatically reduced when subjects tasted the milkshakes wearing noseclips (Tomassini Barbarossa et al., 2017). Moreover, non-taster individuals have been reported to show reduced ability to distinguish fat content in food (Hayes and Duffy, 2007). Indeed, bitter non-tasters appeared to be unable to discriminate fat content in high and low-fat italian salad dressing and also fatty acids in a high-fat food (Nasser et al., 2001; Tepper and Nurse, 1998). Consequently, these subjects are reported to have higher consumption of dietary fat and high energy food as compared to tasters (Keller and Tepper, 2004; Tepper et al., 2011). Whereas, PROP supertasters might avoid high-fat food due to their intense sensations (Duffy and Bartoshuk, 2000).

Melis et al. (2015) showed for the first time a correlation between CD36 SNP and the decreased fat taste perception observed in PROP non-taster subjects. It appeared that subjects exhibited the AVI/AVI TAS2R38 phenotype homozygous for the G-allele of the rs171667 for CD36 had 11-fold lower detection threshod for oleic acid than subjects with the AVI/AVI TAS2R38 genotype but with a homozygous AA genotype of the rs1761667 polymorphism. It also appeared that PROP non-tasters had higher threshod for oleic acid than super-tasters. They observed that AA-genotype of rs1761667 exhibited increased BMI and decreased sensitivity to fat and bitter taste perception.

Several studies showed that PROP non-tasters have a lower density of fungiform papillae than PROP tasters (Melis et al., 2013b; Shahbake et al., 2005; Yeomans et al., 2009). Ebba et al. (2012) hypothesized that the increased ability to taste linoleic acid exhibited by PROP tasters, compared with non-tasters, could be ascribed to the difference in density of fungiform papillae between these two groups. Indeed, Melis et al. (2015) observed a lower density of fungiform papillae in PROP non-tasters, who also exhibit a lower capability to detect oleic acid compared with PROP tasters. They suggest that a high expression level of CD36 in gustatory cells seems to be a determining factor for detecting dietary fat only in subjects who have a low density of taste papillae.

Recently, investigations about orosensory of LCFA and PROP showed that PROP detection threshold was associated with LA detection threshold in obesity in a Tunisian population (Karmous et al., 2018) (Figure 31).

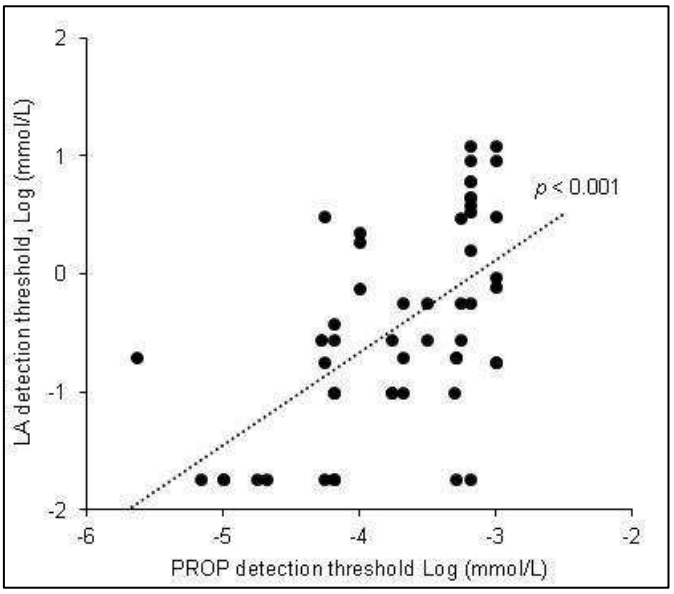

Figure 31. **Relationship between PROP and LA detection thresholds.** The bitter taster status, here, represented by the PROP detection threshold, appeared proportional to the fat taster status, represented by the fat taster status. In other words, individuals non-taster for fat, thus exhibiting a high LA threshold are proned to also exhibit a high PROP threshold and consequently to be bitter non-taster. Adapted from Karmous et al. (2018).

At the cellular and molecular levels, both fat taste, i.e, CD36 and GPR120 (Figure 32-1), and bitter taste, i.e, TAS2R16 and TAS2R38, receptors are expressed in type-II cells (Figure 32-2). Thus, both pathways might activate gustducin for downstream signalling receptors (Roper and Chaudhari, 2017) followed by activation of PLCβ2 which is involved in hydrolyse of phosphatidylinositol4,5-bisphosphate (PIP_2) into inositol trisphosphate (IP_3) (Figure32-3). Then, IP_3 binds to its receptor, IP_3R, on the ER and triggers Ca^{2+} release (Figure 32-4). This

rise in $[Ca^{2+}]_i$ leads to the opening of TRPM5 channels (Figure 32-5) that triggers an increase in free intracellular concentration of Na^+ resulting in cell depolarization and the release of neurotransmitters (Figure 32-5). Thus, Khan et al. (2018) stated that the common mechanism might be "Ca^{2+}-signalling" between the bitter and the fat taste perception.

The elucidation of a cross-talk between fat and bitter taste modalities could find applications in food industry, obesity treatment and pharmaceutical industry for reducing the bitterness of the medicaments in children especially (Mennella et al., 2013).

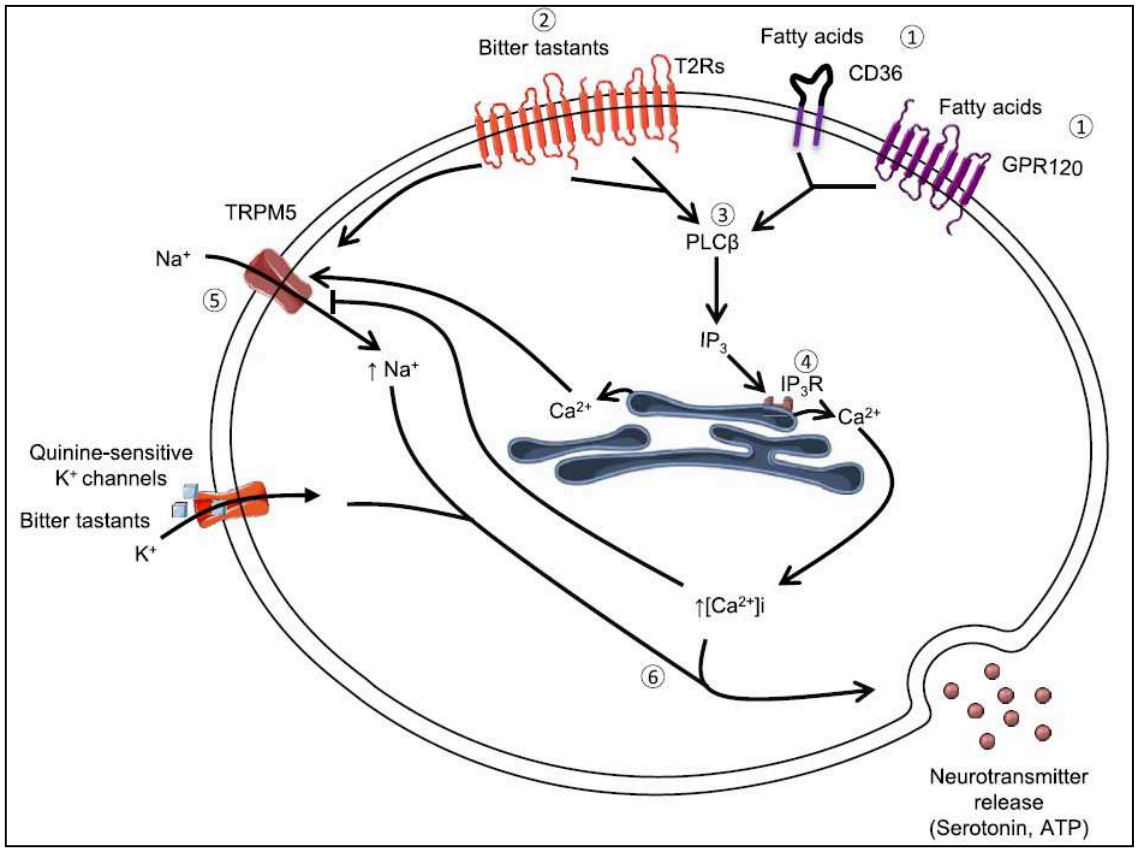

Figure 32. **A cross-talk between fat and bitter taste modalities**. 1. The dietary fatty acids bind to CD36 and/or directly to GPR120. 2. Bitter tastants bind to their receptors belonging to the T2R family. 3. The binding activates the cascade of PLCβ-IP_3. 4. The IP_3 generated binds to IP_3R and triggers the release of Ca^{2+} from the endoplasmic reticulum pool. 5. Increases in $[Ca^{2+}]_i$ activate TRPM5 channels responsible for cell depolarization. Very high increases in $[Ca^{2+}]_i$ inhibit TRPM5. 6. Increases in $[Ca^{2+}]_i$ trigger the release of neurotransmitters such as serotonin and ATP. Adapted from Khan et al. (2018).

Keller (2012) suggested that fat and bitter taste receptors might play different roles. Indeed, TAS2R38 would be involved in textural perception of dietary fat according to the PROP taster status and CD36 would influence chemosensory perception of fat.

Another explanation has been advanced involving the density of taste papillae and the number of taste bud cells (Feeney et al., 2011; Miller, 1986; Zhang et al., 2009). It appears that

individuals with high density of taste papillae exhibit high sensitivity to oro-sensory detection of bitter and fatty acids. However, this assumption is debated as Hayes et al. (2008) found no relation between decreased bitter taste perception and fungiform density.

Otherwise, Khan et al. (2018) hypothesized that simultaneous administration of fat and bitter tasting agents results in very high increases in $[Ca^{2+}]_i$ due to the opening of store-operated calcium channels, following the activation of fat taste receptors. Such increases in $[Ca^{2+}]_i$ much higher than required, may trigger inhibition of TRPM5 channels and, consequently, inhibition of bitter-taste perception.

Working hypotheses

CB_1R involvement in dietary fatty acids perception

The proportions of linoleic acid (18:2) have increased significantly in Western diets since the twentieth century from 1 to 8% of the total energy intake. This increase is correlated with the increase in the prevalence of obesity during this same period (Alvheim et al., 2012). Recently, the hypothesis of a spontaneous preference for lipids associated with the taste of fat has emerged and could explain the behavioural aspect related to obesity. Yoshida et al. (2010) tested the feeding preferences in wild-type and $CB_1R^{-/-}$ mice treated with an endocannabinoid agonist, 2-AG. As a result, only the intake of sugar solutions was increased in wild mice treated with 2-AG, the consumption of bitter, salty, acid and umami solutions were not affected by the same treatment. No change in consumption was observed in $CB_1R^{-/-}$ mice (Yoshida et al., 2010). This study demonstrates a change in palatability perception by endocannabinoids via CB_1R receptors.

Because excess lipid food intake is associated with obesity and the endocannabinoid system via CB_1R receptors enhances food intake (perception of sweetness in particular), we propose to study the role of the CB_1 receptor in the preference dietary lipids. Indeed, the involvement of the endocannabinoid system in the gustatory perception of dietary fats via CB_1R in the lingual epithelium has not been studied yet. We propose to study the role of CB_1R in fat preference in mice using behavioral tests, biochemical and Ca^{2+} studies. We hypothesized that the ECS would affect, via lingual CB_1R, fat taste detection as early as in TBC.

The project aims at elucidating the following tasks:

1) To study the consequences of the deletion of Cb_1r in mice invalidated for the gene ($CB_1R^{-/-}$) on the perception of dietary fatty acids.
2) To study the consequence of the deletion of Cb_1r gene on the expression of CD36 and GPR120 proteins
3) To study the cellular signalling mechanisms, via CD36 and GPR120 receptors in $CB_1R^{-/-}$ mice taste cells.
4) To study the consequences of the deletion of the gene on the GLP-1 release by taste bud cells.

This could enable new anti-obesity treatment involving the synthesis of chemical agents such as “inverse agonists”, agonist for CD36 and GPR120 and antagonist for CB_1R.

Bidirectional communication between bitter and fat gustatory modalities in human gustatory cells

Bitter taste is detected by the lingual T2R38 receptor. It has been proposed that non-tasters for fatty acids are also non-tasters for bitter taste. Besides, CD36 gene polymorphism leads to a decrease in protein expression and, consequently, a decrease in the oro-sensory detection of fat taste. Some subjects who express CD36 polymorphism also exhibit an increase in the bitter detection threshold. So, there might exist an interaction between the two taste modalities, i.e., fat and bitter tastes. Hence the ability to detect fatty acids and the potential cross-talk between fat and bitter tastes remain an important issue to explore as it could constitute new anti-obesity strategies. We hypothesized that bitter and fat taste share a common downstream signalling mechanism. We have investigated the relationship between fat and bitter taste by studying downstream signalling mechanisms in human immortalized fungiform cells, HTC-8.

The project aims at elucidating the following tasks:

1) To study the expression of dietary lipids taste receptors (CD36 and GPR120) and bitter taste receptor (TAS2R38) to investigate whether the 2 receptors are co-expressed by human fungiform taste bud cells.
2) To study cell signalling, particularly Ca^{2+} signal, via CD36/GPR120 and T2R38 to investigate the bidirectional communication between these 2 receptor families.
3) To study the role played by autocrine/paracrine agents like oxytocin that is released from TBC in the modulation of the fat taste modality.

These tasks has been performed on human fungiforms cells (HTC-8) immortalized by BRAIN institute (Zwingenberg, Germany).

The highlighting of the cross-talk between these two taste modalities could allow the possibility to establish new personalized food strategies taking into account of bitter and fat taste receptor polymorphisms (sensitivity threshold) for overweigh or cancer patients.

Second part
Personal contributions

Modulation of fat preference by endocannabinoids

Material & Methods

1. Material & Methods

1.1. Ethical approval

French guidelines for the use and care of laboratory animals were followed, and the experimental protocols were approved by the regional animal ethic committee of the University of Burgundy. In vivo studies were performed on male C57BL/6J wild type (WT) mice (Janvier Labs, Le Genest Saint Isle, France) and $CB_1R^{-/-}$ mice (generous gift from Dr. James Pickel, National Institute of Mental Health, Bethesda, MD, USA) with a C57BL/6J background. Animals were individually housed in a controlled environment (constant temperature and humidity, dark period from 19:00 to 7:00). The mice had free access to standard regular chow and tap water during the experiments, unless otherwise specified.

1.2. Behavioural experiments

1.2.1. Two-bottle preference tests

After being deprived of water for 6 h, mice were offered simultaneously two bottles, containing either control or experimental solution for 12 h. To minimize bias due to textural properties, the two solutions contained 0.3% xanthan gum (w/v, Sigma, Saint Quentin-Fallavier, France), whereas the experimental solutions were added with either 0.2% rapeseed oil (w/v, Fleur de Colza, Lesieur, France) or 0.2% linoleic acid (w/v, LA, Sigma, Saint Quentin-Fallavier, France). At the end of each test, the intake of control and experimental solutions was recorded by weighing the feeders/bottles. The experiments were repeated two times, independently.

In parallel, two groups (n = 5 each) of 10 WT mice, treated daily with rimonabant (SR141716, 10 mg/kg of body weight, Sanofi, Paris, France) or vehicle (0.1% DMSO/0.025% Tween 80 in 0.9% NaCl), by an intraperitoneal injection for 26 days, were subjected to the same two-bottle preference test. Food intake and weight were monitored during the experiment.

1.2.2. Licking tests

The $CB_1R^{-/-}$ (n = 9) and WT (n = 9) mice were deprived of food and water for 6 h before the test. The mice were conditioned to choose between a palatable (4% sucrose) and a control solution. Once the mice were conditioned, they were randomly subjected to two-bottle test, containing either control (0.3% xanthan gum) or a test solution (0.3% xanthan gum + 0.2%

linoleic acid, LA). The number of licks, motivated by each bottle, was recorded using computer-controlled lickometers (Med Associates, Fairfax, VT, USA). Data were analyzed for 5 min from the first lick.

1.3. Papillae and taste buds isolation

The mice were anesthetized with 2% isoflurane gas, and then sacrificed by cervical dislocation. Taste bud cells (TBC) were isolated according to previously published procedure (El-Yassimi et al., 2008). In brief, lingual epithelium was separated from connective tissues by enzymatic dissociation (elastase and dispase mixture, 2 mg/mL each in Tyrode buffer: 120 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM $CaCl_2$, 10 mM glucose, 1 mM $MgCl_2$, 10 mM Na pyruvate, pH 7.4) (Figure 33).

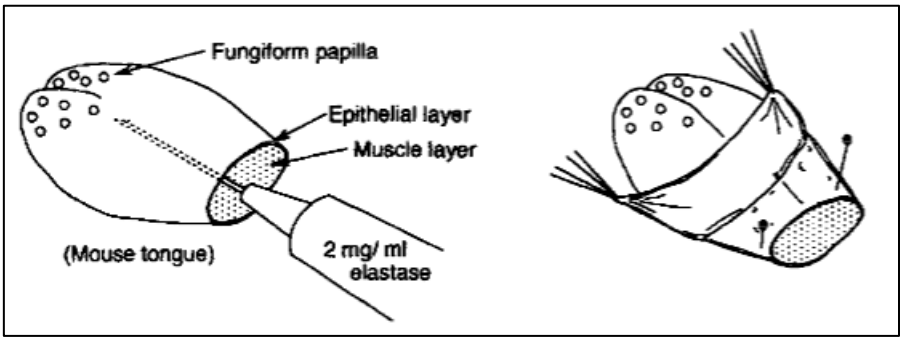

Figure 33. **Tast bud isolation.** From Miyamoto et al. (1996).

Samples were frozen immediately in liquid nitrogen and stored at −80 °C (not exceeding one month) until RNA extraction, or lysed in a buffer for Western blot analyses. For real-time qPCR and Western blot, each point corresponds to a pool of TBC from four mice.

1.4. Real-time qPCR

Total RNA from $CB_1R^{-/-}$ and WT TBC (n = 6) was extracted by using TRIzol method according to the manufacturer's recommendations (Invitrogen, Cergy-Pontoise, France). After purification, mRNA was resuspended in RNase free water. The samples were then analyzed and quantified using Traycell (Hellma Analytics, Müllheim, Germany). Samples having purity (A_{260}/A_{280}) between 1.80 and 2.00 were retained for the rest of the experiment. mRNA (500 ng) was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen, Cergy-Pontoise, France) in a 20 µL of reaction volume containing 5 µL mRNA, 1 µL random primer (100 ng/µL) (Invitrogen, Cergy-Pontoise, France), 0.4 µL dNTP (25 mM), 8.6 µL RNase-free water. After incubation for 5 min at 65 °C, 2 µL 5× First-Strand Buffer, 1 µL DTT, 1 µL M-MLV (200 UI) and 1 µL RNaseOUT were added, and the samples were

incubated for 1 h at 42 °C and then for 15 min at 70 °C. Real time qPCR reactions were performed on 10 ng cDNA in a 20 µL of reaction volume in triplicates with a StepOnePlus (Life Technologies, Saint-Aubin, France) device with the use of SYBR green PCR Master Mix (Life Technologies, Saint-Aubin, France). For each gene (Table 1), a standard curve was established from five cDNA dilutions (50 ng to 0.05 ng per well) and used to determine the PCR efficiency. Real time qPCR reactions were performed with a denaturing step of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primer specificity was checked using the melt curves. The comparative $2^{-\Delta\Delta CT}$ method was used for relative quantification.

Table 1 : **List of primers**

GLP-1R	Forward	GGACACATGAAGTCATCTTTGCCT
	Reverse	CAAGCCCTGGAAGGAAGTGAAGGA
P-GCG	Forward	TGCTGAAGGGACCTTTACCAGTGA
	Reverse	GCCTTTCACCAGCCAAGCAATGAA
β-actin	Forward	TTCTTTGCAGCTCCTTCGTT
	Reverse	ATGGAGGGGAATACAGCCC

1.5. Western Blotting

Freshly isolated mouse TBC were lysed using a micro-potter in 20 µL of TSE buffer (50 mM Tris HCl, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40, 5 µL/mL protease inhibitors (Sigma, Saint Quentin-Fallavier, France)) (Zhang et al., 2003a). Samples were stored on ice for 30 min, and then centrifuged (10,000g, 10 min, 4 °C). Lysates were used immediately or stored at −80 °C until the assay. Protein concentrations in homogenates were assayed using the BCA assay (Sigma, Saint Quentin-Fallavier, France). Denatured proteins (25 µg) were separated by SDS-PAGE (8%) and transferred to a polyvinylidene difluoride membrane. After being blocked for 3 h using a TBS buffer containing 5% BSA and 0.05% Tween-20, the membrane was incubated overnight with the primary antibody (Table 2). The α-gustducin was used as an internal reference protein. After a set of washes, the appropriate peroxidase-conjugated secondary antibody was added. Antibody labeling was detected by chemiluminescence (Clarity, Bio-Rad, Marnes-la-Coquette, France).

Table 2 : List of primary antibodies used in Western blotting

Primary antibody	**Reference**	**Supplier**	**Dilution**
CD36	AF2519	R&D Systems	1:1000
GNAT3/α-gustducin	sc-395	Santa Cruz	1:200
GPR120	ab97272	Abcam	1:500
β-actin	sc-47778	Santa Cruz	1:5000

1.6. Tissue culture of TBC and GLP-1 release

Papillae from WT and $CB_1R^{-/-}$ mice were isolated and incubated at 36 °C. The incubation media contained either 33 µM fatty acid-free BSA alone (control group) or 200 µM linoleic acid (LA) mixed and vortexed with 33 µM fatty acid-free BSA. After 2 h of incubation, the media were collected, and the active GLP-1 release was measured by ELISA (Millipore S.A.S., Molsheim, France). As the secretion of GLP-1 by TBC is very low, to be sure to detect active GLP-1 in the incubation medium, 10 pM of pure GLP-1 was systematically added in each experimental well, but not in standard curve, according to the manufacturers' recommendations. The dipeptidyl peptidase 4 (DPP4) inhibitor (0.1%, Millipore S.A.S., Molsheim, France) was added to the medium to prevent GLP-1 degradation.

1.7. Measurement of Ca^{2+} signalling

TBC were freshly isolated from mouse tongues as described by Dramane et al. (2012). The cells were cultured onto 24-well plates, containing RPMI-1640 medium, supplemented with 10% fetal calf serum, 2 mM glutamine, 50 µg/mL penicillin–streptomycin, and 20 mM HEPES, and incubated overnight at 37 °C. The next day, the supernatant was discarded. The cells were then incubated with Fura-2/AM (Invitrogen, Cergy-Pontoise, France) at 1 µM for 30 min at 37 °C in loading buffer which contained the following: 110 mM NaCl, 5.4 mM KCl, 25 mM $NaHCO_3$, 0.8 mM $MgCl_2$, 0.4 mM KH_2PO_4, 20 mM Hepes, 1.2 mM $CaCl_2$, 10 mM Glucose; pH 7.4 (Figure 34). After adding the test molecules into the wells, the changes in intracellular free Ca^{2+} (F_{340}/F_{380}) were monitored under the Nikon microscope (TiU) by using S-fluor 40× oil immersion objective. NIS-Elements software was used to record the images. The microscope was equipped with Luca-S EM-CCD (Andor technology, Gometz-le-châtel, France) camera for real-time recording of 16-bit digital images. The dual excitation fluorescence imaging system was used to analyze individual cells. The changes in intracellular free Ca^{2+} were expressed as ΔRatio, calculated as the difference between F_{340} and F_{380}. All test molecules were added in small volumes with no interruption in recordings. For Ca^{2+} signalling experiments, the fatty acid was dissolved in ethanol (0.1%, v/v) and added into the experimental cuvette.

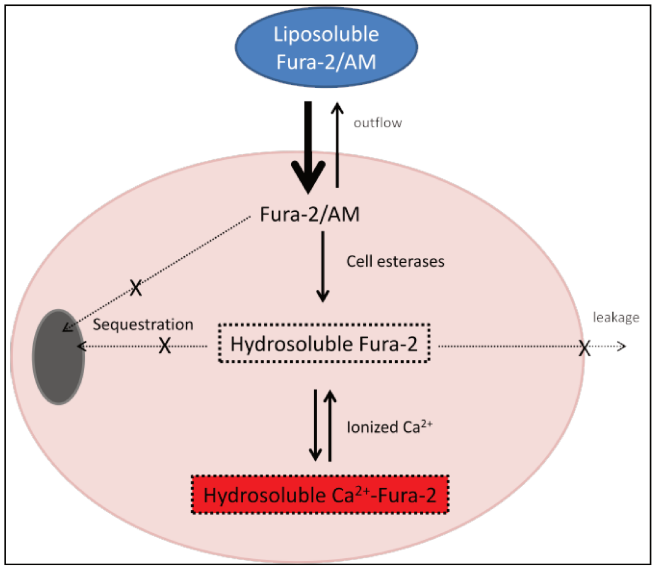

Figure 34. **Cell loading with Fura-2/AM.**

Anandamide (AEA, CB_1R endogenous ligand), arachidonyl-2′-chloroethylamide (ACEA, CB_1R synthetic ligand), LA, DB-cAMP, and U73122 were supplied by Sigma (Saint Quentin-Fallavier, France). A784168, TRPV1 (transient receptor potential vanilloid 1) antagonist, and rimonabant (CB_1R inverse agonist) were provided by Tocris (Bio-Techne, Lille, France) and Sanofi (Paris, France), respectively.

1.8. Statistics

Results are expressed as means ± SEM. The significance of differences between groups was evaluated with GraphPad Prism (GraphPad Software, La Jolla, CA, USA) using two-tailed Student's t-test or two-way ANOVA with Bonferroni correction. A p value of less or equal 0.05 was considered to be statistically significant.

Results

2. Results

2.1. The absence of CB_1R gene induces a low preference for fatty solutions independently of postprandial factors

$CB_1R^{-/-}$ mice displayed a significant decrease in the preference for the fatty solutions (rapeseed oil and LA) compared to wild type mice (Figure 35a, b). Hormonal post-ingestive regulatory feedback greatly influences metabolism and appetite and, consequently, behaviour during long-term two-bottle preference tests (Parker et al., 2014; Sclafani and Ackroff, 2012). Therefore, we performed short-term licking tests to measure fat preference limiting post-ingestive cues. LA was chosen among other LCFA because it showed the best results in two-bottle preference tests (data not shown). As shown in Figure 1c, the number of licks for LA was significantly higher in WT mice than in $CB_1R^{-/-}$ mice confirming the low preference in $CB_1R^{-/-}$ mice for fatty solutions.

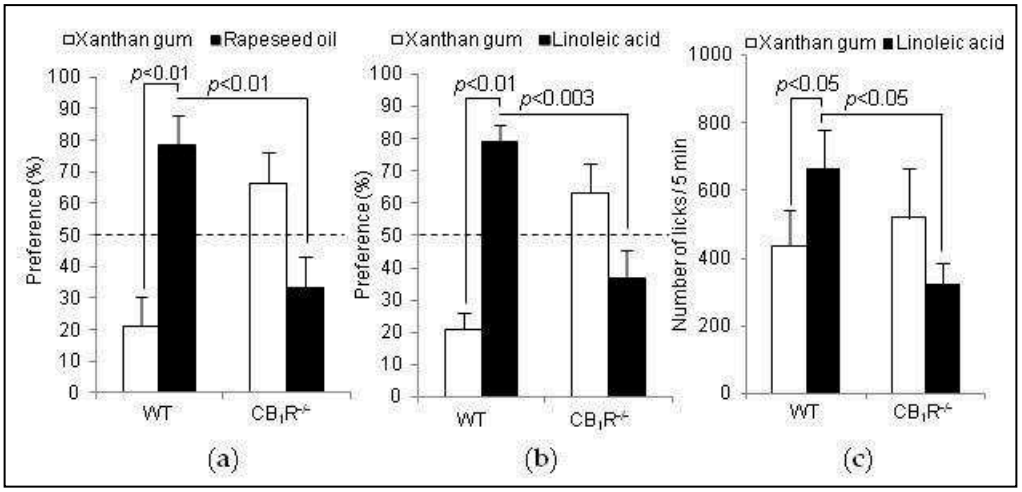

Figure 35. **Effect of CB_1R gene invalidation on preference for lipids**.Two bottles (control and experimental) were simultaneously offered to WT and $CB_1R^{-/-}$ mice for 12 hours. Experimental solution contained: (**a**) 0.2% of rapeseed oil (w/v); (**b**) 0.2% of linoleic acid (w/v) diluted in xanthan gum. Control solution contained 0.3% of xanthan gum (w/v). Values are expressed as mean ± SEM (n=5). Dotted line represents the absence of preference. (**c**) Short-term (5 min) licking tests in wild type and $CB_1R^{-/-}$ mice were performed as described in Materials and methods. Animals were subjected successively to a control solution (xanthan gum) and an experimental solution containing 0.2% of linoleic acid. Values are expressed as mean ± SEM (n=9).

2.2. Treatment with rimonabant induces a low preference for fatty solutions and does not alter feeding behaviour or body weight

The mice treated with rimonabant for 26 days exhibited the same behaviour as $CB_1R^{-/-}$ mice, i.e. a low preference for fatty solutions (Figure 36a, b). Interestingly, lean mice fed with standard diet and treated with rimonabant did not show reduced food intake or reduction in body weight (Figure 36c). As the rimonabant induces an early and transient effect, we monitored these parameters for 5 days before starting two-bottle preference tests. Thus, no bias interfered with the behavioural experiments.

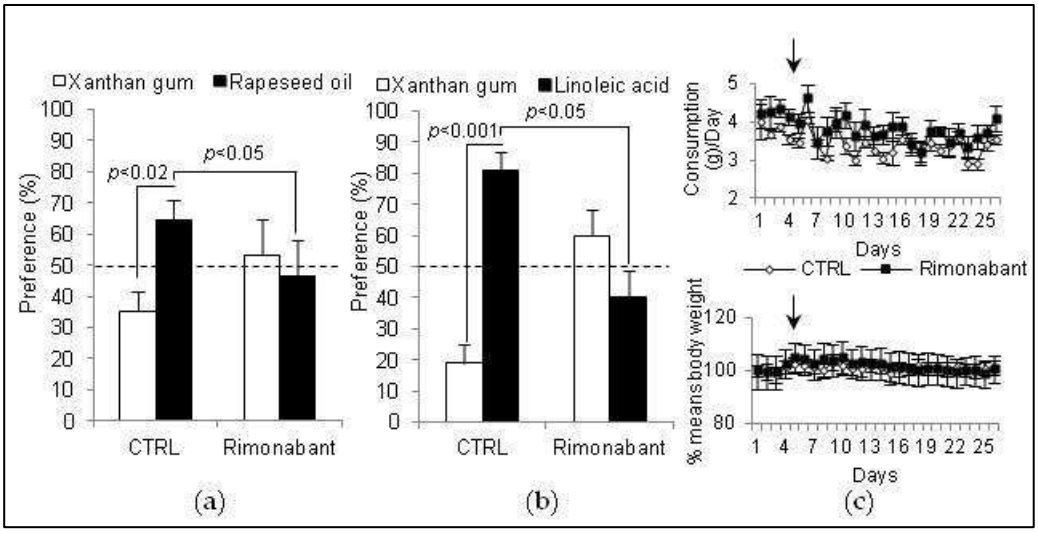

Figure 36. **Effect of rimonabant on preference for lipids, body weight and feeding behaviour**. (**a**, **b**) WT mice, treated with either rimonabant (10 $mg.kg^{-1}.day^{-1}$) or vehicle (CTRL), were simultaneously offered two bottles, a control one and an experimental one. The latter bottle contained either 0.2% of rapeseed oil (w/v) in (**a**) or 0.2% of linoleic acid (w/v) in (**b**) diluted in xanthan gum. Control solution contained 0.3% of xanthan gum. Values are expressed as mean ± SEM (n=5). Dotted line represents the absence of preference. (**c**) Food intake and body weight variations in mice treated or not with rimonabant and fed a standard chow. Values are expressed as mean ± SEM (n=5). Black arrows indicate the beginning of the treatment with rimonabant.

2.3. CD36 and GPR120 protein expressions are not altered in TBC of $CB_1R^{-/-}$ mice

CB_1R gene invalidation did not interfere with CD36 and GPR120 expression levels in taste buds (Figure 37) from $CB_1R^{-/-}$ and WT mice. α-gustducin, a marker of type II TBC, remained

stable (Figure 37). It seems that low preference for fatty solutions observed in $CB_1R^{-/-}$ mice was not due to altered expression of CD36 and GPR120.

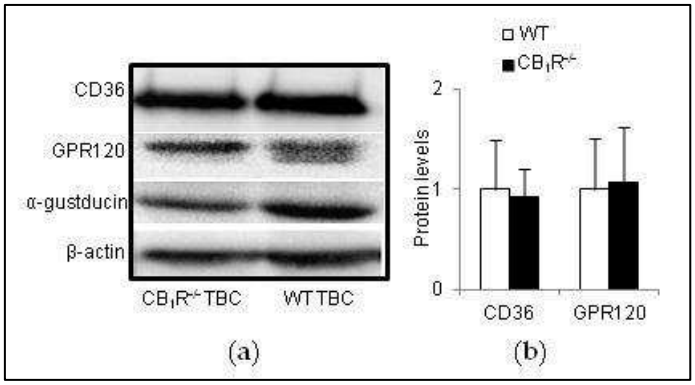

Figure 37. **Impact of CB_1R gene invalidation on CD36 and GPR120 protein expressions**. CD36 and GPR120 protein levels were measured by Western blotting in TBC (n=2) from WT and $CB_1R^{-/-}$ mice. (**a**) A representative blot corresponding to a pool of total proteins from four mice TBC is shown. (**b**) The corresponding histogram shows CD36 and GPR120 protein levels.

2.4. Cb_1r gene invalidation induces a decrease in Pro-glucagon and Glp-1r mRNA and a low GLP-1 basal level

According to previous published data (Ozdener et al., 2014), LCFA induces active GLP-1 release by mouse TBC. Pro-glucagon and Glp-1r mRNA levels were significantly lower in $CB_1R^{-/-}$ TBC than in WT TBC (Figure 38a). To measure the release of active GLP-1 in TBC, freshly isolated mouse TBC were incubated for 2 hours in an oxygenized medium containing anti-DPP4, to prevent GLP-1 degradation, and exposed or not to 200 µM LA. Notably in $CB_1R^{-/-}$ mice TBC, GLP-1 release in the culture medium was significantly lower than that in WT TBC in both conditions. As expected, LA induced a small but significant rise in active GLP-1 levels in culture medium of WT TBC (Figure 38b). Interestingly, the rise elicited by LA in WT TBC was abrogated in $CB_1R^{-/-}$ mice TBC (Figure 38b). These data suggest the absence of CB_1R may alter GLP-1 production and explain the low preference in $CB_1R^{-/-}$ mice for fatty solutions.

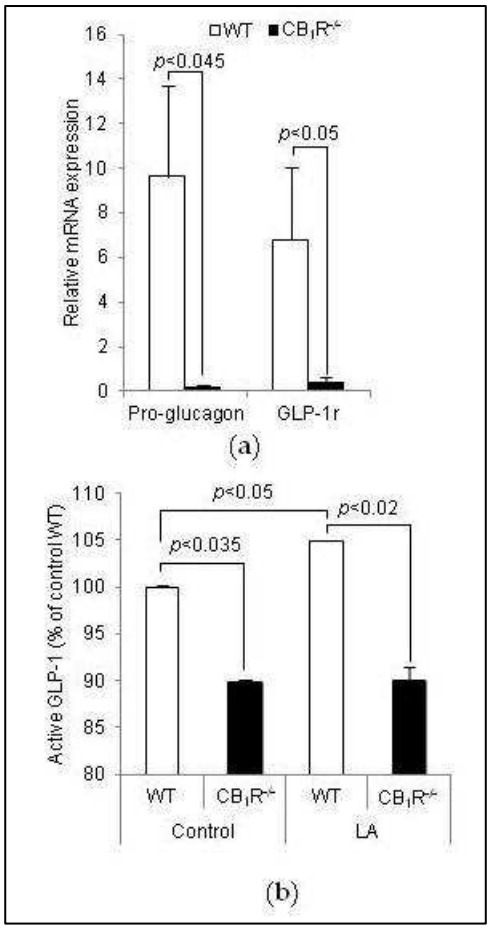

Figure 38. **Effect of CB_1R gene invalidation on GLP-1.** (**a**) Pro-Glucagon and GLP-1r mRNA levels were assayed by real-time qPCR in mouse TBC from WT and $CB_1R^{-/-}$ mice. Values are expressed as mean ± SEM (n=6). (**b**) GLP-1 release by freshly isolated mouse TBC incubated for 2 hours in the presence of 33 μM Fatty Acid-free BSA alone (CTRL) or with 200 μM linoleic acid (LA). Each value corresponds to the GLP-1 released by cultured TBC from three mice. Values are expressed as mean ± SD (n=2).

2.5. Both LA and cannabinoids induce CB_1R-dependent calcium responses in TBC

In TBC, LCFA evokes increases in Ca^{2+} signalling (Dramane et al., 2012a). Here, we investigated the role played by CB_1R on Ca^{2+} signalling using freshly isolated TBC from $CB_1R^{-/-}$ and WT mice. As expected, LA triggered a higher rise in $[Ca^{2+}]_i$ in WT TBC (Gaillard et al., 2008) (Figure 39a, f) than that in $CB_1R^{-/-}$ TBC (Figure 38b, f). Similarly, arachidonyl-2'-chloroethylamide (ACEA, a specific CB_1R agonist), induced a rise in $[Ca^{2+}]_i$ in WT TBC (Figure 39f). However, ACEA triggered a significant lower increase in $[Ca^{2+}]_i$ in $CB_1R^{-/-}$ TBC than that in WT TBC (Figure 39f). Finally, the combination of LA and ACEA also induced a

strong rise in $[Ca^{2+}]_i$ in WT TBC (Figure 39c, f) corresponding to the combined effect of the two molecules that was not apparent in $CB_1R^{-/-}$ TBC (Figure 39d, f). Interestingly, when tested on $CB_1R^{-/-}$ TBC, ACEA still triggered a rise in $[Ca^{2+}]_i$, suggesting a role for other receptors in this model (Figure 38f). In line with this, blockade of TRPV1 with the specific antagonist, A784168, in $CB_1R^{-/-}$ TBC curtailed the action of ACEA on calcium response (Figure 39e).

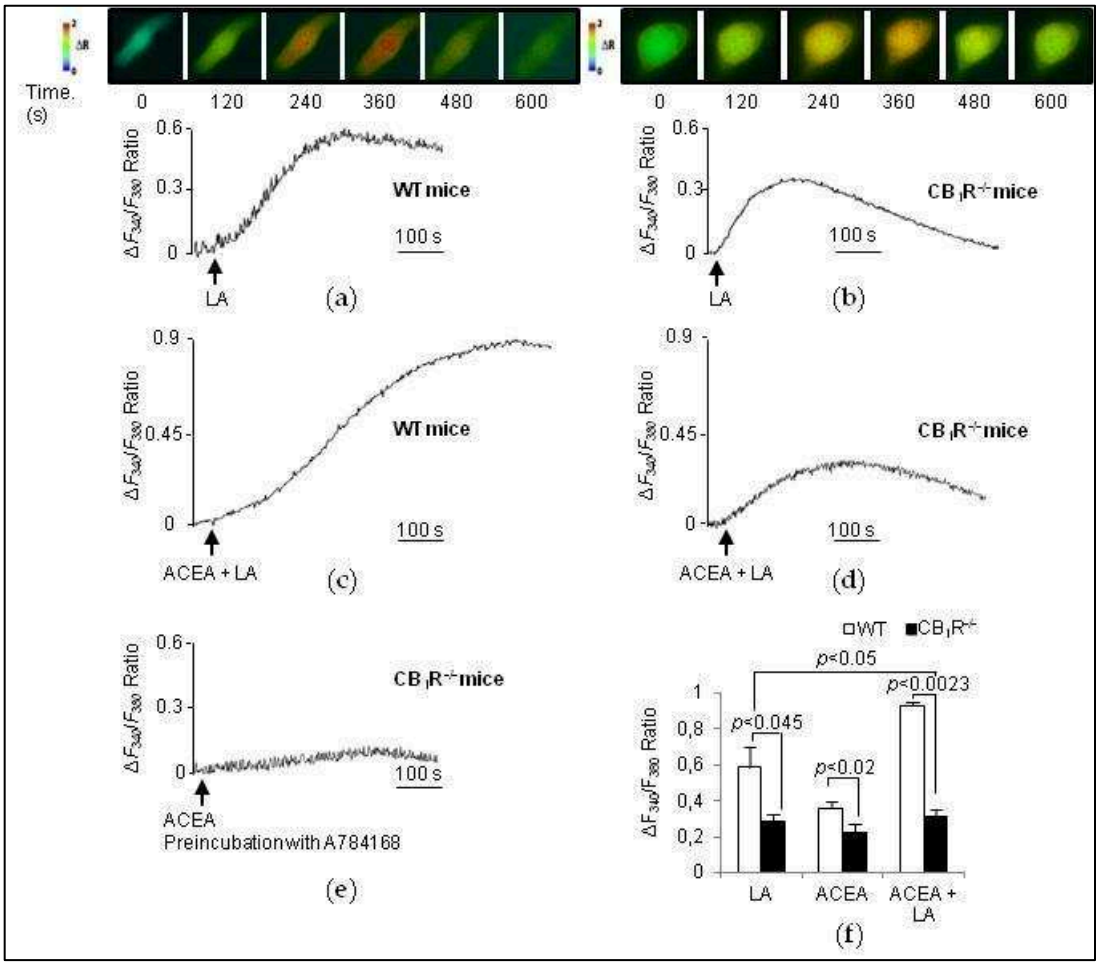

Figure 39. **Effects of linoleic acid (LA) and cannabinoids on Ca^{2+} signalling in mouse TBC**. Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in taste bud cells freshly isolated from WT mice (**a**) and $CB_1R^{-/-}$ mice (**b**) following addition of LA (25 μM) and the corresponding graphs below. Changes in $[Ca^{2+}]_i$ evoked by combined addition of ACEA (1.5 μM) and LA (25 μM) in WT (**c**) and $CB_1R^{-/-}$ TBC (**d**), respectively. Changes in $[Ca^{2+}]_i$ evoked by ACEA (1.5 μM) after a 15 minutes preincubation with A784168, a TRPV1 antagonist, in $CB_1R^{-/-}$ TBC (**e**). The arrow heads indicate when the test molecules were added. Variations in $\Delta F_{340}/F_{380}$ Ratio induced by LA (25 μM), ACEA (1.5 μM) and ACEA (1.5 μM) in combination with LA (25 μM) in WT and $CB_1R^{-/-}$ mice TBC (**f**). Values are expressed as mean ± SEM (n=5).

2.6. CB_1R blockade significantly decreases calcium responses triggered by LA, AEA and ACEA in WT TBC

To further explore the role of CB_1R on calcium signalling, we preincubated WT TBC with the specific CB_1R antagonist rimonabant. As observed in Figure 40, rimonabant significantly abrogated $[Ca^{2+}]_i$ responses, induced by LA, AEA and ACEA (Figure 40c) corroborating the previous results observed in $CB_1R^{-/-}$ TBC.

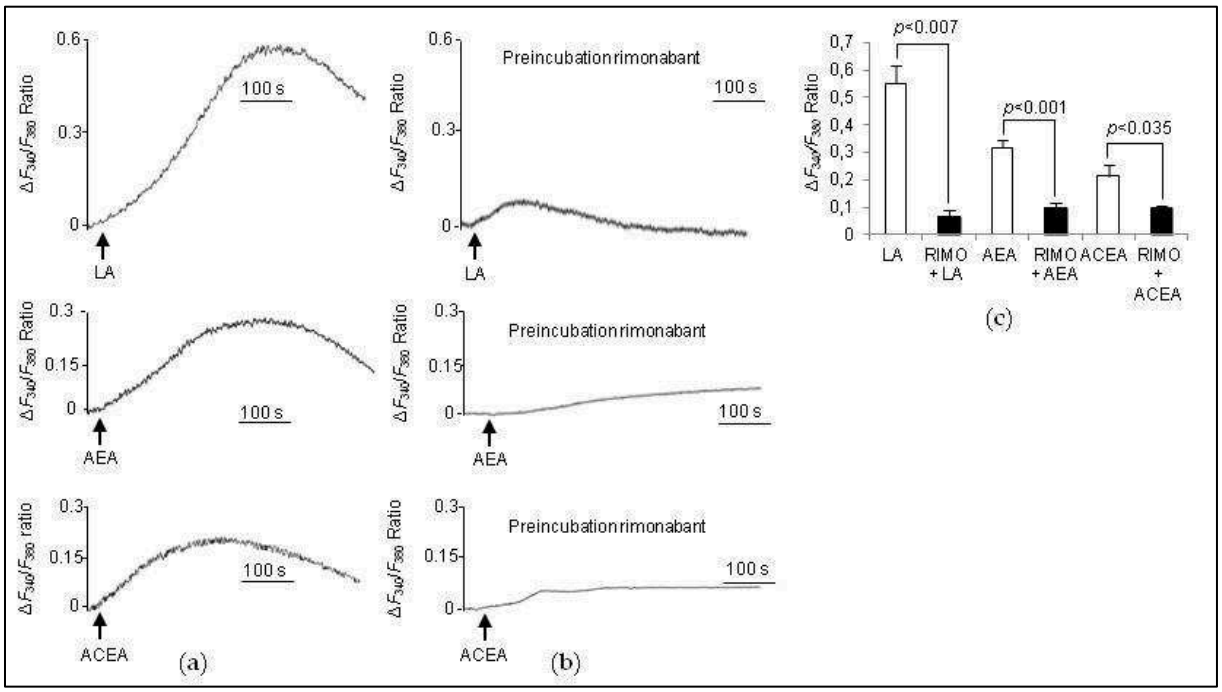

Figure 40. **Effects of rimonabant on linoleic acid in mTBC (LA) and cannabinoids-induced Ca^{2+} signalling in mTBC.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. Graphs show the increase in $[Ca^{2+}]_i$ in taste bud cells freshly isolated from WT mice following addition of LA (25 μM), anandamide (AEA, 5 μM) and ACEA (1.5 μM) (**a**). WT TBC before the addition of LA (25 μM), AEA (5 μM) and ACEA (1.5 μM) were preincubated (15 minutes) with rimonabant (50 μM) (**b**). The arrow heads indicate when the test molecules were added. Changes in $\Delta F_{340}/F_{380}$ Ratio induced by LA (25 μM), AEA (5 μM), and ACEA (1.5 μM) in WT mice TBC after a preincubation with or without rimonabant (RIMO, 50 μM) (**c**). Values are expressed as mean ± SEM (n=5).

2.7. AEA-induced Ca^{2+}-signalling is PLC dependent

Finally, in order to validate our model, we investigated the downstream CB_1R pathway elicited by AEA treatment. Here, we used DB-cAMP, a cAMP analog and a phosphodiesterase inhibitor, and U73122, a phospholipase C inhibitor. We observed that DB-

cAMP did not significantly alter the Ca^{2+} response induced by AEA. Conversely, a pretreatment with U73122 significantly decreased the Ca^{2+} response, indicating that the Ca^{2+} response triggers a PLC-dependent Ca^{2+} signalling (Figure 41).

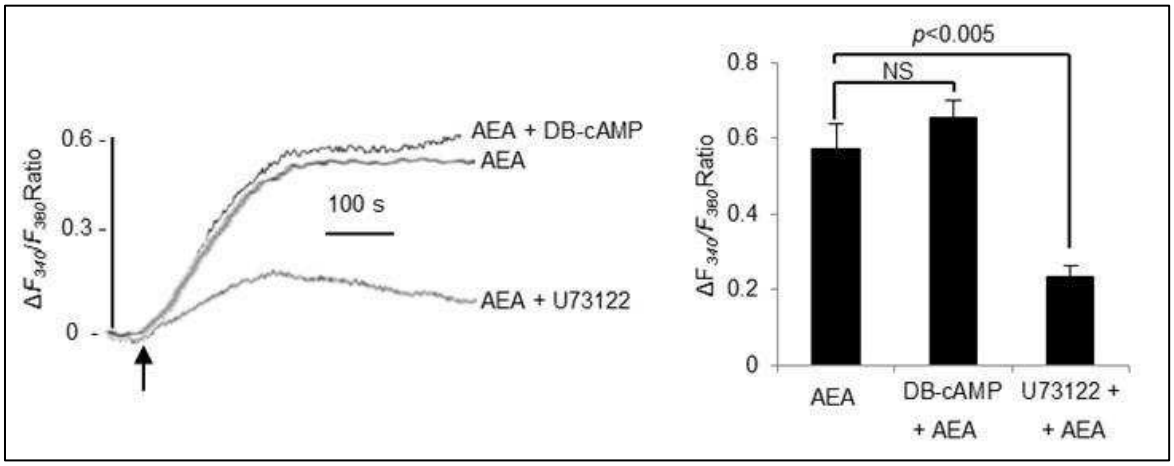

Figure 41. **Effects of DB-cAMP and U73122 on anandamide (AEA, 5 μM)-induced Ca^{2+} signalling in TBC.** Ca^{2+} imaging studies were performed in calcium-containing buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40× oil immersion objectives. Graphs show the increase in $[Ca^{2+}]_i$ in taste bud cells freshly isolated from WT mice following addition of anandamide (AEA, 5 μM) with or without preincubation (20 min) with DB-cAMP (1 mM) or preincubation (20 min) with U73122 (10 μM) (left panel). The arrowhead indicates when the test molecules were added. Changes as histograms (right panel) in $\Delta F_{340}/F_{380}$ Ratio induced by anandamide (AEA, 5 μM) in WT mice TBC after a preincubation with or without DB-cAMP (1 mM) or U73122 (10 μM). Values are expressed as mean ± SEM (n = 6).

Discussion

3. Discussion

Williams and Kirkham (1999) demonstrated that CB_1R is responsible for increased food intake, induced by an endocannabinoid agonist. Later on, Yoshida et al. (2010) revealed that endocannabinoids enhanced the gustatory responses to sweet tastants via CB_1R. Indeed, the activation of endocannabinoid system (ECS) appears to be associated with hyperphagia and a preference for palatable food. Interestingly, CB_1R are also expressed in a subset of taste bud cells (Yoshida et al., 2010). We report here that $CB_1R^{-/-}$ mice displayed no preference for fat solutions compared to WT mice. The same behaviour was also observed when WT mice were treated with rimonabant, a CB_1R blocker, confirming the role for this receptor in the detection of dietary lipids. We have employed LA as a candidate for LCFA because this fatty acid is abundantly present in Western food; however, it is possible that the saturated fatty acids like palmitic acid (PA) might also initiate the same gustatory response. Indeed, we have shown previously that LA and PA triggered the same increases in $[Ca^{2+}]_i$ in mouse taste bud cells (Gaillard et al., 2008).

In the present study, for behavioural experiments, we used whole body knockout mice for CB_1R, and it is possible that the hypothalamic cannabinoid system, via the dopaminergic area, might be involved in fat taste preference (Melis et al., 2013c). Nonetheless, we sought to elucidate cellular mechanisms in the modulation of fat preference. We first tested the hypothesis whether there is an alteration in CD36 and GPR120 protein in TBC of $CB_1R^{-/-}$ mice. In our study, CD36 and GPR120 protein expressions were not altered by the absence of CB_1R, suggesting that the absence of preference for fatty solutions may be due to altered downstream signalling. Moreover, we checked the delivery of linoleic acid under both conditions, and we observed identical uptake of exogenous fatty acid.

Previous studies indicated that both CD36 and GPR120 activation by a LCFA triggered mobilization of $[Ca^{2+}]_i$ from the intracellular endoplasmic reticulum Ca^{2+} pool during fat taste perception (Galindo et al., 2012; Ozdener et al., 2014). In our study, we show, for the first time, that LA-mediated increase in $[Ca^{2+}]_i$ was altered when CB_1R was inactivated by rimonabant or by the absence of CB_1R. In addition, the CB_1R agonist ACEA also increased calcium flux per se in TBC, albeit with lower potency than LA. However, the effect of ACEA was maintained in TBC from $CB_1R^{-/-}$ mice, raising the possibility that the increase in $[Ca^{2+}]_i$ could be mediated by the receptors other than CB_1R, for example, TRPV1. Indeed, it has been shown that activation of TRPV1 by endocannabinoids induces calcium signalling (Kentish and Page, 2015; Ryskamp et al., 2014). Besides, blockade of TRPV1 with A784168 totally

abolished $[Ca^{2+}]_i$ response induced by ACEA, indicating that the residual calcium signal observed in $CB_1R^{-/-}$ TBC with ACEA may be due to TRPV1 activity. Furthermore, it appears that the CB_1R-coupled downstream signalling is PLC-dependent, in accordance with the observations of De Petrocellis et al. (2007). However, it remains to be elucidated in future whether anandamide, employed in the present study, activates the $G_{\beta\delta}$ subunit of CB_1R, and activates PLC via PI-3-kinase pathway. As a whole, our data indicate that CB_1R may play a crucial role in fat taste perception by modulating calcium signalling.

As previously described, GLP-$1^{-/-}$ mice have reduced taste responses to dietary fat, suggesting that orosensory detection of LCFA could be associated to the secretion of lingual GLP-1 (Martin et al., 2012). Data reported herein showed that the secretion of active GLP-1 induced by LA is strongly decreased in $CB_1R^{-/-}$ mice suggesting the existence of a link between CB_1R signalling and GLP-1 production. Hence, CB_1R activation may stimulate proglucagon and GLP-1r production and, therefore, modulate perception threshold of LCFA. Further investigations are needed to explore the possibility whether GLP-1 secretion is stimulated via $[Ca^{2+}]_i$ signalling in TBC or by other mechanisms (Takai et al., 2015).

In conclusion, the present report shows that CB_1R influences fat taste perception via regulating calcium signalling in TBC (Figure 38). It is proposed that CB_1R activation induces a $[Ca^{2+}]_i$ response that strengthens fat perception, that is mediated by CD36. Activation of ECS could, thereby, increase sensory stimuli relaying palatability of foods and, ultimately, stimulate food intake. The physiopathological relevance of such a regulatory pathway is supported by the fact that ECS tone is increased in obesity.

These results suggest that by correcting the effects of the ECS as early as in the oral cavity we could modulate the orosensory detection of dietary lipids. We proposed the following schematic model summarizing the results obtained and the potential lingual CB_1R involvement (Figure 42). Hence, a local action specifically targeting the lingual CB_1R (enhancing or antagonizing), thus avoiding side effects that occurred with rimonabant treatment, would be likely to improve overweight or cancer patient care.

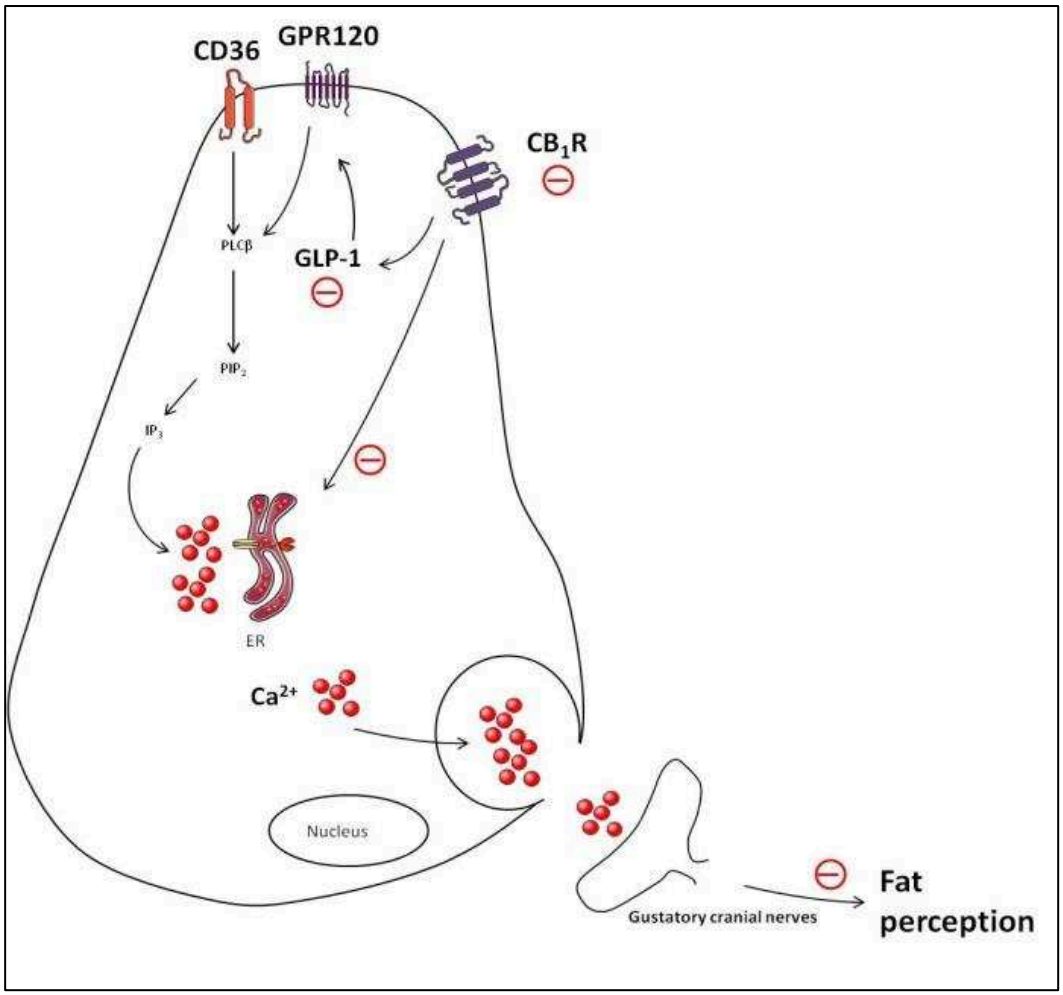

Figure 42. **Schematic model of the proposed CB_1R involvement in TBC**. Modified from Gilbertson and Khan (2014) including the obtained results regarding the CB_1 receptor and its interaction with CD36 and GPR120.

Publication

Orosensory Detection of Dietary Fatty Acids Is Altered in $CB_1R^{-/-}$ Mice

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Article

Orosensory Detection of Dietary Fatty Acids Is Altered in $CB_1R^{-/-}$ Mice

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Abstract: Obesity is one of the major public health issues, and its prevalence is steadily increasing all the world over. The endocannabinoid system (ECS) has been shown to be involved in the intake of palatable food via activation of cannabinoid 1 receptor (CB_1R). However, the involvement of lingual CB_1R in the orosensory perception of dietary fatty acids has never been investigated. In the present study, behavioral tests on $CB_1R^{-/-}$ and wild type (WT) mice showed that the invalidation of *Cb1r* gene was associated with low preference for solutions containing rapeseed oil or a long-chain fatty acid (LCFA), such as linoleic acid (LA). Administration of rimonabant, a CB_1R inverse agonist, in mice also brought about a low preference for dietary fat. No difference in CD36 and GPR120 protein expressions were observed in taste bud cells (TBC) from WT and $CB_1R^{-/-}$ mice. However, LCFA induced a higher increase in $[Ca^{2+}]_i$ in TBC from WT mice than that in TBC from $CB_1R^{-/-}$ mice. TBC from $CB_1R^{-/-}$ mice also exhibited decreased *Proglucagon* and *Glp-1r* mRNA and a low GLP-1 basal level. We report that CB_1R is involved in fat taste perception via calcium signaling and GLP-1 secretion.

Keywords: nutrition; lipids; fat taste; CD36; feeding behavior; cannabinoids; CB_1R; GLP-1

1. Introduction

Due to the abundance of food resources in the modern era, the Western diet is comprised of more than 40% of fat, thereby contributing to the increase in the prevalence of obesity that is associated with a number of pathologies (type 2 diabetes mellitus, hypertension, cancer, and others).

The taste modalities represent an essential factor involved in food intake. It is now well established that obese subjects exhibit higher spontaneous preference for fat than lean subjects [1,2]. Recent studies have proposed the existence of a sixth taste modality dedicated to the orosensory perception of dietary fat. The CD36 (cluster of differentiation 36) has been suggested to act as lingual lipid receptor [3]. The binding of a fatty acid to lingual CD36 in taste bud cells (TBC) leads to modifications in the membrane potential and to an increase in free intracellular calcium concentrations, $[Ca^{2+}]_i$, followed by the release of neurotransmitters [4,5]. These gustatory signals are transmitted from the oral cavity, through the cranial nerve IX (lingual branch of the glossopharyngeal), to the nucleus of the solitary tract (NST) [6]. The NST is connected to different brain areas associated with food intake, rewarding, memory, and processes integrating visceral signals [7,8]. Hence, the integration of the gustatory signals in brain triggers a behavioral and metabolic response [8].

GPR120 (G protein-coupled receptor 120) has also been proposed to play a role in fat-related regulation of satiation [8,9]. Nevertheless, GPR120 does not seem to have a major role in oral fat detection. Indeed, contradictory results have been reported for behavioral tests in GPR120$^{-/-}$ mice [10–12]. However, the implication of GPR120 in the release of the incretin hormone glucagon-like peptide-1 (GLP-1) has been highlighted in mouse taste bud cells [9,13]. Thus, CD36 is likely to play a role in the detection of dietary fatty acids in TBC, whereas GPR120 would be implicated in the modulation of postprandial fat taste sensitivity.

The presence of GLP-1 and its receptor in the gustatory mucosa has been demonstrated [9,14,15], suggesting that taste bud cells may modulate taste perception in an autocrine or a paracrine manner. Indeed, linoleic acid (LA) has been reported to induce GLP-1 release in human TBC in a GPR120-dependent manner [9]. Martin et al. [13] have suggested that GLP-1 is locally active and might affect the basic functions in mouse taste buds. Besides, Shin et al. [15] showed that local GLP-1 signaling could enhance sweet-taste sensitivity, supporting the existence of a paracrine mechanism for the regulation of taste function.

The implication of the endocannabinoid system in the regulation of food intake is well documented [16–20]. Several studies have demonstrated that exogenous cannabinoids, like delta 9-tetrahydrocannabinol (Δ9-THC) or anandamide (AEA), induce hyperphagia and preference for palatable food [18,19] via cannabinoid-1 receptors (CB_1R) [21]. Therefore, the CB_1R blocker/inverse agonist, rimonabant, has been used in the treatment of obesity [22–24].

Being largely expressed in the central and peripheral nervous system, CB_1R has also been detected in TBC, and it has been shown that the activation of these receptors by cannabinoids enhances sweet taste [7]. However, the involvement of lingual CB_1R in fat taste perception has never been investigated. Considering that the increase in dietary fat intake plays an important role in the prevalence of obesity, the present investigation was designed to assess whether the activation of CB_1R in TBC is associated with the altered orosensory perception of dietary lipids in $CB_1R^{-/-}$ and wild type (WT) mice.

2. Materials and Methods

2.1. Ethical Approval

French guidelines for the use and care of laboratory animals were followed, and the experimental protocols were approved by the regional animal ethic committee of the University of Burgundy. In vivo studies were performed on male C57BL/6J wild type (WT) mice (Janvier Labs, Le Genest Saint Isle, France) and $CB_1R^{-/-}$ mice (generous gift from Dr. James Pickel, National Institute of Mental Health, Bethesda, MD, USA) with a C57BL/6J background. Animals were individually housed in a controlled environment (constant temperature and humidity, dark period from 19:00 to 7:00). The mice had free access to standard regular chow and tap water during the experiments, unless otherwise specified.

2.2. Behavioral Experiments

2.2.1. Two-Bottle Preference Tests

After being deprived of water for 6 h, mice were offered simultaneously two bottles, containing either control or experimental solution for 12 h. To minimize bias due to textural properties, the two solutions contained 0.3% xanthan gum (*w*/*v*, Sigma, Saint Quentin-Fallavier, France), whereas the experimental solutions were added with either 0.2% rapeseed oil (*w*/*v*, Fleur de Colza, Lesieur, France) or 0.2% linoleic acid (*w*/*v*, LA, Sigma). At the end of each test, the intake of control and experimental solutions was recorded by weighing the feeders/bottles. The experiments were repeated two times, independently.

In parallel, two groups (n = 5 each) of 10 WT mice, treated daily with rimonabant (SR141716, 10 mg/kg of body weight, Sanofi, Paris, France) or vehicle (0.1% DMSO/0.025% Tween 80 in 0.9%

NaCl), by an intraperitoneal injection for 26 days, were subjected to the same two-bottle preference test. Food intake and weight were monitored during the experiment.

2.2.2. Licking Tests

The $CB_1R^{-/-}$ (n = 9) and WT (n = 9) mice were deprived of food and water for 6 h before the test. The mice were conditioned to choose between a palatable (4% sucrose) and a control solution. Once the mice were conditioned, they were randomly subjected to two-bottle test, containing either control (0.3% xanthan gum) or a test solution (0.3% xanthan gum + 0.2% linoleic acid, LA). The number of licks, motivated by each bottle, were recorded using computer-controlled lickometers (Med Associates, Fairfax, VT, USA). Data were analyzed for 5 min from the first lick.

2.3. Papillae and Taste Buds Isolation

The mice were anesthetized with 2% isoflurane gas, and then sacrificed by cervical dislocation. Taste bud cells (TBC) were isolated according to previously published procedure [4]. In brief, lingual epithelium was separated from connective tissues by enzymatic dissociation (elastase and dispase mixture, 2 mg/mL each in Tyrode buffer: 120 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM $CaCl_2$, 10 mM glucose, 1 mM $MgCl_2$, 10 mM Na pyruvate, pH 7.4). Samples were frozen immediately in liquid nitrogen and stored at −80 °C (not exceeding one month) until RNA extraction, or lysed in a buffer for Western blot analyses. For real-time qPCR and Western blot, each point corresponds to a pool of TBC from four mice.

2.4. Real-Time qPCR

Total RNA from $CB_1R^{-/-}$ and WT TBC (n = 6) was extracted by using TRIzol method according to the manufacturer's recommendations (Invitrogen, Cergy-Pontoise, France). After purification, mRNA was resuspended in RNase free water. The samples were then analyzed and quantified using Traycell (Hellma Analytics, Müllheim, Germany). Samples having a purity (A_{260}/A_{280}) between 1.80 and 2.00 were retained for the rest of the experiment. mRNA (500 ng) was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen) in a 20 µL of reaction volume containing 5 µL mRNA, 1 µL random primer (100 ng/µL) (Invitrogen), 0.4 µL dNTP (25 mM), 8.6 µL RNase-free water. After incubation for 5 min at 65 °C, 2 µL 5× First-Strand Buffer, 1 µL DTT, 1 µL M-MLV (200 UI) and 1 µL RNaseOUT were added, and the samples were incubated for 1 h at 42 °C and then for 15 min at 70 °C. Real time qPCR reactions were performed on 10 ng cDNA in a 20 µL of reaction volume in triplicates with a StepOnePlus (Life Technologies, Saint-Aubin, France) device with the use of SYBR green PCR Master Mix (Life Technologies, Saint-Aubin, France). For each gene, a standard curve was established from five cDNA dilutions (50 ng to 0.05 ng per well) and used to determine the PCR efficiency. Forward and reverse primer sequences used for amplification were 5′-GGACACATGAAGTCATCTTTGCCT-3′ and 5′-CAAGCCCTGGAAGGAAGTGAAGGA-3′ for *Glp-1r* (NM_021332), 5′-TGCTGAAGG GACCTTTACCAGTGA-3′ and 5′-GCCTTTCACCAGCCAAGCAATGAA-3′ for *Gcg* (NM_008100), and 5′- TTCTTTGCAGCTCCTTCGTT-3′ and 5′-ATGGAGGGGAATACAGCCC-3′ for *β-actin* (NM_007393). The amplicon size for *Glp-1r* is 107 bp and is located in the exon 11 and 12; for *Gcg* is 85 bp and is located in the exon 4; for *β-actin* is 149 bp and is located in the exon 1 and 2. Real time qPCR reactions were performed with a denaturing step of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primer specificity was checked using the melt curves. The PCR efficiency was calculated as follow $10^{-1/\text{slope}} - 1$. The parameters for *GLP-1r* were as follows: slope −3.241, y intercept 33.848, R^2 0.98, and PCR efficiency 1.03; *Gcg*: slope −3.527, y intercept 29.296, R^2 0.92, and PCR efficiency 0.92; and *β-actin*: slope −2.837, y intercept 29.296, R^2 0.996, and PCR efficiency 1.25. The comparative $2^{-\Delta\Delta CT}$ method was used for relative quantification.

2.5. Western Blotting

Freshly isolated mouse TBC were lysed using a micro-potter in 20 µL of TSE buffer (50 mM Tris HCl, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40, 5 µL/mL protease inhibitors (Sigma)) [25]. Samples were stored on ice for 30 min, and then centrifuged (10,000 *g*, 10 min, 4°C). Lysates were used immediately or stored at −80 °C until the assay. Protein concentrations in homogenates were assayed using the BCA assay (Sigma, Saint Quentin-Fallavier, France). Denatured proteins (25 µg) were separated by SDS-PAGE (8%) and transferred to a polyvinylidene difluoride membrane. After being blocked for 3 h using a TBS buffer containing 5% BSA and 0.05% Tween-20, the membrane was incubated overnight with either of the antibodies: anti-CD36 antibody (R&D Systems, AF2519; 1:1000), anti-GPR120 antibody (Abcam, Paris, France, ab97272; 1:500), anti-α-gustducin antibody (Santa Cruz, Heidelberg, Germany, sc-395; 1:200) and anti-β-actin antibody (Santa Cruz, Heidelberg, Germany, sc-47778; 1:5000). The α-gustducin was used as an internal reference protein. After a set of washes, the appropriate peroxidase-conjugated secondary antibody was added. Antibody labeling was detected by chemiluminescence (Clarity, Bio-Rad, Marnes-la-Coquette, France).

2.6. Tissue Culture of TBC and GLP-1 Release

Papillae from WT and $CB_1R^{-/-}$ mice were isolated and incubated at 36 °C. The incubation media contained either 33 µM fatty acid-free BSA alone (control group) or 200 µM linoleic acid (LA) mixed and vortexed with 33 µM fatty acid-free BSA. After 2 h of incubation, the media were collected, and the active GLP-1 release was measured by ELISA (Millipore S.A.S., Molsheim, France). As the secretion of GLP-1 by TBC is very low, to be sure to detect active GLP-1 in the incubation medium, 10 pM of pure GLP-1 was systematically added in each experimental well, but not in standard curve, according to the manufacturers' recommendations. The dipeptidyl peptidase 4 (DPP4) inhibitor (0.1%, Millipore) was added to the medium to prevent GLP-1 degradation.

2.7. Measurement of Ca^{2+} Signaling

TBC were freshly isolated from mouse tongues as described by Dramane et al. [26]. The cells were cultured onto 24-well plates, containing RPMI-1640 medium, supplemented with 10% fetal calf serum, 2 mM glutamine, 50 µg/mL penicillin–streptomycin, and 20 mM HEPES, and incubated overnight at 37 °C. The next day, the supernatant was discarded. The cells were then incubated with Fura-2/AM (Invitrogen) at 1 µM for 30 min at 37 °C in loading buffer which contained the following: 110 mM NaCl, 5.4 mM KCl, 25 mM $NaHCO_3$, 0.8 mM $MgCl_2$, 0.4 mM KH_2PO_4, 20 mM Hepes, 1.2 mM $CaCl_2$, 10 mM Glucose; pH 7.4. After adding the test molecules into the wells, the changes in intracellular free Ca^{2+} (F_{340}/F_{380}) were monitored under the Nikon microscope (TiU) by using S-fluor 40× oil immersion objective. NIS-Elements software was used to record the images. The microscope was equipped with Lucas EM-CCD (Andor Technology, Gometz-le-châtel, France) camera for real-time recording of 16-bit digital images. The dual excitation fluorescence imaging system was used to analyze individual cells. The changes in intracellular free Ca^{2+} were expressed as ΔRatio, calculated as the difference between F_{340} and F_{380}. All test molecules were added in small volumes with no interruption in recordings. For Ca^{2+} signaling experiments, the fatty acid was dissolved in ethanol (0.1%, v/v) and added into the experimental cuvette.

Anandamide (AEA, CB_1R endogenous ligand), arachidonyl-2′-chloroethylamide (ACEA, CB_1R synthetic ligand), LA, DB-cAMP, and U73122 were supplied by Sigma (Saint Quentin-Fallavier, France). A784168, TRPV1 (transient receptor potential vanilloid 1) antagonist, and rimonabant (CB_1R inverse agonist) were provided by Tocris (Bio-Techne, Lille, France) and Sanofi (Paris, France), respectively.

2.8. Statistics

Results are expressed as means ± SEM. The significance of differences between groups was evaluated with GraphPad Prism (GraphPad Software, La Jolla, CA, USA) using two-tailed Student's

t-test or two-way ANOVA with Bonferroni correction. A p value of less or equal 0.05 was considered to be statistically significant.

3. Results

3.1. The Absence of CB_1R Gene Induces a Low Preference for Fatty Solutions Independently of Postprandial Factors

$CB_1R^{-/-}$ mice displayed a significant decrease in the preference for the fatty solutions (rapeseed oil and LA) compared to wild type mice (Figure 1a,b). Hormonal post-ingestive regulatory feedback greatly influences metabolism and appetite and, consequently, behavior during long-term two-bottle preference tests [27] under CB_1R activation [28]. Therefore, we performed short-term licking tests to measure fat preference limiting post-ingestive cues. LA was chosen among other LCFAs because it showed the best results in two-bottle preference tests. As shown in Figure 1c, the number of licks for LA was significantly higher in WT mice than in $CB_1R^{-/-}$ mice, confirming the low preference in $CB_1R^{-/-}$ mice for fatty solutions.

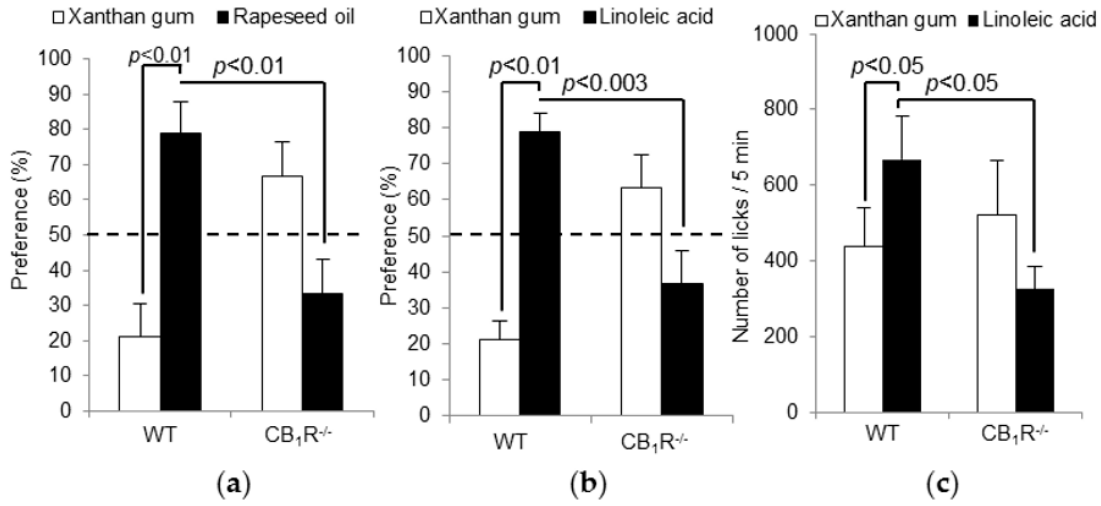

Figure 1. Effect of CB_1R gene invalidation on preference for lipids. Two bottles (control and experimental) were simultaneously offered to wild type (WT) and $CB_1R^{-/-}$ mice for 12 h. The experimental solution contained (**a**) 0.2% of rapeseed oil (w/v); (**b**) 0.2% of linoleic acid (w/v) diluted in xanthan gum. The control solution contained 0.3% of xanthan gum (w/v). Values are expressed as mean ± SEM (n = 10). Dotted line represents the absence of preference in $CB_1R^{-/-}$ mice (less than 50% of preference). (**c**) Short-term (5 min) licking tests in WT and $CB_1R^{-/-}$ mice were performed as described in Materials and Methods. Animals were subjected to a control solution (xanthan gum) and an experimental solution containing 0.2% of linoleic acid. Values are expressed as mean ± SEM (n = 9).

3.2. Treatment with Rimonabant Induces a Low Preference for Fat Solutions and Does Not Alter Feeding Behavior

The mice treated with rimonabant for 26 days exhibited the same behavior as $CB_1R^{-/-}$ mice, i.e., a low preference for fatty solutions (Figure 2a,b). Interestingly, the mice treated with rimonabant did not show reduced food intake or body weight (Figure 2c). As the rimonabant induces an early and transient effect, we monitored these parameters for 5 days before starting two-bottle preference tests. Thus, no bias interfered with the behavioral experiments.

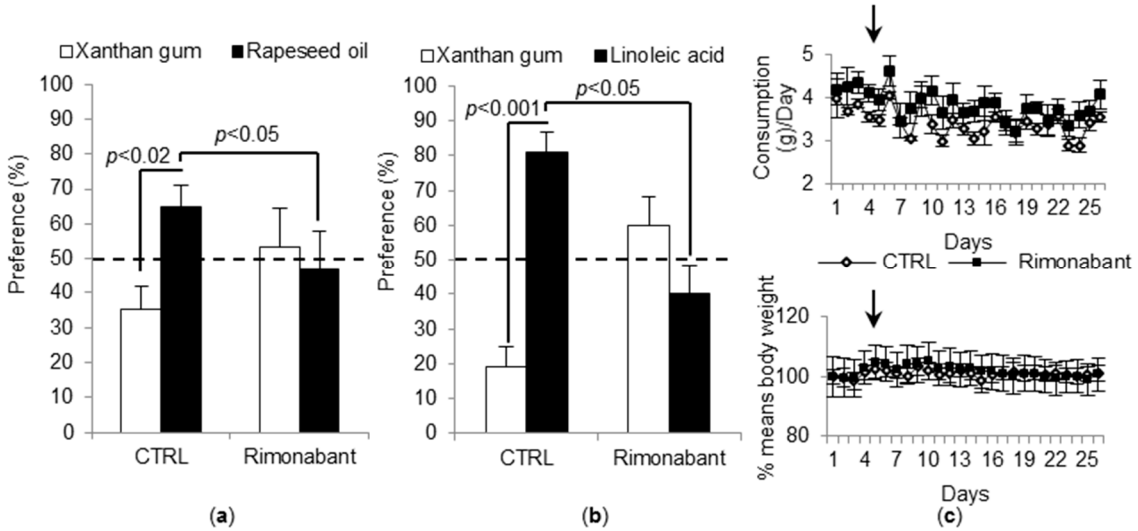

Figure 2. Effect of rimonabant on preference for lipids, body weight, and feeding behavior. (**a**,**b**) WT mice, treated with either rimonabant (10 mg·kg^{-1}·day^{-1}) or vehicle (CTRL), were simultaneously offered two bottles, a control one and an experimental one. The latter bottle contained either 0.2% of rapeseed oil (*w*/*v*) (**a**) or 0.2% of linoleic acid (*w*/*v*) (**b**) diluted in xanthan gum. Control solution contained 0.3% of xanthan gum. Values are expressed as mean ± SEM (*n* = 5). Dotted line represents the absence of preference in rimonabant-treated mice (less than 50% of preference). (**c**) Food intake and body weight variations in mice treated or not with rimonabant and fed a standard chow. Values are expressed as mean ± SEM (*n* = 5). Black arrows indicate the beginning of the treatment with rimonabant.

3.3. CD36 and GPR120 Protein Expressions Are Not Altered in TBC of CB$_1$R$^{-/-}$ Mice

CB_1R gene invalidation did not interfere with CD36 and GPR120 protein expression in taste buds from $CB_1R^{-/-}$ (Figure 3). α-Gustducin, a marker of type II TBC, remained stable (Figure 3). It seems that the low preference for fatty solutions observed in $CB_1R^{-/-}$ mice was not due to altered expression of CD36 and GPR120.

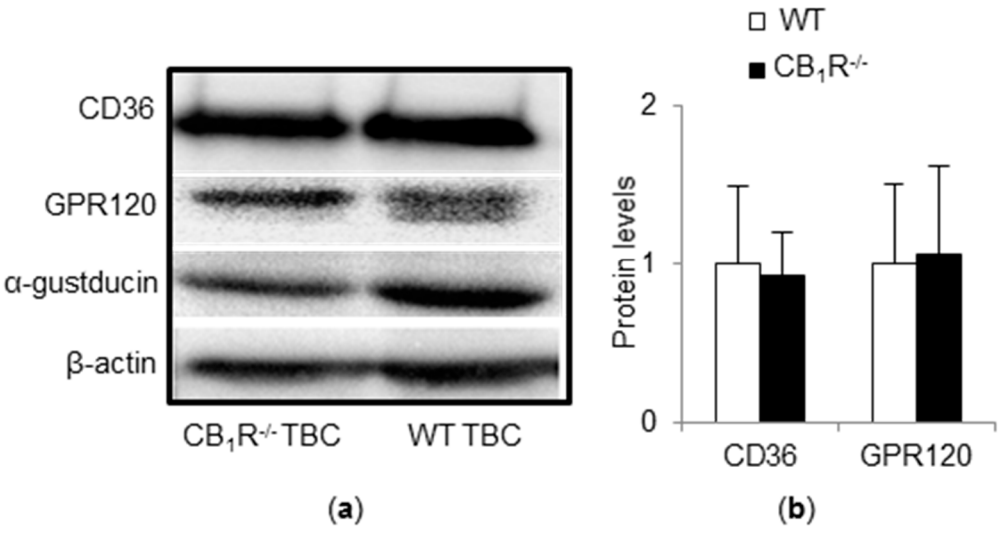

Figure 3. Impact of CB_1R gene invalidation on CD36 and GPR120 protein expressions. CD36 and GPR120 protein levels were measured by Western blotting in taste bud cells (TBC) (*n* = 2) from WT and $CB_1R^{-/-}$ mice. (**a**) A representative blot corresponding to a pool of total proteins from four mice TBC is shown. (**b**) The corresponding histogram shows CD36 and GPR120 protein levels. Values are expressed as mean ± SD (*n* = 2).

3.4. CB_1R Gene Invalidation Induces a Decrease in Proglucagon and GLP-1r mRNA and Basal GLP-1 Level

Proglucagon and *GLP-1r* mRNA levels were significantly lower in $CB_1R^{-/-}$ TBC than WT TBC (Figure 4a). According to previously published data [9], LA induces the release of GLP-1 from mouse TBC. To measure the release of active GLP-1, the mouse TBC were incubated for 2 h in an oxygenized medium containing anti-DPP4, to prevent GLP-1 degradation, and exposed, or not, to 200 µM LA. In $CB_1R^{-/-}$ mice TBC, GLP-1 release in the culture medium was significantly lower than that in WT TBC, in both basal and LA-stimulated conditions. As expected, LA induced a small, but significant, release of active GLP-1 in culture medium of WT TBC (Figure 4b).

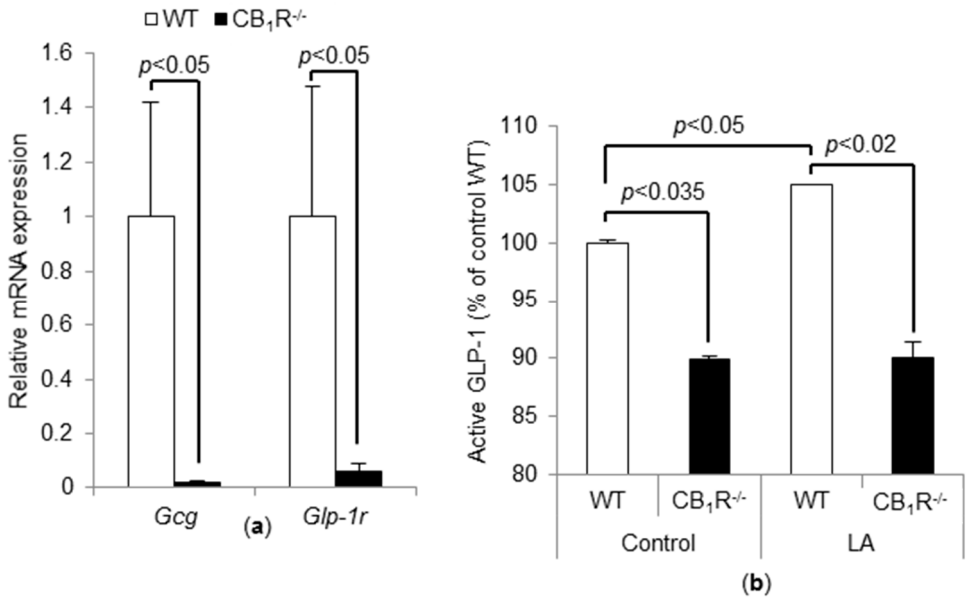

Figure 4. Effect of CB_1R gene invalidation on GLP-1. (**a**) *Proglucagon (Gcg)* and *GLP-1r* mRNA levels were assayed by real-time qPCR in mouse TBC from WT and $CB_1R^{-/-}$ mice. Values are expressed as mean ± SEM (n = 6). (**b**) ELISA results showing GLP-1 release by freshly isolated mouse TBC incubated for 2 h in the presence of 33 µM fatty acid-free BSA alone (CTRL) or with 200 µM linoleic acid (LA). Each value corresponds to the GLP-1 released by cultured TBC. We independently reproduced the results twice, by using, each time, TBC from three mice. We observed identical results, and we pooled them. Each point represents values as mean ± SD (n = 6).

3.5. Both LA and Cannabinoids Induce CB_1R-Dependent Ca^{2+} Responses in TBC

In mouse TBC, LCFA evokes increases in Ca^{2+} signaling [26]. As expected, LA triggered a higher rise in $[Ca^{2+}]_i$ in WT TBC [6] (Figure 5a,f) than that in $CB_1R^{-/-}$ TBC (Figure 5b,f). Similarly, arachidonyl-2′-chloroethylamide (ACEA, a specific CB_1R agonist), induced a rise in $[Ca^{2+}]_i$ in WT TBC (Figure 5f). However, ACEA triggered a significantly lower increase in $[Ca^{2+}]_i$ in $CB_1R^{-/-}$ TBC than that in WT TBC (Figure 5f). Finally, the combination of LA and ACEA also induced a strong rise in $[Ca^{2+}]_i$ in WT TBC (Figure 5c,f) corresponding to the combined effect of the two molecules that was not apparent in $CB_1R^{-/-}$ TBC (Figure 5d,f). Interestingly, when tested on $CB_1R^{-/-}$ TBC, ACEA still triggered a rise in $[Ca^{2+}]_i$ (Figure 5f). Furthermore, blockade of TRPV1 with a specific antagonist, A784168, in $CB_1R^{-/-}$ TBC curtailed the action of ACEA on calcium response (Figure 5e).

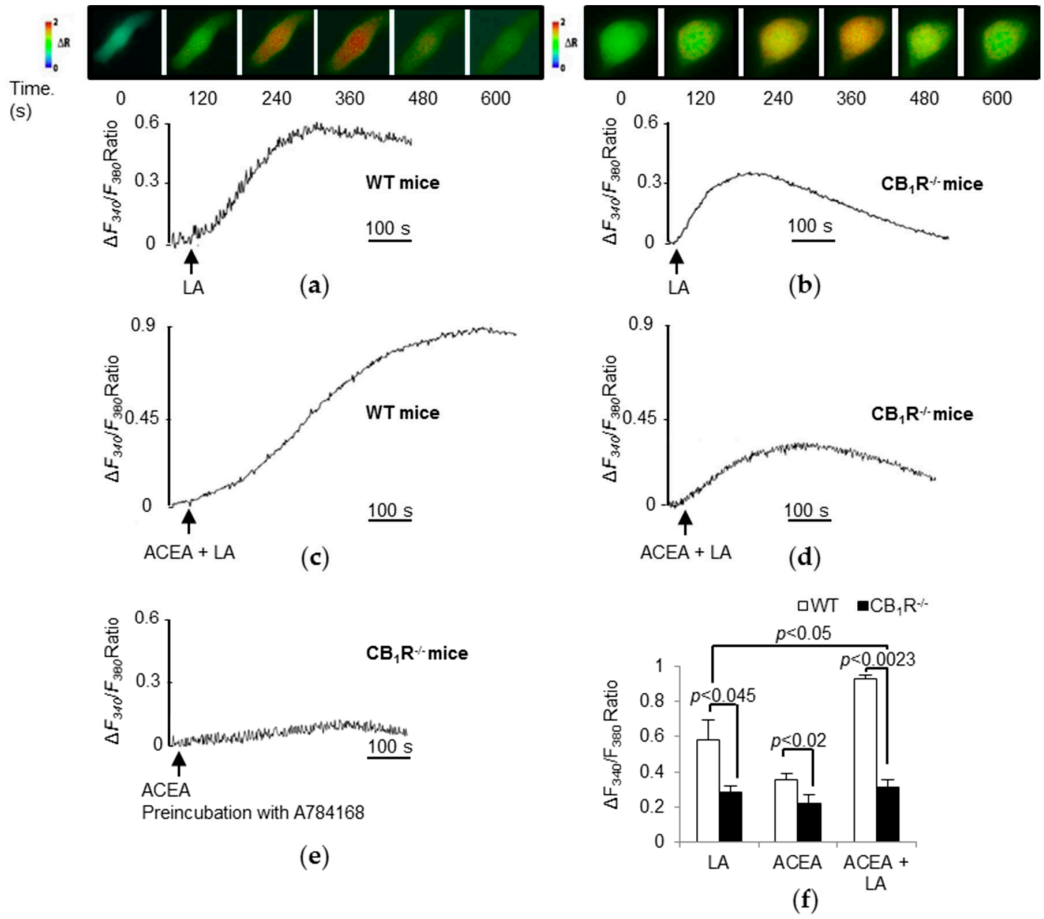

Figure 5. Effects of linoleic acid (LA) and cannabinoids on Ca^{2+} signaling in mouse TBC. Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40× oil immersion objectives. Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in taste bud cells freshly isolated from WT mice (**a**) and $CB_1R^{-/-}$ mice (**b**) following addition of LA (25 μM) and the corresponding graphs below. Changes in $[Ca^{2+}]_i$ evoked by combined addition of ACEA (1.5 μM) and LA (25 μM) in WT (**c**) and $CB_1R^{-/-}$ TBC (**d**), respectively. Changes in $[Ca^{2+}]_i$ evoked by ACEA (1.5 μM) after a 15 min preincubation with A784168, a TRPV1 antagonist, in $CB_1R^{-/-}$ TBC (**e**). The arrowheads indicate when the test molecules were added. Variations in $\Delta F_{340}/F_{380}$ Ratio induced by LA (25 μM), ACEA (1.5 μM), and ACEA (1.5 μM), in combination with LA (25 μM), in WT and $CB_1R^{-/-}$ mice TBC (**f**). Values are expressed as mean ± SEM (n = 5).

3.6. CB_1R Blockade Significantly Decreases Ca^{2+} Responses Triggered by LA, AEA, and ACEA in WT TBC

To further explore the role of CB_1R on calcium signaling, we preincubated WT TBC with a specific CB_1R inverse agonist rimonabant. As observed in Figure 6, rimonabant significantly abrogated $[Ca^{2+}]_i$ responses, induced by LA, AEA, and ACEA (Figure 6c), corroborating the previous results observed in $CB_1R^{-/-}$ TBC.

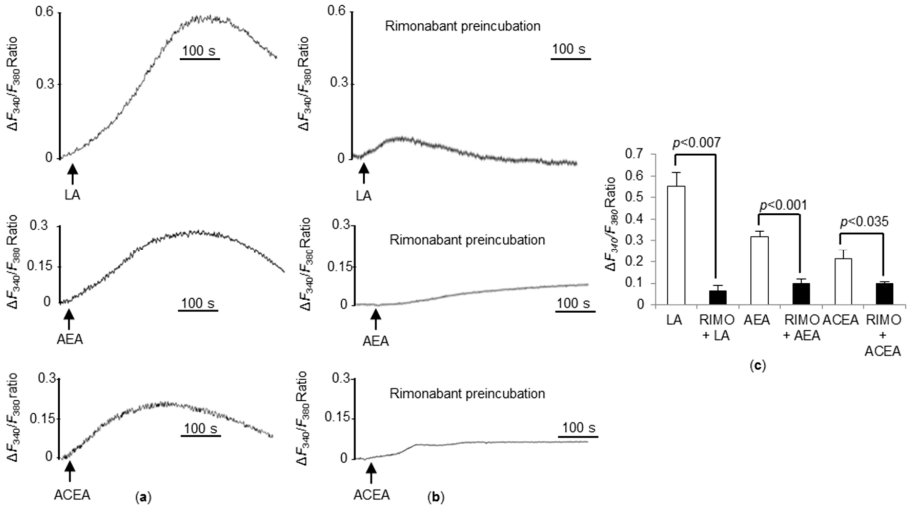

Figure 6. Effects of rimonabant on linoleic acid (LA) and cannabinoid-induced Ca^{2+} signaling in TBC. Ca^{2+} imaging studies were performed in calcium-containing buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40× oil immersion objectives. Graphs show the increase in $[Ca^{2+}]_i$ in taste bud cells freshly isolated from WT mice following addition of LA (25 μM), anandamide (AEA, 5 μM), and ACEA (1.5 μM) (**a**). WT TBC before the addition of LA (25 μM), AEA (5 μM) and ACEA (1.5 μM) were preincubated (15 min) with rimonabant (50 μM) (**b**). The arrowheads indicate when the test molecules were added. Changes in $\Delta F_{340}/F_{380}$ Ratio induced by LA (25 μM), AEA (5 μM), and ACEA (1.5 μM) in WT mice TBC after a preincubation with or without rimonabant (RIMO, 50 μM) (**c**). Values are expressed as mean ± SEM (n = 5).

3.7. AEA-Induced Ca^{2+}-Signaling Is PLC Dependent

Finally, in order to validate our model, we investigated the downstream CB_1R pathway elicited by AEA treatment. Here, we used DB-cAMP, a cAMP analog and a phosphodiesterase inhibitor, and U73122, a phospholipase C inhibitor. We observed that DB-cAMP did not significantly alter the Ca^{2+} response induced by AEA. Conversely, a pretreatment with U73122 significantly decreased the Ca^{2+} response, indicating that the Ca^{2+} response triggers a PLC-dependent Ca^{2+} signaling (Figure 7).

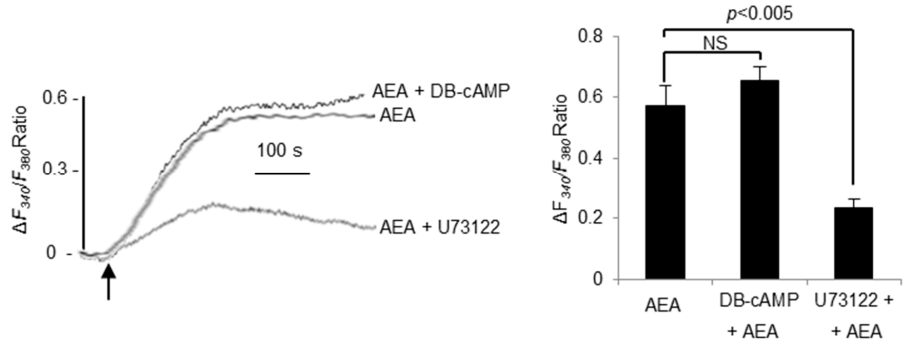

Figure 7. Effects of DB-cAMP and U73122 on anandamide (AEA, 5 μM)-induced Ca^{2+} signaling in TBC. Ca^{2+} imaging studies were performed in calcium-containing buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40× oil immersion objectives. Graphs show the increase in $[Ca^{2+}]_i$ in taste bud cells freshly isolated from WT mice following addition of anandamide (AEA, 5 μM) with or without preincubation (20 min) with DB-cAMP (1 mM) or preincubation (20 min) with U73122 (10 μM) (left panel). The arrowhead indicates when the test molecules were added. Changes as histograms (right panel) in $\Delta F_{340}/F_{380}$ Ratio induced by anandamide (AEA, 5 μM) in WT mice TBC after a preincubation with or without DB-cAMP (1 mM) or U73122 (10 μM). Values are expressed as mean ± SEM (n = 6).

4. Discussion

Williams and Kirkham [19] demonstrated that CB_1R is responsible for increased food intake, induced by an endocannabinoid agonist. Later on, Yoshida et al. [7] revealed that endocannabinoids enhanced the gustatory responses to sweet tastants via CB_1R. Indeed, the activation of endocannabinoid system (ECS) appears to be associated with hyperphagia and a preference for palatable food. Interestingly, CB_1R are also expressed in a subset of taste bud cells [7]. We report here that $CB_1R^{-/-}$ mice displayed no preference for fat solutions compared to WT mice. The same behavior was also observed when WT mice were treated with rimonabant, a CB_1R blocker, confirming the role for this receptor in the detection of dietary lipids. We have employed LA as a candidate for LCFA because this fatty acid is abundantly present in Western food; however, it is possible that the saturated fatty acids like palmitic acid (PA) might also initiate the same gustatory response. Indeed, we have shown previously that LA and PA triggered the same increases in $[Ca^{2+}]_i$ in mouse taste bud cells [6].

In the present study, for behavioral experiments, we used whole body knockout mice for CB_1R, and it is possible that the hypothalamic cannabinoid system, via the dopaminergic area, might be involved in fat taste preference [28]. Nonetheless, we sought to elucidate cellular mechanisms in the modulation of fat preference. We first tested the hypothesis whether there is an alteration in CD36 and GPR120 protein in TBC of $CB_1R^{-/-}$ mice. In our study, CD36 and GPR120 protein expressions were not altered by the absence of CB_1R, suggesting that the absence of preference for fatty solutions may be due to altered downstream signaling. Moreover, we checked the delivery of linoleic acid under both conditions, and we observed identical uptake of exogenous fatty acid.

Previous studies indicated that both CD36 and GPR120 activation by a LCFA triggered mobilization of $[Ca^{2+}]_i$ from the intracellular endoplasmic reticulum Ca^{2+} pool during fat taste perception [9,29]. In our study, we show, for the first time, that LA-mediated increase in $[Ca^{2+}]_i$ was altered when CB_1R was inactivated by rimonabant or by the absence of CB_1R. In addition, the CB_1R agonist ACEA also increased calcium flux per se in TBC, albeit with lower potency than LA. However, the effect of ACEA was maintained in TBC from $CB_1R^{-/-}$ mice, raising the possibility that the increase in $[Ca^{2+}]_i$ could be mediated by the receptors other than CB_1R, for example, TRPV1. Indeed, it has been shown that activation of TRPV1 by endocannabinoids induces calcium signaling [30,31]. Besides, blockade of TRPV1 with A784168 totally abolished $[Ca^{2+}]_i$ response induced by ACEA, indicating that the residual calcium signal observed in $CB_1R^{-/-}$ TBC with ACEA may be due to TRPV1 activity. Furthermore, it appears that the CB_1R-coupled downstream signaling is PLC-dependent, in accordance with the observations of De Petrocellis et al. [32]. However, it remains to be elucidated in future whether anadamide, employed in the present study, activates the $G\beta\gamma$ subunit of CB_1R, and activates PLC via PI-3-kinase pathway. As a whole, our data indicate that CB_1R may play a crucial role in fat taste perception by modulating calcium signaling.

As previously described, GLP-$1^{-/-}$ mice have reduced taste responses to dietary fat, suggesting that orosensory detection of LCFA could be associated to the secretion of lingual GLP-1 [13]. Data reported herein showed that the secretion of active GLP-1 induced by LA is strongly decreased in $CB_1R^{-/-}$ mice suggesting the existence of a link between CB_1R signaling and GLP-1 production. Hence, CB_1R activation may stimulate proglucagon and GLP-1r production and, therefore, modulate perception threshold of LCFA. Further investigations are needed to explore the possibility whether GLP-1 secretion is stimulated via $[Ca^{2+}]_i$ signaling in TBC or by other mechanisms [33].

In conclusion, the present report shows that CB_1R influences fat taste perception via regulating calcium signaling in TBC. It is proposed that CB_1R activation induces a $[Ca^{2+}]_i$ response that strengthens fat perception, that is mediated by CD36. Activation of ECS could, thereby, increase sensory stimuli relaying palatability of foods and, ultimately, stimulate food intake. The physiopathological relevance of such a regulatory pathway is supported by the fact that ECS tone is increased in obesity. Hence, the ECS seems to emerge as a key modulator of oral sweet and fat detection and may represent a potential target for developing new anti-obesity strategies or, conversely, for enhancing food intake in the case of loss of appetite as it occurs in cachexia.

Author Contributions: Conceptualization, N.A.K.; Data curation, P.P.-D. and L.D.; Formal analysis, L.B., J.L., A.H. and N.A.K.; Funding acquisition, N.A.K.; Investigation, L.B. and P.D.; Methodology, J.L., P.P.-D., G.M. and P.D.; Project administration, N.A.K.; Supervision, N.A.K.; Validation, N.A.K.; Visualization, N.A.K.; Writing—original draft, L.B. and N.A.K.; Writing—review & editing, L.B.

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References

1. Drewnowski, A.; Brunzell, J.D.; Sande, K.; Iverius, P.H.; Greenwood, M.R. Sweet tooth reconsidered: Taste responsiveness in human obesity. *Physiol. Behav.* **1985**, *35*, 617–622. [CrossRef]
2. Mela, D.J.; Sacchetti, D.A. Sensory preferences for fats: Relationships with diet and body composition. *Am. J. Clin. Nutr.* **1991**, *53*, 908–915. [CrossRef] [PubMed]
3. Laugerette, F.; Passilly-Degrace, P.; Patris, B.; Niot, I.; Febbraio, M.; Montmayeur, J.-P.; Besnard, P. CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *J. Clin. Investig.* **2005**, *115*, 3177–3184. [CrossRef] [PubMed]
4. El-Yassimi, A.; Hichami, A.; Besnard, P.; Khan, N.A. Linoleic acid induces calcium signaling, Src kinase phosphorylation, and neurotransmitter release in mouse CD36-positive gustatory cells. *J. Biol. Chem.* **2008**, *283*, 12949–12959. [CrossRef] [PubMed]
5. Gilbertson, T.A.; Khan, N.A. Cell signaling mechanisms of oro-gustatory detection of dietary fat: Advances and challenges. *Prog. Lipid Res.* **2014**, *53*, 82–92. [CrossRef] [PubMed]
6. Gaillard, D.; Laugerette, F.; Darcel, N.; El-Yassimi, A.; Passilly-Degrace, P.; Hichami, A.; Khan, N.A.; Montmayeur, J.-P.; Besnard, P. The gustatory pathway is involved in CD36-mediated orosensory perception of long-chain fatty acids in the mouse. *FASEB J.* **2008**, *22*, 1458–1468. [CrossRef] [PubMed]
7. Yoshida, R.; Ohkuri, T.; Jyotaki, M.; Yasuo, T.; Horio, N.; Yasumatsu, K.; Sanematsu, K.; Shigemura, N.; Yamamoto, T.; Margolskee, R.F.; et al. Endocannabinoids selectively enhance sweet taste. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 935–939. [CrossRef] [PubMed]
8. Besnard, P.; Passilly-Degrace, P.; Khan, N.A. Taste of fat: A Sixth Taste Modality? *Physiol. Rev.* **2016**, *96*, 151–176. [CrossRef] [PubMed]
9. Ozdener, M.H.; Subramaniam, S.; Sundaresan, S.; Sery, O.; Hashimoto, T.; Asakawa, Y.; Besnard, P.; Abumrad, N.A.; Khan, N.A. CD36- and GPR120-mediated Ca^{2+} signaling in human taste bud cells mediates differential responses to fatty acids and is altered in obese mice. *Gastroenterology* **2014**, *146*, 995–1005. [CrossRef] [PubMed]
10. Cartoni, C.; Yasumatsu, K.; Ohkuri, T.; Shigemura, N.; Yoshida, R.; Godinot, N.; le Coutre, J.; Ninomiya, Y.; Damak, S. Taste preference for fatty acids is mediated by GPR40 and GPR120. *J. Neurosci.* **2010**, *30*, 8376–8382. [CrossRef] [PubMed]
11. Sclafani, A.; Zukerman, S.; Ackroff, K. GPR40 and GPR120 fatty acid sensors are critical for postoral but not oral mediation of fat preferences in the mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2013**, *305*, R1490–R1497. [CrossRef] [PubMed]
12. Ancel, D.; Bernard, A.; Subramaniam, S.; Hirasawa, A.; Tsujimoto, G.; Hashimoto, T.; Passilly-Degrace, P.; Khan, N.-A.; Besnard, P. The oral lipid sensor GPR120 is not indispensable for the orosensory detection of dietary lipids in mice. *J. Lipid Res.* **2015**, *56*, 369–378. [CrossRef] [PubMed]
13. Martin, C.; Passilly-Degrace, P.; Chevrot, M.; Ancel, D.; Sparks, S.M.; Drucker, D.J.; Besnard, P. Lipid-mediated release of GLP-1 by mouse taste buds from circumvallate papillae: Putative involvement of GPR120 and impact on taste sensitivity. *J. Lipid Res.* **2012**, *53*, 2256–2265. [CrossRef] [PubMed]
14. Baggio, L.L.; Drucker, D.J. Biology of incretins: GLP-1 and GIP. *Gastroenterology* **2007**, *132*, 2131–2157. [CrossRef] [PubMed]
15. Shin, Y.-K.; Martin, B.; Golden, E.; Doston, C.D.; Maudsley, S.; Kim, W.; Jang, H.-J.; Mattson, M.P.; Drucker, D.J.; Egan, J.M.; et al. Modulation of taste sensitivity by GLP-1 signaling. *J. Neurochem.* **2008**, *106*, 455–463. [CrossRef] [PubMed]

16. Cota, D.; Marsicano, G.; Lutz, B.; Vicennati, V.; Stalla, G.K.; Pasquali, R.; Pagotto, U. Endogenous cannabinoid system as a modulator of food intake. *Int. J. Obes.* **2003**, *27*, 289–301. [CrossRef] [PubMed]
17. Engeli, S.; Böhnke, J.; Feldpausch, M.; Gorzelniak, K.; Janke, J.; Bátkai, S.; Pacher, P.; Harvey-White, J.; Luft, F.C.; Sharma, A.M.; Jordan, J. Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* **2005**, *54*, 2838–2843. [CrossRef] [PubMed]
18. Jamshidi, N.; Taylor, D.A. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Br. J. Pharmacol.* **2001**, *134*, 1151–1154. [CrossRef] [PubMed]
19. Williams, C.M.; Kirkham, T.C. Anandamide induces overeating: Mediation by central cannabinoid (CB1) receptors. *Psychopharmacology* **1999**, *143*, 315–317. [CrossRef] [PubMed]
20. Williams, C.M.; Kirkham, T.C. Observational analysis of feeding induced by delta 9-THC and anandamide. *Physiol. Behav.* **2002**, *76*, 241–250. [CrossRef]
21. Di Marzo, V.; Goparaju, S.K.; Wang, L.; Liu, J.; Bátkai, S.; Járai, Z.; Fezza, F.; Miura, G.I.; Palmiter, R.D.; Sugiura, T.; Kunos, G. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **2001**, *410*, 822–825. [CrossRef] [PubMed]
22. Després, J.-P.; Golay, A.; Sjöström, L. Rimonabant in obesity-lipids study group effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N. Engl. J. Med.* **2005**, *353*, 2121–2134.
23. Van Gaal, L.; Pi-Sunyer, X.; Després, J.-P.; McCarthy, C.; Scheen, A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: Pooled 1-year data from the rimonabant in obesity (RIO) program. *Diabetes Care* **2008**, *31* (Suppl. 2), S229–S240. [CrossRef]
24. Van Gaal, L.F.; Rissanen, A.M.; Scheen, A.J.; Ziegler, O.; Rössner, S. RIO-Europe study group effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet Lond. Engl.* **2005**, *365*, 1389–1397. [CrossRef]
25. Zhang, X.; Fitzsimmons, R.L.; Cleland, L.G.; Ey, P.L.; Zannettino, A.C.W.; Farmer, E.-A.; Sincock, P.; Mayrhofer, G. CD36/fatty acid translocase in rats: Distribution, isolation from hepatocytes, and comparison with the scavenger receptor SR-B1. *Lab. Investig. J. Tech. Methods Pathol.* **2003**, *83*, 317–332. [CrossRef]
26. Dramane, G.; Abdoul-Azize, S.; Hichami, A.; Vögtle, T.; Akpona, S.; Chouabe, C.; Sadou, H.; Nieswandt, B.; Besnard, P.; Khan, N.A. STIM1 regulates calcium signaling in taste bud cells and preference for fat in mice. *J. Clin. Investig.* **2012**, *122*, 2267–2282. [CrossRef] [PubMed]
27. Parker, H.E.; Gribble, F.M.; Reimann, F. The role of gut endocrine cells in control of metabolism and appetite. *Exp. Physiol.* **2014**, *99*, 1116–1120. [CrossRef] [PubMed]
28. Melis, M.; Scheggi, S.; Carta, G.; Madeddu, C.; Lecca, S.; Luchicchi, A.; Cadeddu, F.; Frau, R.; Fattore, L.; Fadda, P.; et al. PPARα regulates cholinergic-driven activity of midbrain dopamine neurons via a novel mechanism involving α7 nicotinic acetylcholine receptors. *J. Neurosci.* **2013**, *33*, 6203–6211. [CrossRef] [PubMed]
29. Galindo, M.M.; Voigt, N.; Stein, J.; van Lengerich, J.; Raguse, J.-D.; Hofmann, T.; Meyerhof, W.; Behrens, M. G Protein—Coupled receptors in human fat taste perception. *Chem. Sens.* **2012**, *37*, 123–139. [CrossRef] [PubMed]
30. Kentish, S.J.; Page, A.J. The role of gastrointestinal vagal afferent fibres in obesity. *J. Physiol.* **2015**, *593*, 775–786. [CrossRef] [PubMed]
31. Ryskamp, D.A.; Redmon, S.; Jo, A.O.; Križaj, D. TRPV1 and endocannabinoids: Emerging molecular Signals that modulate mammalian vision. *Cells* **2014**, *3*, 914–938. [CrossRef] [PubMed]
32. De Petrocellis, L.; Marini, P.; Matias, I.; Moriello, A.S.; Starowicz, K.; Cristino, L.; Nigam, S.; Di Marzo, V. Mechanisms for the coupling of cannabinoid receptors to intracellular calcium mobilization in rat insulinoma beta-cells. *Exp. Cell Res.* **2007**, *313*, 2993–3004. [CrossRef] [PubMed]
33. Takai, S.; Yasumatsu, K.; Inoue, M.; Iwata, S.; Yoshida, R.; Shigemura, N.; Yanagawa, Y.; Drucker, D.J.; Margolskee, R.F.; Ninomiya, Y. Glucagon-like peptide-1 is specifically involved in sweet taste transmission. *FASEB J.* **2015**, *29*, 2268–2280. [CrossRef] [PubMed]

Modulation of fat preference by bitter agonists

Material & Methods

1. Material & Methods

1.1. Cell culture

HTC-8 cells were kindly provided by BRAIN (Zwingenberg, Germany). HFFC and Ulduz-1 cell lines are human TBC that escaped death, not immortalized cells, and were provided by Mehmet Hakan Ozdener (USA).

HTC-8, HFFC and Ulduz-1 cells were cultured in HTC-medium ,i.e., Basal Iscove Medium supplemented with GlutaMAX (Gibco) containing 20% MCDB 153 Basal Medium (Sigma), 10% FCS (Dutsher), 1% antibiotic/antimicotic 100X (Sigma), 2.5 µg/ml Gentamycin (Gibco) and 10 µg/ml Insulin (Sigma).

TAS2R38 overexpressing cells (HTC-8:TAS2R38) were cultured in HTC-medium added with 0.5 µg/ml Puromycin (Sigma).

1.2. Immunocytochemistry

Cells were fixed in glacial ethanol 95% for 20 min. cells were blocked for 1 h with PBS containing 5% BSA and 0.2% Triton X-100. Next, the cells were incubated overnight at 4°C with a primary antibody. After washing, cells were incubated for 2 h at room temperature with a fluorescent anti-rabbit secondary antibody (Invitrogen, Alexa 488; 1:500). Regarding the co-expression assay, the cells were washed and blocked again before adding an anti-TAS2R16 primary antibody (Osenses, OST00468W; 1:300) or an anti-TAS2R38 primary antibody (Osenses, OST00440W; 1:300). Cells were next incubated with a fluorescent anti-rabbit secondary antibody (Invitrogen, Alexa 568; 1:500) and then all the cells were counterstained with DAPI (Sigma, 0.1µg/ml) to stain the nuclei. Fluorescence was analyzed under a Cell Observer (Zeiss).

Table 3 : Liste of primary antibodies used in immunocytochemistry

Primary antibody	Reference	Supplier	Dilution
CD36	HPA002018	Atlas Antibodies	1:50
GNAT3/α-gustducin	PA5-23986	ThermoFisher	1:50
GPR120	sc-99105	Santa Cruz	1:50
PLCβ2	sc-206	Santa Cruz	1:50
TAS2R16	OST00468W	Osenses	1:300
TAS2R38	OST00440W	Osenses	1:300

1.3. Real-time qPCR

Total RNA from $2x10^6$ were extracted by using TRIzol method according to the manufacturer's recommendations (Invitrogen, Cergy-Pontoise, France). After purification, mRNA was resuspended in RNase free water. The samples were then analyzed and quantified using Traycell (Hellma Analytics, Müllheim, Germany). Samples having purity (A_{260}/A_{280}) between 1.80 and 2.00 were retained for the rest of the experiment. mRNA (1 µg) was reverse-transcribed into cDNA using High Capacity RNA-to-cDNA kit (Applied Biosystems) and the samples were incubated for 1 h at 37 °C and then for 5 min at 95 °C.

Real time qPCR reactions were performed on 10 ng cDNA in a 20 µL of reaction volume in triplicates with a StepOnePlus (Life Technologies, Saint-Aubin, France) device with the use of SYBR green PCR Master Mix (Life Technologies, Saint-Aubin, France). For each gene (Table 4), a standard curve was established from five cDNA dilutions (100 ng to 0.1 ng per well) and used to determine the PCR efficiency. Real time qPCR reactions were performed with a denaturing step of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primer specificity was checked using the melt curves. The comparative $2^{-\Delta\Delta CT}$ method was used for relative quantification.

Table 4 : **List of primers**

Taste receptors		
CD36	Forward	TCTGTGCCTGTTTTAACCCAA
	Reverse	GCCAGTTGGAGACCTGCTTA
GPR120	Forward	CTTCTTCTCCGACGTCAAGG
	Reverse	GAGGGATAGCGCTGATGAA
TAS2R16	Forward	GGCATCTCTCGCTTCTGTCT
	Reverse	CCAGGGAAACAACCTCAAAA
TAS2R38	Forward	ACAGTGATTGTGTGCTGCTG
	Reverse	GCTCTCCTCAACTTGGCATT
Type I cell markers		
GLAST	Forward	CGAAGCCATCATGAGACTGGTA
	Reverse	TCCCAGCAATCAGGAAGAGAA
Type II cell markers		
GNAT3	Forward	ATGAGGACCAACGACAAC
	Reverse	GCGTAAGCTGCTGAGTCATTG
PLCβ2	Forward	TCCAGCCCACCAAGTTCGTCT
	Reverse	CGAGGCCTTGGAGAGCAGGT
Type III cell markers		
SNAP-25	Forward	GGACGAACGGGAGCAGATG
	Reverse	CGCTCACCTGCTCTAGGTTTC
Hormones and receptors		
CCK	Forward	TCGCAGAGAACGGATGGCGA

	Reverse	GGGGTCCAGGTTCTGCAGGT
CCK-AR	Forward	GCGATTTGCAAACCCTTACAG
	Reverse	CACCTTCAAAGCATGGGATTTT
GHRL	Forward	CACAGCAACAAAGCTGCACC
	Reverse	AAGTCCAGCCAGAGCATGCC
GHSR	Forward	AGCGCTACTTCGCCATC
	Reverse	CCGATGAGACTGTAGAG
Leptin	Forward	AACCCTGTGCGGATTCTTGT
	Reverse	TCTTGGACTTTTTGGATGGGC
Ob-R	Forward	CATTTTATCCCCATTGAGAAGTA
	Reverse	CTGAAAATTAAGTCCTTGTGCCCAG
P-GCG	Forward	TGCTGAAGGGACCTTTACCAGTGA
	Reverse	GCCTTTCACCAGCCAAGCAATGAA
GLP-1R	Forward	TTGGGGTGAACTTCCTCATC
	Reverse	CTTGGCAAGTCTGCATTTGA
OXT	Forward	GCTGAAACTTGATGGCTCCG
	Reverse	TTCTGGGGTGGCTATGGG
OTR	Forward	GTGCAGATGTGGAGCGTCT
	Reverse	GTTGAGGCTGGCCAAGAG
Housekeeping genes		
β-actin	Forward	GTACCACTGGCATCGTGATGGACT
	Reverse	CCGCTCATTGCCAATGGTGAT
GAPDH	Forward	GACAGTCAGCCGCATCTTCT
	Reverse	TTAAAAGCAGCCCTGGTGAC

OXT and OTR amplified cDNA were then deposited into an agarose gel electrophoresis: TAE 1X containing 1.5% agarose (Invitrogen).

1.4. Western Blotting

$2x10^6$ cells were lysed using 100 µL of RIPA buffer (25 mM Tris HCl, 75 mM NaCl, 0.5 mM EDTA, 0.25% Sodium Deoxycholate, 0.05% SDS, 0.5% Nonidet P40, 10 µl/ml protease inhibitors (Sigma) (Zhang et al., 2003a). Samples were stored on ice for 20 min and then centrifuged (12,000 g, 15 min, 4°C). Lysates were used immediately or stored at −80°C until the assay. Protein concentrations in homogenates were assayed using the BCA assay (Sigma). Denaturated proteins (20 µg) were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. After having blocked for 3 hours using a TBS buffer containing 5% BSA and 0.05% Tween-20, the membrane was incubated overnight with a primary antibody (Table 5).

Table 5 : List of primary antibodies used in Western blotting

Primary antibody	Reference	Supplier	Dilution
Taste receptors			
CD36	HPA002018	Atlas Antibodies	1:200
GPR120	sc-390752	Santa Cruz	1:200
TAS2R16	OST00468W	Osenses	1:300
TAS2R38	OST00440W	Osenses	1:300
Type I cell markers			
GLAST	sc-515839	Santa Cruz	1:500
Type II cell markers			
$G_{\alpha t1}$	sc-136143	Santa Cruz	1:500
GNAT3/α-gustducin	PA5-23986	ThermoFisher	1:1000
PLCβ2	sc-206	Santa Cruz	1:250
PLCδ4	sc-373875	Santa Cruz	1:500
Type III cell markers			
SNAP25	sc-20038	Santa Cruz	1:500
Hormone receptors			
CCK-AR	sc-514303	Santa Cruz	1:500
GHS-R1	sc-374515	Santa Cruz	1:500
GLP-1R	sc-390774	Santa Cruz	1:500
Ob-R	sc-8391	Santa Cruz	1:500
OTR	sc-515709	Santa Cruz	1:300
Calcium channels			
Orai1	AS-54653	AnaSpec	1:400
Orai3	54688	AnaSpec	1:500
β-actin	sc-47778	Santa Cruz	1:5000

After a set of washes, the appropriate peroxidase-conjugated secondary antibody was added. Antibody labeling was detected by chemiluminescence (Clarity, Bio-Rad, Marnes-la-Coquette, France).

1.5. Measurement of Ca^{2+} signalling

Cells were cultured into 24-well plates, containing HTC-medium and incubated overnight at 37°C. The next day, the supernatant was discarded. The cells were then incubated with Fura-2/AM (1 µM) (Invitrogen) for 30 min at 37°C in loading buffer (100% Ca^{2+}) which contained the following: 110 mM NaCl; 5.4 mM KCl; 25 mM $NaHCO_3$; 0.8 mM $MgCl_2$; 0.4 mM KH_2PO_4; 20 mM Hepes; 1.2 mM $CaCl_2$; 10 mM Glucose, pH 7.4. The rest of the protocol has

been described p. 85 section 1.7. All test molecules were added in small volumes with no interruption in recordings.

All chemicals used for the measurement of Ca^{2+} signalling were supplied by Sigma, except for D-Salicin, Sinigrin and ML-9 (Santa Cruz), Sulfosuccinimidyl Oleate and AH 7614 (Cayman Chemical).

Functional EC_{50} studies were performed as described above, agonists were added at 5 different concentrations starting at 25 to 400 µM for LA, at 100 µM to 10mM for Sinigrin and PTC and at 1 mM to 20 mM for Salicin. Data were normalized and analyzed using GraphPad Prism (GraphPad Software).

1.6. siRNA knockdown of Orai1 and Orai3

HTC-8 cells were transfected with the siRNA ON-target plus Smart pool (100 µM) designed against Orai1 and Orai3 or non-targeting siRNA as a control (Dharmacon). 24 hours before treatment, the cells were cultured in Accell siRNA Delivery Media (Dharmacon) and further transfected with siRNA (Figure 43). After 72-hour incubation before Ca^{2+} measurements or Western blot analysis.

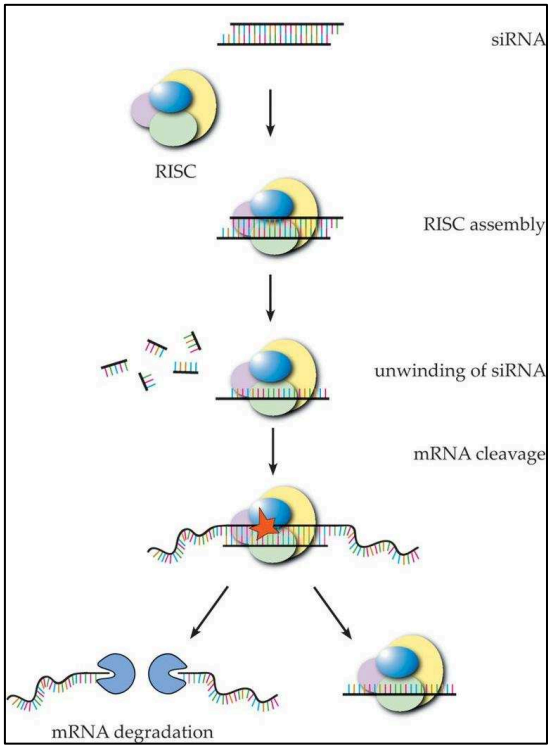

Figure 43. **siRNA machinery**. Synthetic siRNA is transfected into cells. siRNA is incorporated into RISC, followed by unwinding of the double-stranded molecule by the helicase activity of RISC. The sense strand of siRNA is removed and the antisense strand recruits targeted mRNA, which is cleaved by RISC and subsequently degraded by cellular nucleases. Modified from Cejka et al. (2006).

1.7. Oxytocin mRNA essay and Oxytocin release

HTC-8 cells were seeded into 6-weel plates, containing HTC-medium and incubated 3 days at 37°C. The cells were then incubated with either HTC medium containing LA 100 µM, LA 100 µM and SSO 1 µM, LA 100 µM and AH7614 30 µM, LA 100 µM and SSO 1 µM and AH7614 30 µM or with HTC medium alone. After 2 hours of incubation, the medium was collected and the oxytocin release was measured by ELISA (Fine Test, China). In parallel, the cells were extracted by using TRIzol (Invitrogen, Cergy-Pontoise, France) and qPCR were carried out (see section 1.3).

1.8. Statistics

Results are expressed as Means ± SEM. The significance of differences between groups was evaluated with ANOVA and Mann Whitney tests. A p value of less than 0.05 was considered to be statistically significant.

Results

2. Results

2.1. Expression of bitter and fat receptor genes and proteins in HTC-8 cells

We assessed gene and protein expressions of fat and bitter taste receptors. We observed that the HTC-8 cells expressed CD36, GPR120, TAS2R38 and TAS2R16 (Figure 44A) and genes (Figure 44B). Here, we compared protein expression from HTC-8 cells to HTC-8 cells overexpressing TAS2R38 (HTC-8:TAS2R38), Ulduz-1 cells and HFFC cells.

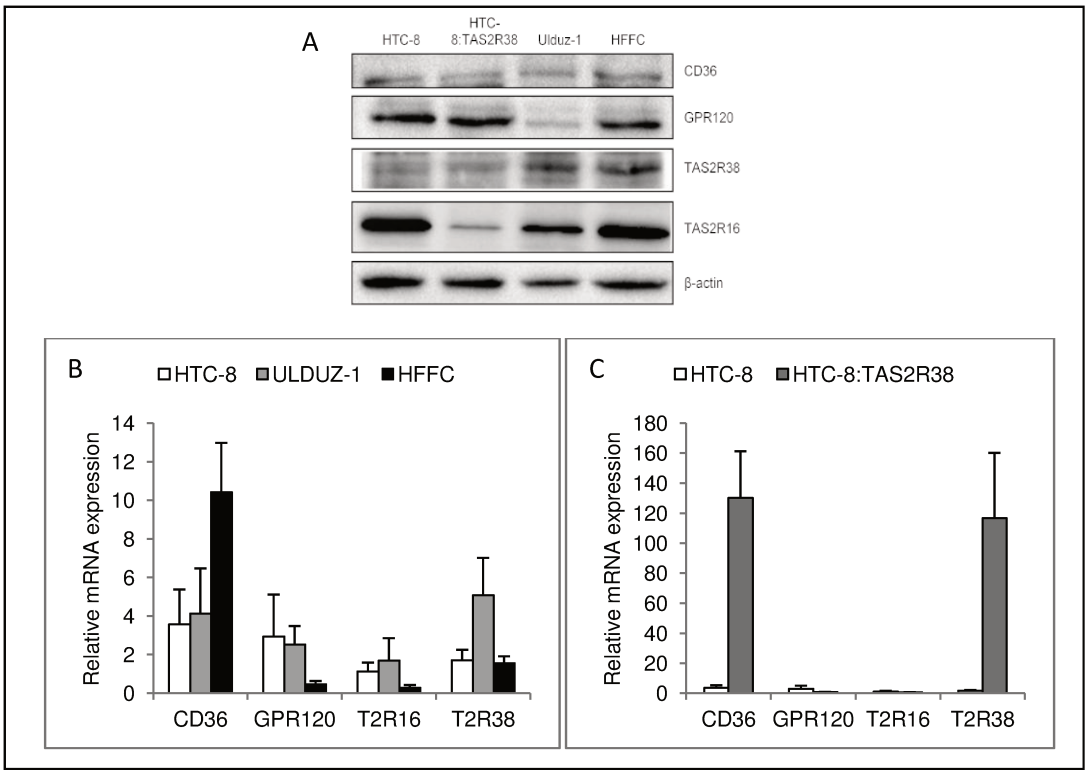

Figure 44. **Fat and bitter taste receptors expression.** A- CD36, GPR120, TAS2R38 and TAS2R16 protein levels were measured by Western blotting in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells (n=5). B- CD36, GPR120, TAS2R16 and TAS2R38 mRNA levels assayed by real-time PCR in HTC-8, Ulduz-1 and HFFC cells. C- CD36 and TAS2R38 mRNA levels assayed by real-time PCR in HTC-8 and HTC-8:TAS2R38 cells. Values are expressed as mean ± SEM (n=5).

It seems noteworthy to emphasize that HTC-8:TAS2R38 cells overexpress TAS2R38 mRNA as expected. But, interestingly, HTC-8:TAS2R38 cells also overexpress CD36 mRNA (Figure 44C). This result supports the hypothesis of the existence of a link between TAS2R38 and CD36.

2.2. Expression of bitter and fat receptor in HTC-8 cells

In a second time, we explored the localization of fat and bitter receptors in these cell lines. We observed that in the four TBC cell lines, CD36, GPR120, TAS2R16 and TAS2R38 are widely expressed at the plasma membrane level (Figures 45 A-D).

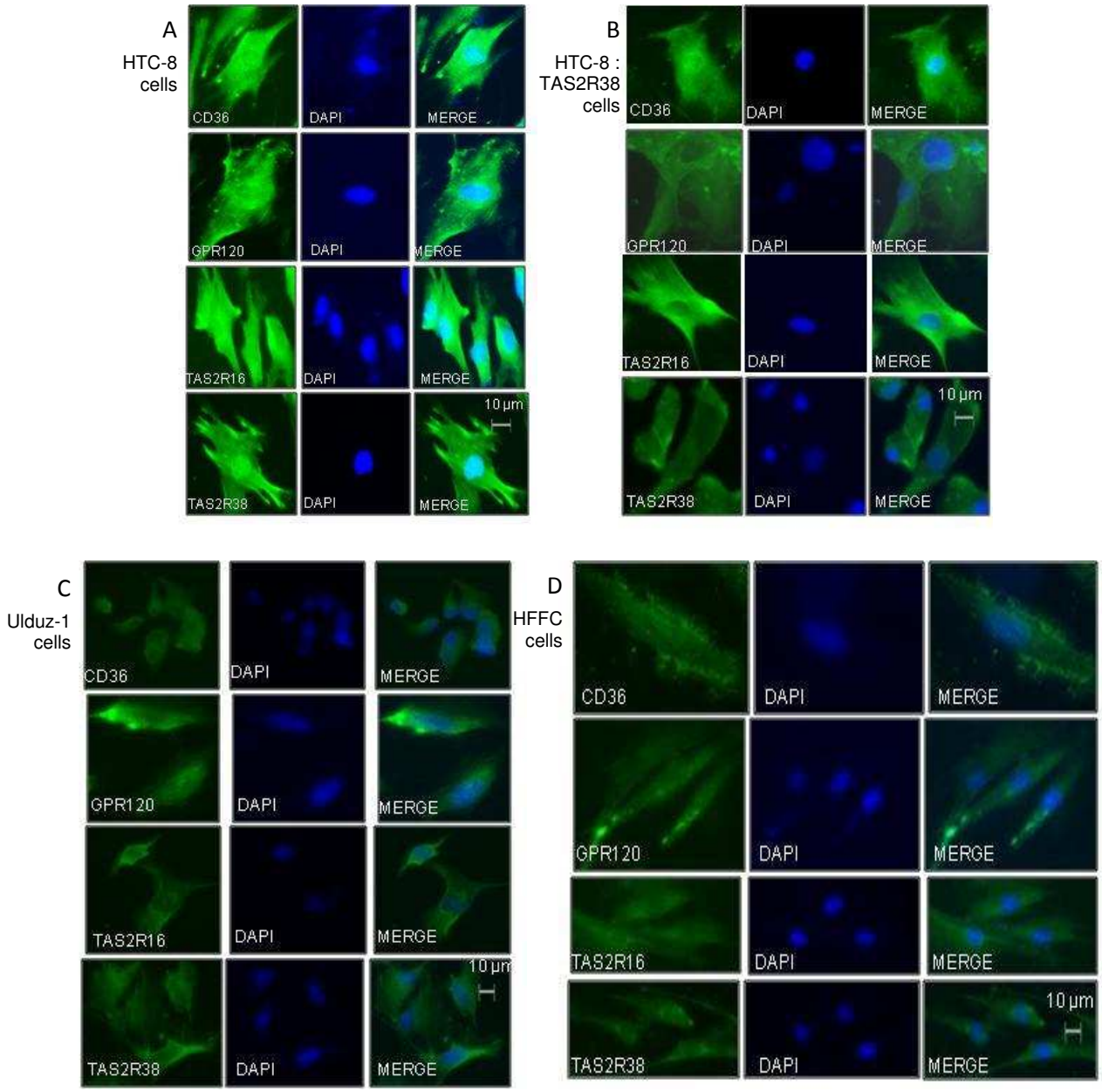

Figure 45. **Immunostaining of fat taste receptors (CD36 and GPR120) and bitter taste receptors (TAS2R16 and TAS2R38).** Images were acquired with Zeiss Cell Observer station (Zeiss, Oberkochen, Germany) (A-D). Immunoreactivity for CD36, GPR120, TAS2R16 and TAS2R38 was observed in cultured HTC-8 (A), in cultured HTC-8:TAS2R38 (B), in cultured Ulduz-1 (C) and in cultured HFFC cells (D).

2.3 Expression of type I cell marker genes and proteins in HTC-8 cells

To further characterize the HTC-8 cells, we investigated the gene and protein expression of GLAST, a type I cell marker. We obtained a weak signal in Western Blotting (Figure 46A). We, then, observed that GLAST mRNA is expressed in the three cell lines, i.e., HTC-8:TAS2R38, Ulduz-1 and HFFC, and, to a lesser extent, in HTC-8 cells (Figure 46B).

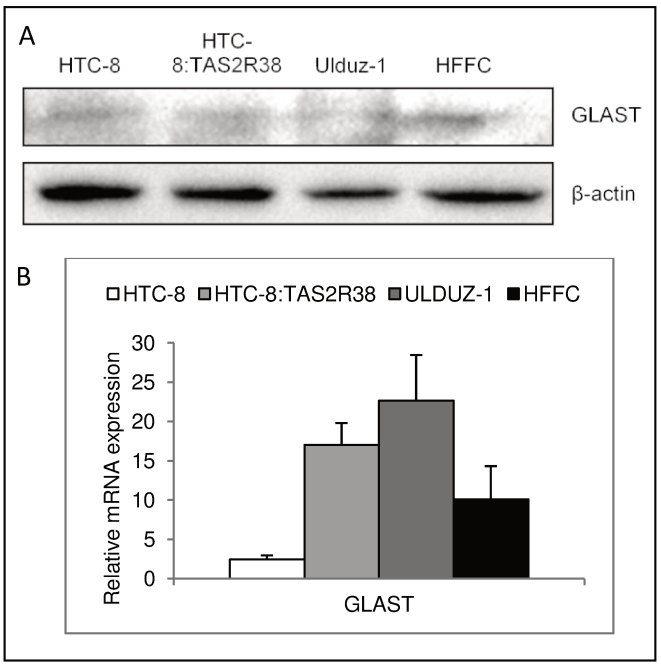

Figure 46. **Type I cell marker expression.** A- GLAST protein levels were measured by Western blotting in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells (n=5). B- GLAST mRNA levels assayed by real-time PCR in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells. Values are expressed as mean ± SEM (n=5).

2.4 Expression of type II cell marker genes and proteins in HTC-8 cells

Regarding type II cell markers, we monitored α-gustducin (GNAT3), $G_{\alpha t1}$ (GNAT1), PLCβ2 and PLCδ4. We noticed that the four markers were expressed in the four TBC cell lines (Figure 47 A and B). These results suggest that the HTC-8 cells are mixed type expressing type I and also type II markers or they might be another subtype of cells.

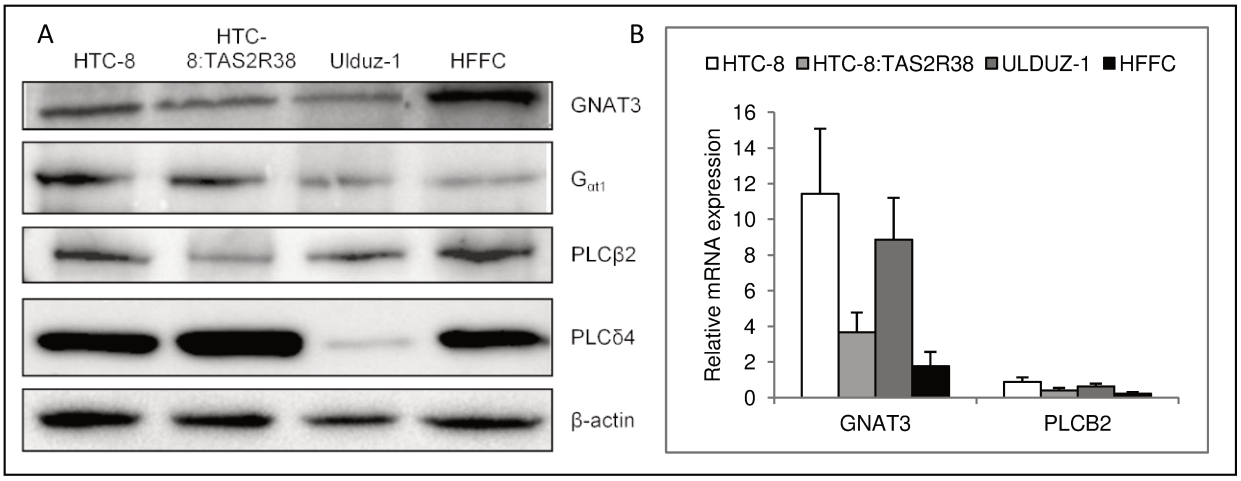

Figure 47. **Type II cell marker expression.** A- GNAT3, $G_{\alpha t1}$, PLCβ2 and PLCδ4 protein levels were measured by Western blotting in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells (n=5). B- GNAT3, $G_{\alpha t1}$, PLCβ2 and PLCδ4 mRNA levels assayed by real-time PCR in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells. Values are expressed as mean ± SEM (n=5).

2.5 Expression of type II cell markers

We also performed immnoblotting and observed that GNAT3 and PLCβ2 are distributed the same way in the cells, at the plasma membrane level (Figures 48 A-D).

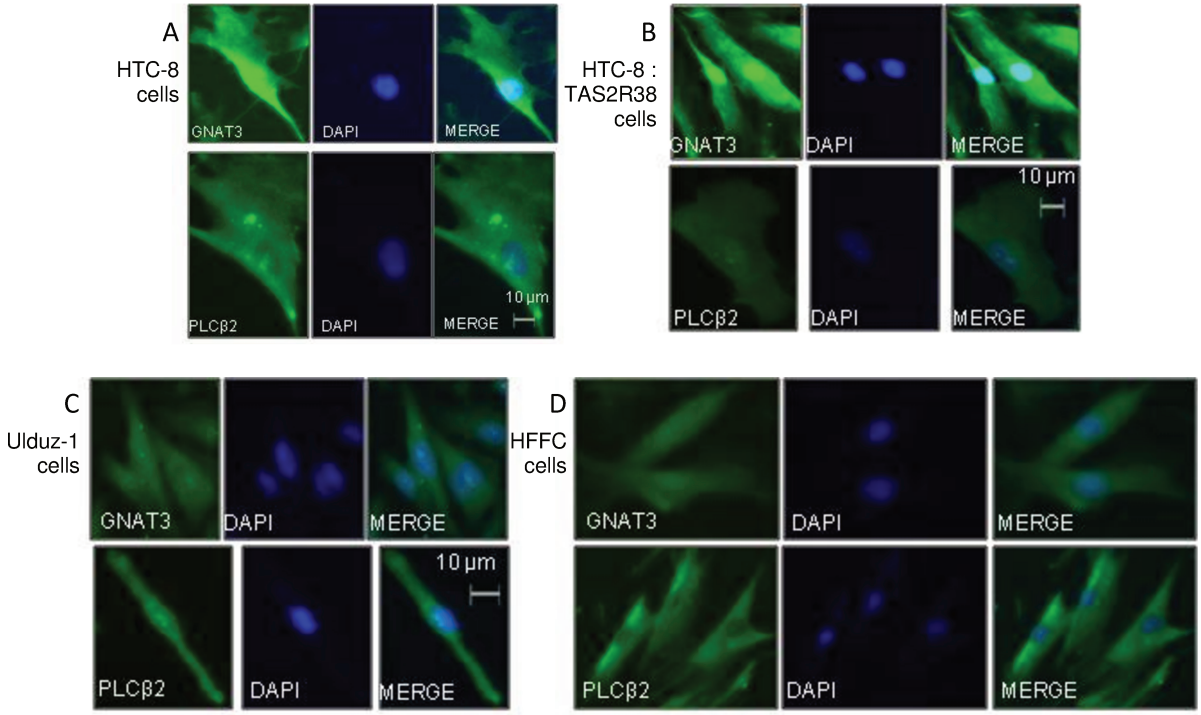

Figure 48. **Immunostaining of GNAT3 and PLCβ2.** Images were acquired with Zeiss Cell Observer station (Zeiss, Oberkochen, Germany) (A-D). Immunoreactivity for GNAT3 and PLCβ2 was observed in cultured HTC-8 (A), in cultured HTC-8:TAS2R38 (B), in cultured Ulduz-1 (C) and in cultured HFFC cells (D).

2.6 Expression of type III cell marker genes and proteins in HTC-8 cells

Finally, we assessed the expression of a type III cell marker, i.e., SNAP25. We detected a very weak signal regarding protein expression levels (Figure 49A). Nevertheless, SNAP25 mRNA seems to be expressed in all the different TBC cell lines (Figure 49B). These findings, taken together, seem to highlight that the four human TBC lines express type I, II and III cell markers.

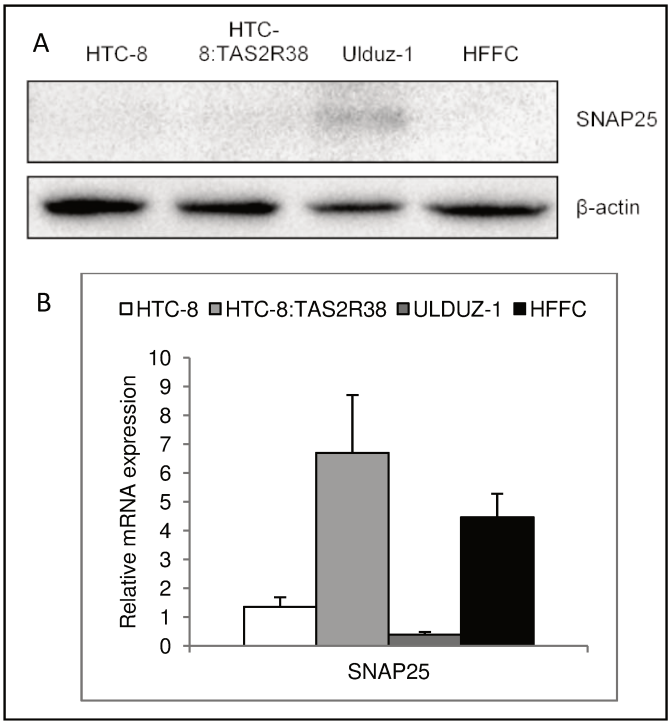

Figure 49. **Type III cell marker expression.** A- SNAP25 protein levels were measured by Western blotting in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells (n=5). B- SNAP25 mRNA levels assayed by real-time PCR in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells. Values are expressed as mean ± SEM (n=5).

2.7. Expression of hormones and their cognate receptor genes and proteins in HTC-8 cells

Additionally, we investigated the presence of various hormones and their cognate receptor. We assayed protein expressions of receptors and mRNA expressions of hormones and receptors. We obtained weak signals for CCK-AR, GHS-R and GLP-1R protein expression for the four cell lines. OTR, oxytocin receptor, protein is expressed in all the different cell lines, whereas, Ob-r protein is expressed only in Ulduz-1 cells (Figure 50A). Otherwise,

CCK-A and CCK-AR, GHRL (ghrelin) and GHSR, P-GCG, GLP-1 precursor, and GLP-1r, OXT and OTR and leptin and Ob-R, its receptor are all express in the four cell lines.

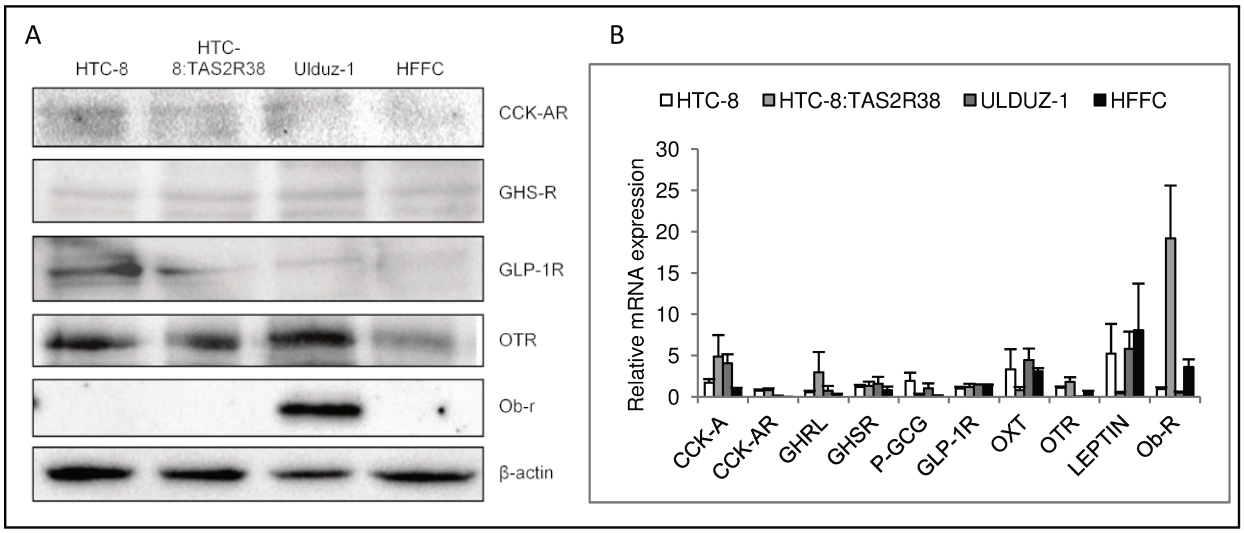

Figure 50. **Hormones and receptors expression.** A- CCK-AR, GHS-R, GLP-1R, OTR and Ob-R protein levels were measured by Western blotting in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells (n=5). B- CCK-AR, GHS-R, GLP-1R, OTR and Ob-R mRNA levels assayed by real-time PCR in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells. Values are expressed as mean ± SEM (n=5).

2.8 Co-expression of bitter and fat receptors in HTC-8 cells

HTC-8 cells co-expressed fat and the bitter taste receptor TAS2R38 as well as HTC-8:TAS2R38, Ulduz-1 and HFFC cells. Indeed, CD36-positive cells are also positive for TAS2R38 (Figure 51).

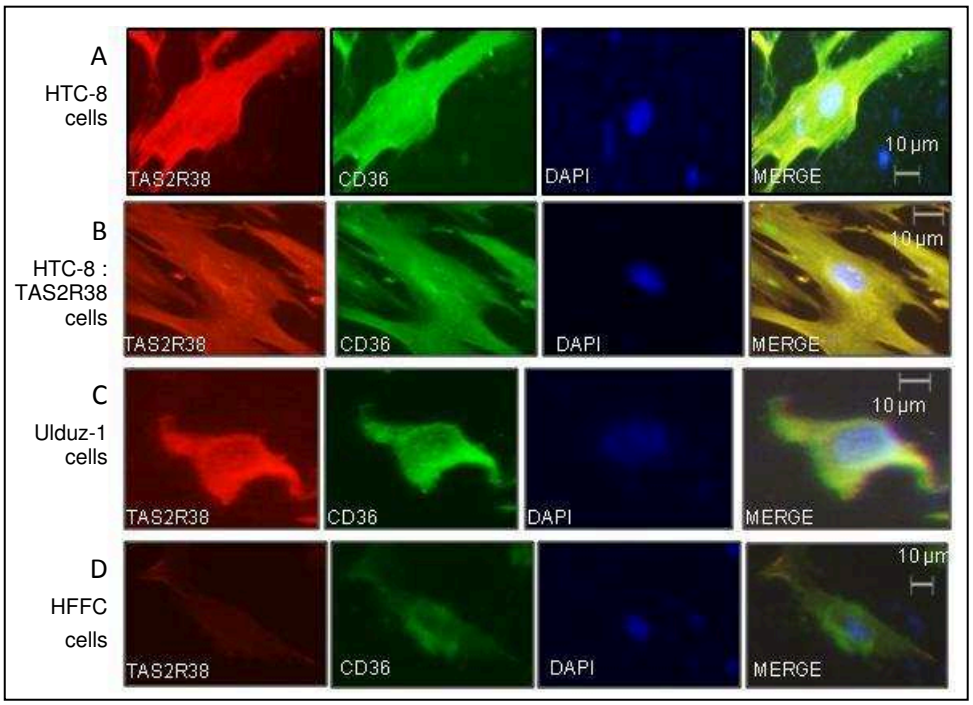

Figure 51. **Immunostaining of the bitter taste receptor TAS2R38 and the fatty taste receptor CD36.** Images were acquired with Zeiss Cell Observer station (Zeiss, Oberkochen, Germany) (A-B). Immunoreactivity for TAS2R38 (red) and CD36 (green) was observed in cultured HTC-8 (A), in cultured HTC-8:TAS2R38 (B), in cultured Ulduz-1 (C) and in cultured HFFC cells (D).

HTC-8 cells co-expressed fat and the bitter taste receptor TAS2R16 as well as the other human cell lines. Indeed, CD36-positive cells are also positive for TAS2R16 (Figure 52).

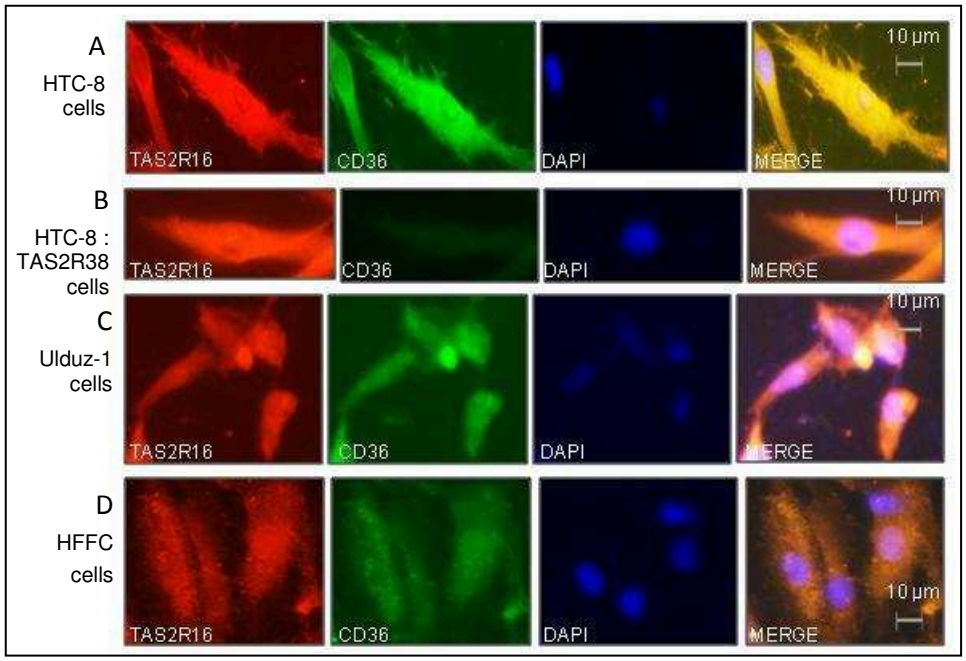

Figure 52. **Immunostaining of the bitter taste receptor TAS2R16 and the fatty taste receptor CD36.** Images were acquired with Zeiss Cell Observer station (Zeiss, Oberkochen, Germany) (A-B). Immunoreactivity for TAS2R16 (red) and CD36 (green) was observed in cultured HTC-8 (A), in cultured HTC-8:TAS2R38 (B), in cultured Ulduz-1 (C) and in cultured HFFC cells (D).

We observed that no significant differences appeared regarding fat and bitter receptors between the different cell lines. We therefore decided to conduct the study only on HTC-8 cells.

2.9 Linoleic acid triggered Ca^{2+} signalling in HTC-8 cells via CD36 and GPR120

The gene and protein expression profile of HTC-8 cells revealed that the cells express fat taste receptors CD36 and GPR120 (Figures 44-45). HTC-8 cells were stained with the Ca^{2+} dye Fura-2/AM and stimulated with increasing concentration of LA. LA evoked increases in Ca^{2+} signalling (Figure 53A). Concentration eliciting half-maximal responses (EC_{50}) were determined at 67.44 µM (Figure 53B).

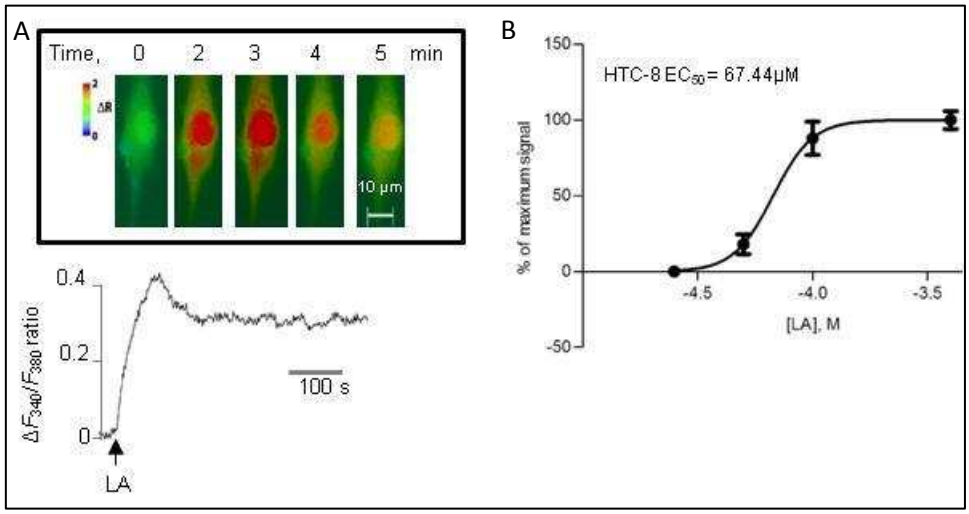

Figure 53. **LA induces increases in $[Ca^{2+}]_i$ in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A-Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 µM) and the corresponding graphs below. B-Graphs represent concentration-response curve obtained with HTC-8 cells and the corresponding EC_{50} value. Values are expressed as mean ± SEM (n=5).

2.10 Sinigrin, an agonist of TAS2R16, triggered Ca^{2+} signalling in HTC-8 cells

The gene and protein expression profile of HTC-8 cells revealed that the cells express the bitter taste receptors TAS2R16 and TAS2R38 (Figures 44-45). HTC-8 cells were stained with the Ca^{2+} dye Fura-2/AM and stimulated with increasing concentration of sinigrin. Sinigrin evoked increases in Ca^{2+} signalling (Figure 54A). Concentration eliciting half-maximal responses (EC_{50}) were determined at 526.8 µM (Figure 54B).

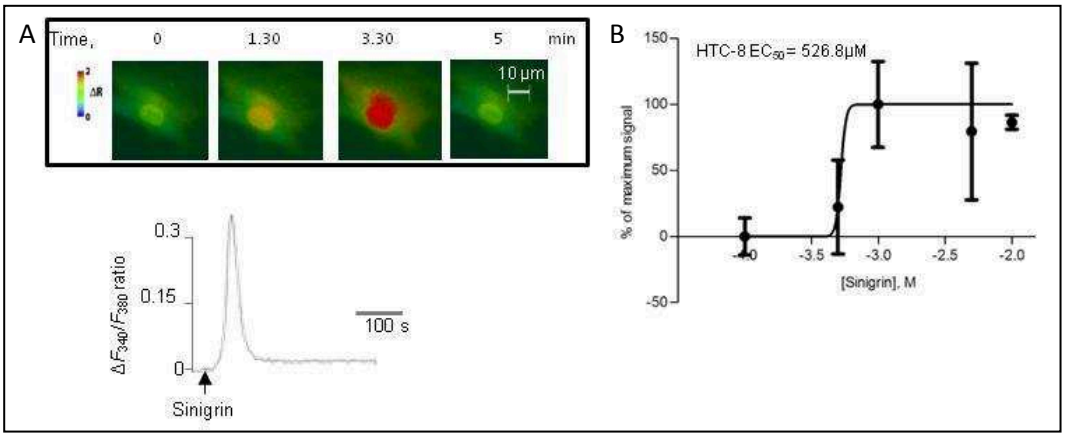

Figure 54. **Sinigrin induces increases in $[Ca^{2+}]_i$ in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A-Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) and the corresponding graphs below. B-Graphs represent concentration-response curve obtained with HTC-8 cells and the corresponding EC_{50} value. Values are expressed as mean ± SEM (n=5).

2.11 PTC, an agonist of TAS2R16, triggered Ca^{2+} signalling in HTC-8 cells

The gene and protein expression profile of HTC-8 cells revealed that the cells express the bitter taste receptor TAS2R38 (Figures 44-45). HTC-8 cells were stained with the Ca^{2+} dye Fura-2/AM and stimulated with increasing concentration of PTC. PTC evoked increases in Ca^{2+} signalling (Figure 55A). Concentration eliciting half-maximal responses (EC_{50}) were determined at 5.589 mM (Figure 55B).

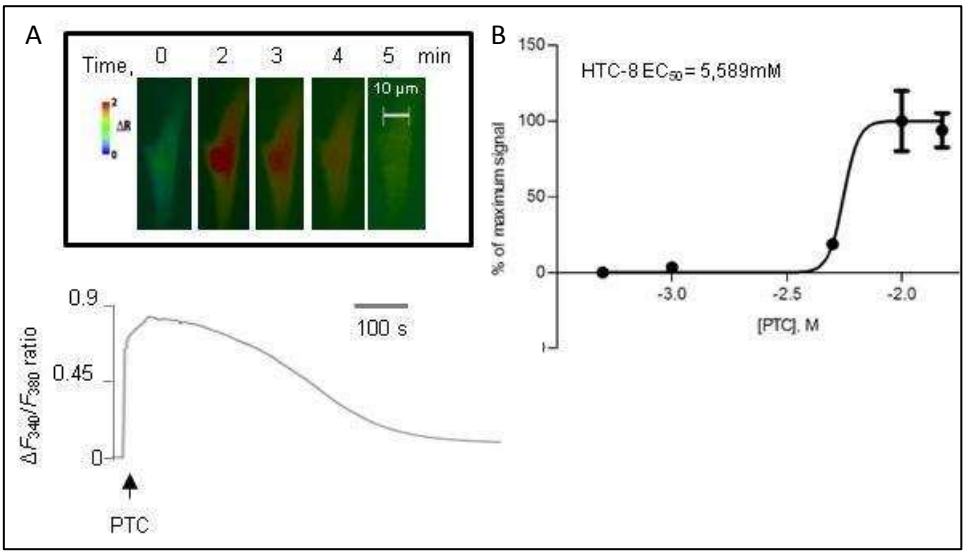

Figure 55. **PTC induces increases in $[Ca^{2+}]_i$ in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A-Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) and the corresponding graphs below. B-Graphs represent concentration-response curve obtained with HTC-8 cells and the corresponding EC_{50} value. Values are expressed as mean ± SEM (n=5).

2.12 Salicin, an agonist of TAS2R16 and TAS2R38, triggered Ca^{2+} signalling in HTC-8 cells

The gene and protein expression profile of HTC-8 cells revealed that the cells express the bitter taste receptor TAS2R16 (Figures 44-45). HTC-8 cells were stained with the Ca^{2+} dye Fura-2/AM and stimulated with increasing concentration of salicin. Salicin evoked increases in Ca^{2+} signalling (Figure 56A). Concentration eliciting half-maximal responses (EC_{50}) were determined at 13.21 mM (Figure 56B).

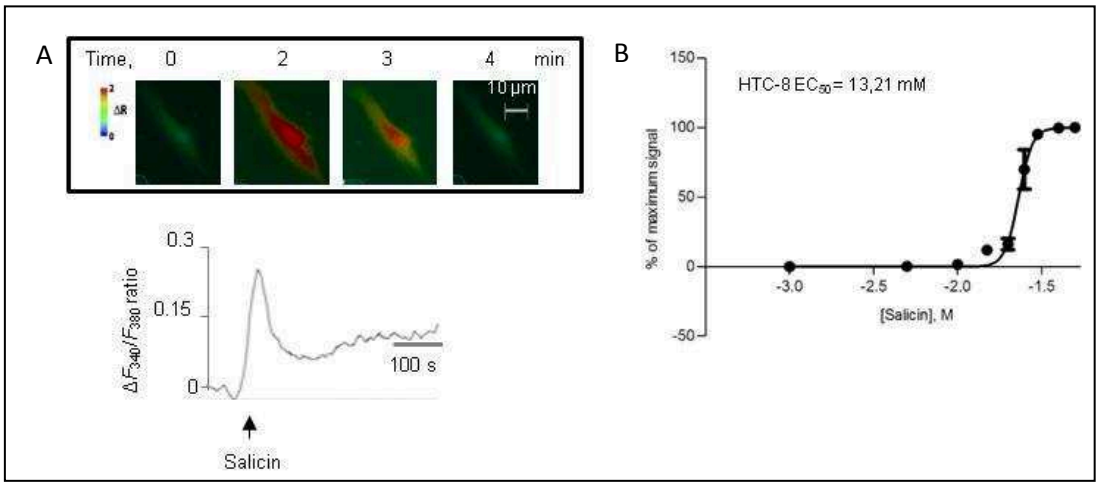

Figure 56. **Salicin induces increases in $[Ca^{2+}]_i$ in HTC-8 cells**. Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A-Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) and the corresponding graphs below. B-Graphs represent concentration-response curve obtained with HTC-8 cells and the corresponding EC_{50} value. Values are expressed as mean ± SEM (n=5).

2.13 CD36 and/or GPR120 inhibitors decrease fat and bitter Ca^{2+} signalling in HTC-8 cells

As expected, AH7614, a GPR120 inhibitor, and SSO, a CD36 inhibitor, significantly abrogated $[Ca^{2+}]_i$ responses induced by LA. Furthermore, synergistic blockade with AH7614 and SSO in HTC-8 cells drastically curtailed the action of LA on calcium response (Figure 57A). Interestingly, AH7614 and SSO added together, significantly abrogated $[Ca^{2+}]_i$ responses induced by sinigrin (Figure 57B). In the same way, AH7614 and SSO added together, significantly abrogated $[Ca^{2+}]_i$ responses induced by PTC (Figure 57C). Similarly, AH7614 and SSO added together, significantly abrogated $[Ca^{2+}]_i$ responses induced by salicin (Figure 57D).

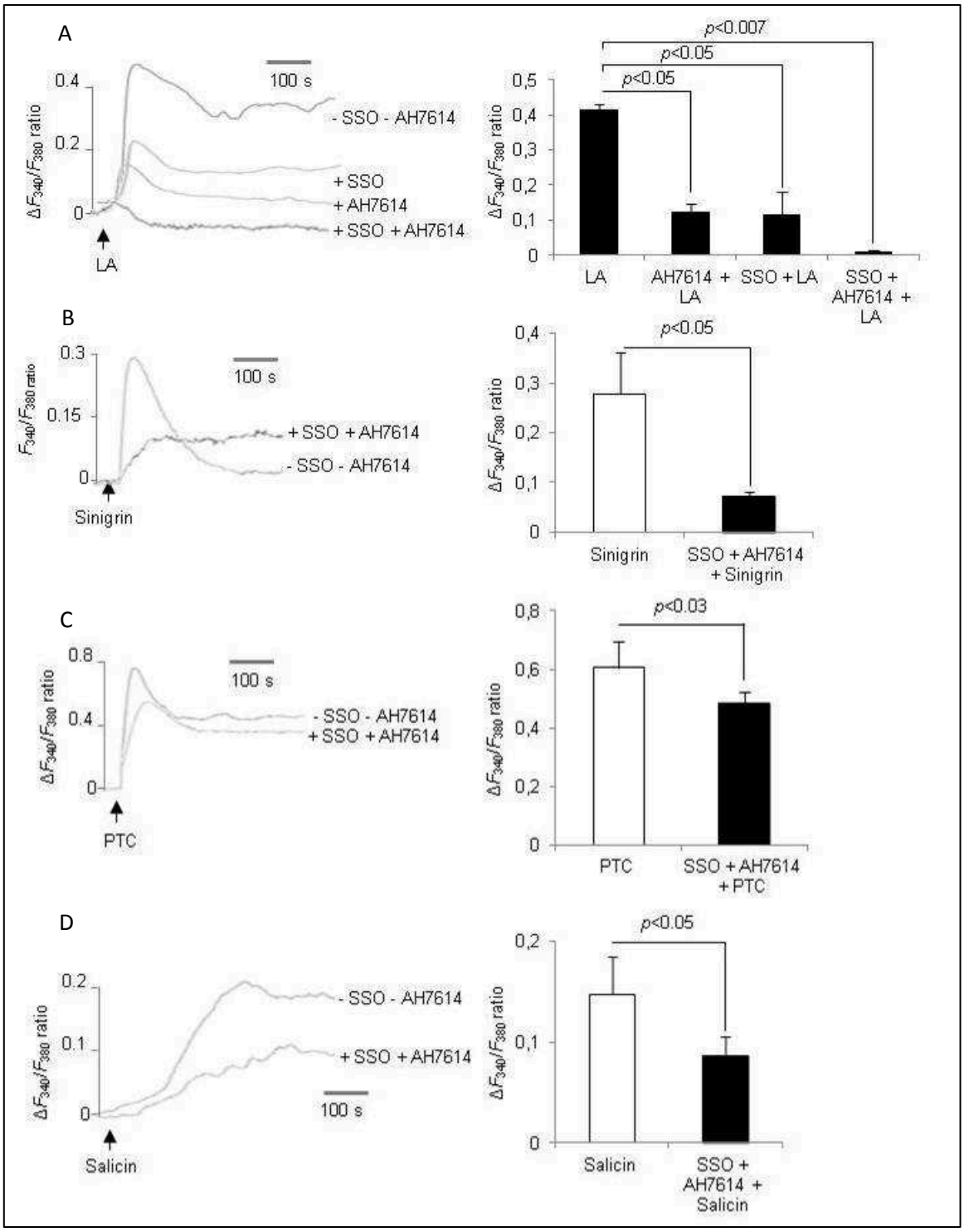

Figure 57. **Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer**. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the variations in $\Delta F_{340}/F_{380}$ Ratio induced by LA (100 μM) after, or not, preincubation with SSO and/or AH7614. B- shows the variations in $\Delta F_{340}/F_{380}$ Ratio induced by sinigrin (1 mM) after, or not, preincubation with SSO and AH7614. C- shows the variations in $\Delta F_{340}/F_{380}$ Ratio induced by PTC (10 mM) after, or not, preincubation with SSO and AH7614. D- shows the variations in $\Delta F_{340}/F_{380}$ Ratio induced by salicin (25 mM) after, or not, preincubation with SSO and AH7614. Values are expressed as mean ± SEM (n=5).

2.14 PLC inhibitor decreases fat and bitter Ca^{2+} signalling in HTC-8 cells

To strengthen our findings, we finally tested the effect of a PLC inhibitor, U73122, on fat and bitter transduction signalling. It appeared that U73122 induced a significant decrease in $[Ca^{2+}]_i$ signalling elicited by LA (Figure 58A). The PLC inhibitor also disrupted bitter taste $[Ca^{2+}]_i$ signalling. Indeed, after a 20-minute preincubation with U73122, the rises triggered by PTC, salicin and sinigrin are significantly lower than in absence of U73122 (Figure 58B, C and D).

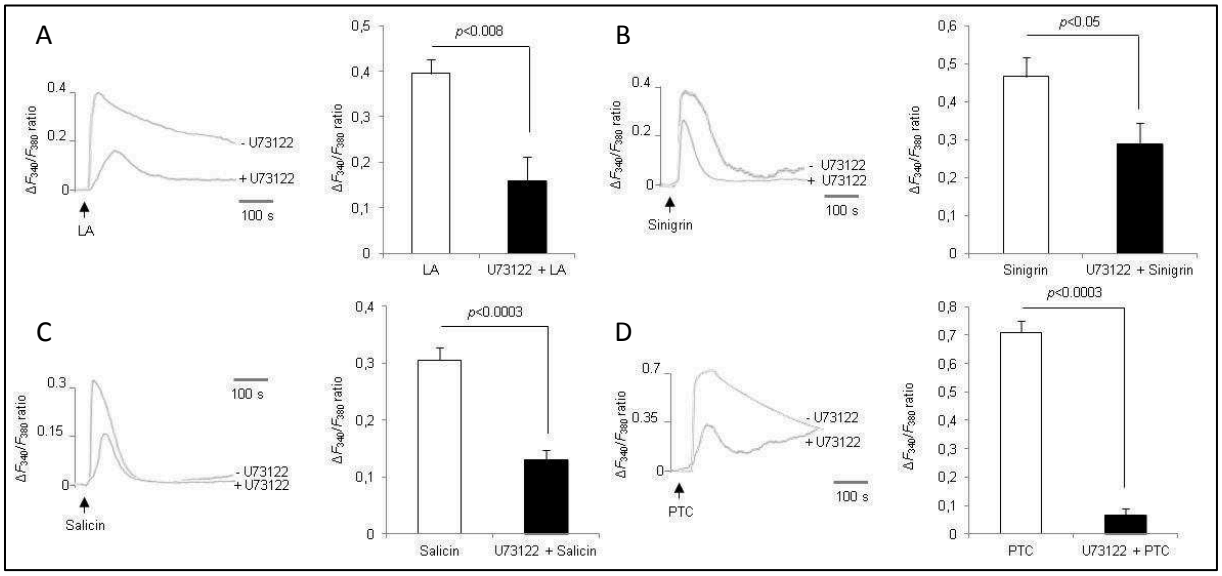

Figure 58. **U73122 induces reduced $[Ca^{2+}]_i$ responses in HTC-8 cells**. Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 μM) after a 20 minutes preincubation with U73122 (0.5 μM) or not and the corresponding graphs beside. B- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) after a 20 minutes preincubation with U73122 (0.5 μM) or not and the corresponding graphs beside. C- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) after a 20 minutes preincubation with U73122 (0.5 μM) or not and the corresponding graphs beside. D- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) after a 20 minutes preincubation with U73122 (0.5 μM) or not and the corresponding graphs beside.

2.15 Fat and bitter agonists mobilize Ca^{2+} extra and intracellular pool in HTC-8 cells

We further studied the origin of the Ca^{2+}. To do so, we used Ca^{2+}-containing and Ca^{2+}-free medium. LA-induced increases in $[Ca^{2+}]_i$ were higher in Ca^{2+}-containing as compared with Ca^{2+}-free medium (Figure 59A). Similarly, we observed the same responses with the bitter

agonists, PTC, sinigrin and salicin, i.e., a strong decrease in $[Ca^{2+}]_i$ responses (Figure 59B, C and D) suggesting a Ca^{2+}-extracellular dependent pathway.

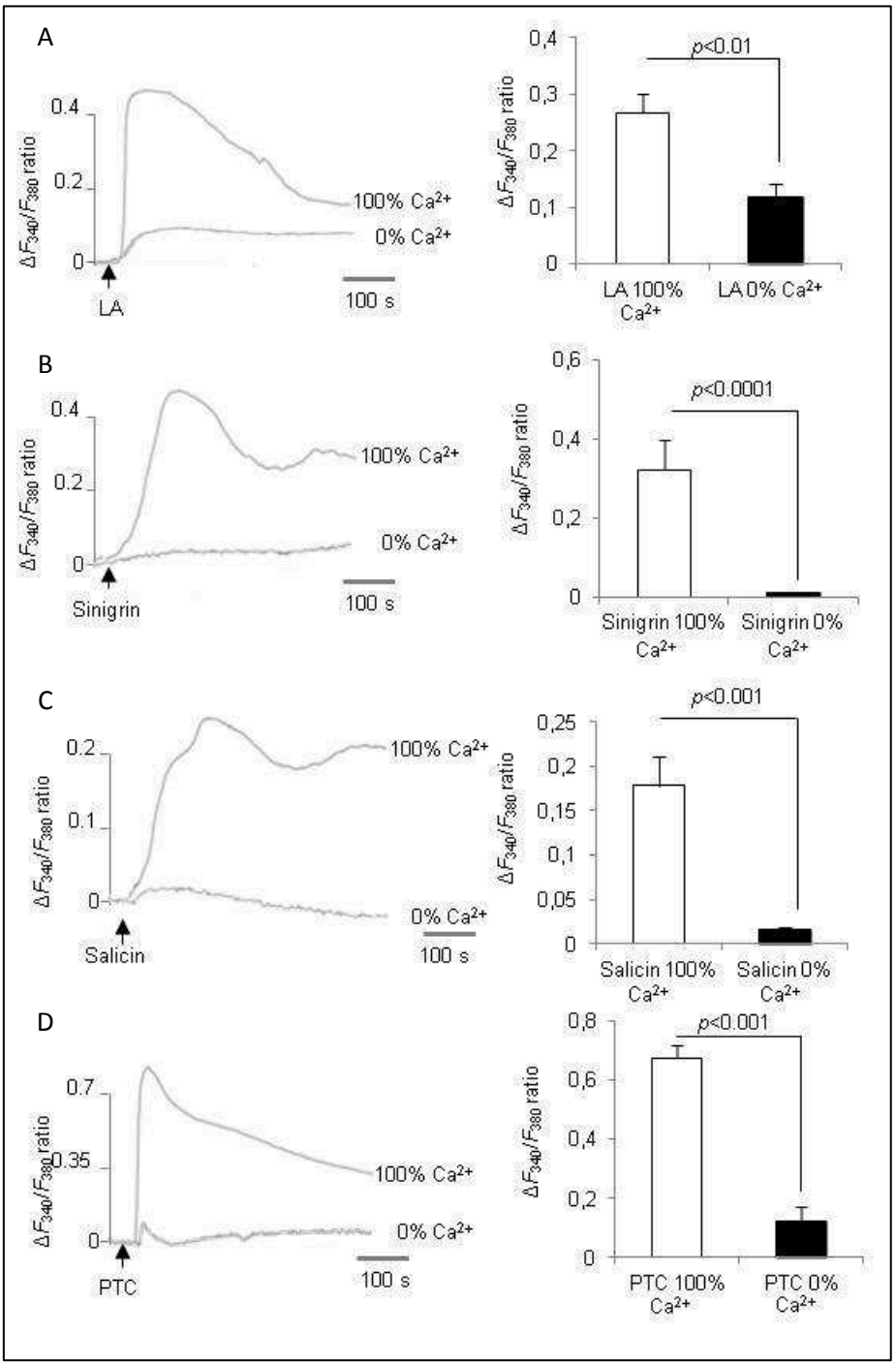

Figure 59. **Effects of LA, PTC, Sinigrin and Salicin on Ca^{2+} signalling in HTC-8 cells.** The experiments were performed in Ca^{2+}-containing and in Ca^{2+}-free media. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A-shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 μM) and the corresponding graph. B- shows the changes in $[Ca^{2+}]_i$ evoked by PTC (10 mM) and the corresponding graph. C- shows the changes in $[Ca^{2+}]_i$ evoked by Sinigrin (1 mM) and the corresponding graph. D- shows the changes in $[Ca^{2+}]_i$ evoked by Salicin (25 mM) and the corresponding graph. Values are expressed as mean ± SEM (n=5).

2.16 Fat and bitter agonists trigger additive Ca^{2+} signalling in HTC-8 cells

The addition of LA and then sinigrin triggered an additive response (Figure 60A). Likewise, sinigrin added before LA induced an additive response (Figure 60B). Finally, when added in the same time, LA and sinigrin induced a huge rise equivalent to the amplitude obtained with the agonists added alone (Figure 60C). Similarly, PTC and salicin displayed the same responses. Indeed, PTC/salicin and LA added one after another induced additive responses (Figure 60D, E, G and H) and added in the same time, generated a response corresponding to the combined effect of the two agonists (Figure 60F and I). These results are in line with LA primarily interacting with CD36 while the bitter agonists activate its cognate TAS2R receptor.

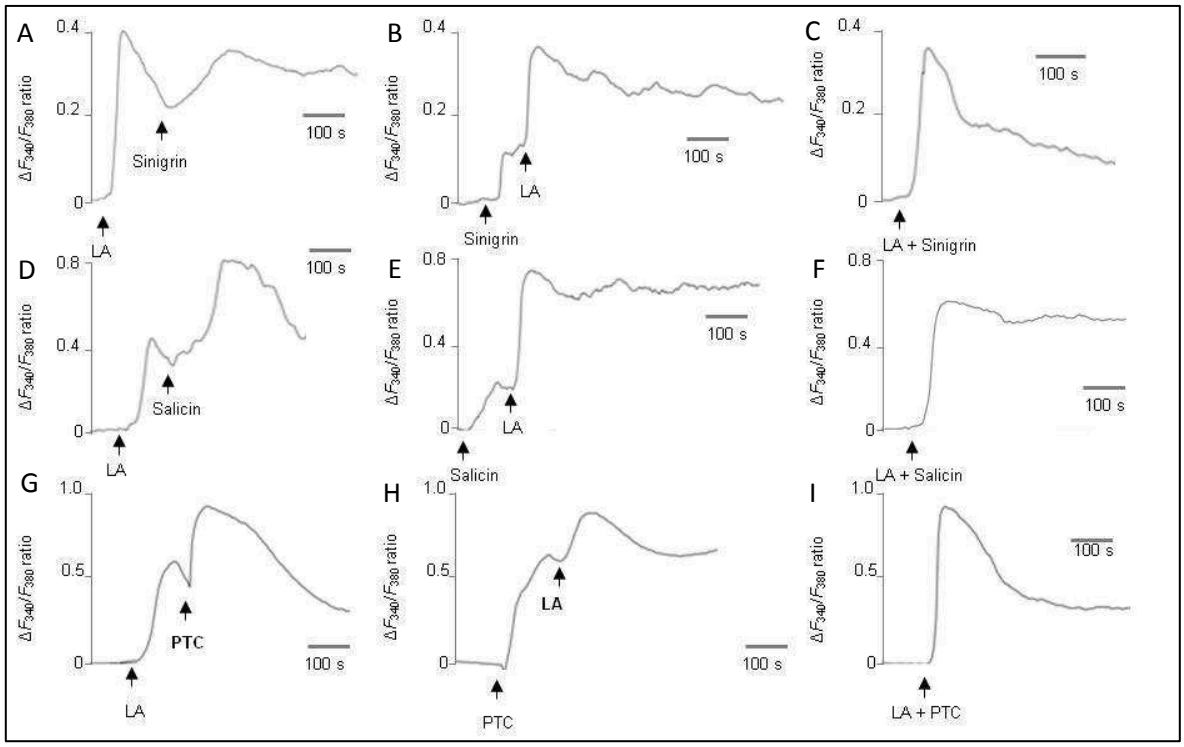

Figure 60. **LA and bitter agonists induce additive $[Ca^{2+}]_i$ responses in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 µM) following by Sinigrin (1 mM). B- shows the changes in $[Ca^{2+}]_i$ evoked by Sinigrin (1 mM) following by LA (100 µM). C- shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 µM) and Sinigrin (1 mM). D- shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 µM) following by PTC (10 mM). E- shows the changes in $[Ca^{2+}]_i$ evoked by PTC (10 mM) following by LA (100 µM). F- shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 µM) and PTC (10 mM). G- shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 µM) following by Salicin (25 mM). H- shows the changes in $[Ca^{2+}]_i$ evoked by Salicin (25 mM) following by LA (100 µM). I- shows the changes in $[Ca^{2+}]_i$ evoked by LA (100 µM) and Salicin (25 mM).

2.17 Capacitative calcium influx blocker decreases fat and bitter Ca^{2+} signalling in HTC-8 cells

Next, we investigated the downstream signalling using inhibitors known to inhibit fat taste signal transduction. We first used a capacitative calcium influx blocker, econazole. The capacitative calcium influx is a calcium current activated by the depletion of internal calcium. As expected, after a preincubation with econazole. LA evoked a significantly lower $[Ca^{2+}]_i$ response than in absence of econazole (Figure 61A). Regarding bitter taste agonists, PTC induced a reduced $[Ca^{2+}]_i$ response in presence of econazole (Figure 61B). Interestingly, salicin and sinigrin failed to raise $[Ca^{2+}]_i$ in HTC-8 cells after a 30-minute preincubation with econazole (Figure 61C and D).

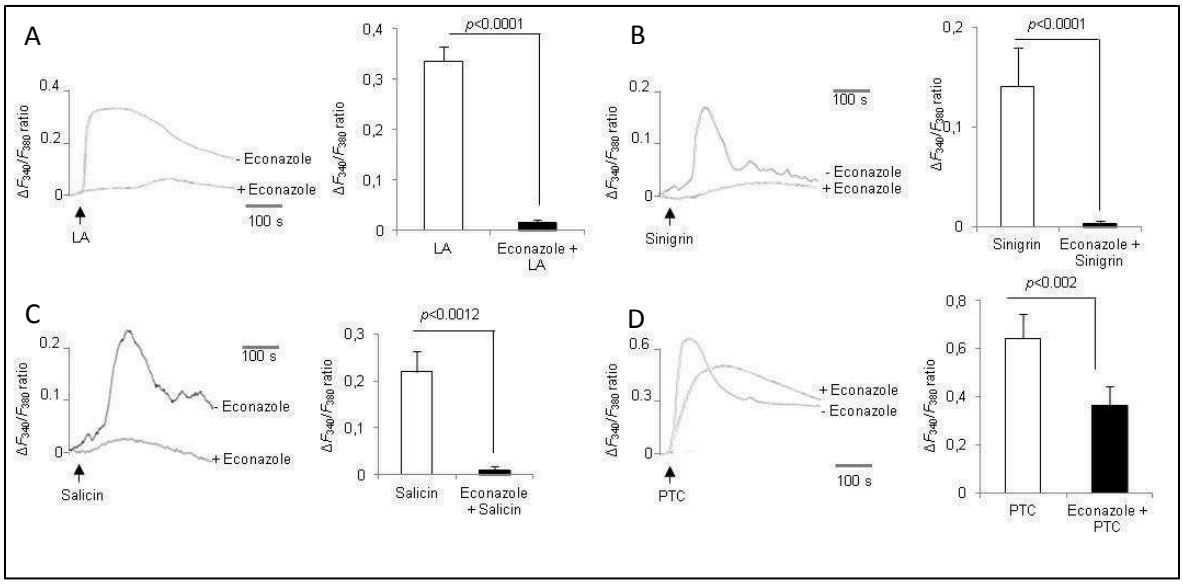

Figure 61. **Econazole induces reduced $[Ca^{2+}]_i$ responses in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 µM) after a 30 minutes preincubation with Econazole (30 µM) or not and the corresponding graphs beside. B- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) after a 30 minutes preincubation with Econazole (30 µM) or not and the corresponding graphs beside. C- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) after a 30 minutes preincubation with Econazole (30 µM) or not and the corresponding graphs beside. D- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) after a 30 minutes preincubation with Econazole (30 µM) or not and the corresponding graphs beside.

2.18 Receptor-mediated and voltage gated Ca^{2+} entry inhibitor decreases fat and bitter Ca^{2+} signalling in HTC-8 cells

Then, we used a receptor-mediated Ca^{2+} entry and voltage gated Ca^{2+} entry inhibitor, SKF96365. We observed that the rise in $[Ca^{2+}]_i$ induced by LA in HTC-8 cells was significantly diminished after a 30-minute preincubation with SKF96365 (Figure 62A). Similarly, $[Ca^{2+}]_i$ response, in presence of SKF96365, after PTC stimulation was decreased (Figure 62B) and highly curtailed after salicin and sinigrin stimulation (Figure 62C and D).

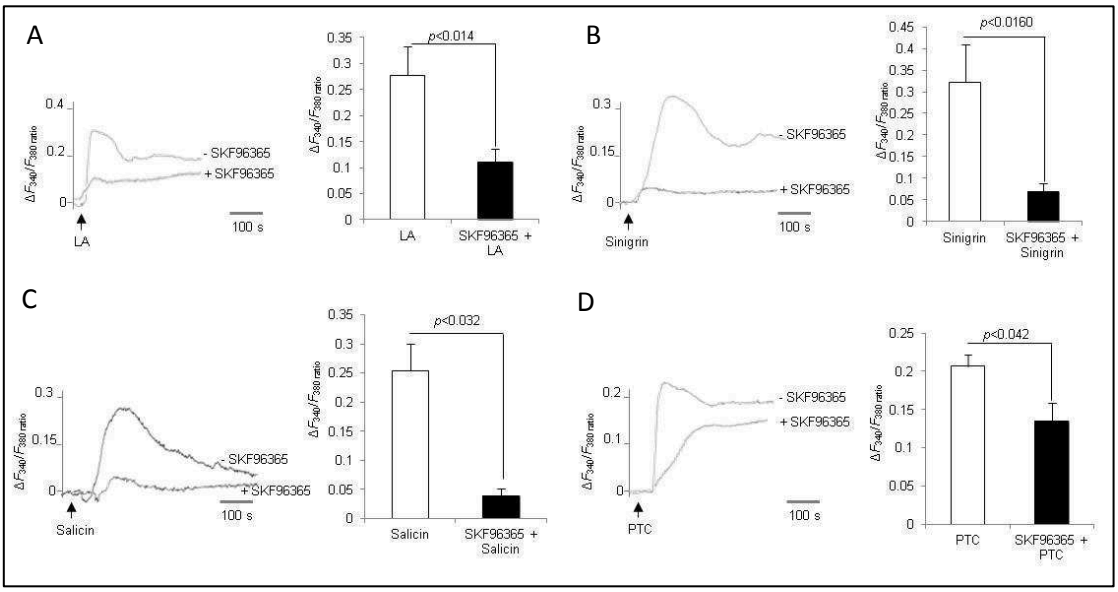

Figure 62. **SKF96365 induces reduced $[Ca^{2+}]_i$ responses in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 μM) after a 30 minutes preincubation with SKF96365 (1 μM) or not and the corresponding graphs beside. B- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) after a 30 minutes preincubation with SKF96365 (1 μM) or not and the corresponding graphs beside. C- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) after a 30 minutes preincubation with SKF96365 (1 μM) or not and the corresponding graphs beside. D- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) after a 30 minutes preincubation with SKF96365 (1 μM) or not and the corresponding graphs beside.

2.19 CALHM1 inhibitor decreases fat and bitter Ca^{2+} signalling in HTC-8 cells

The use of 2-APB, a inhibitor of CALHM1, induced significant decrease in the rise in $[Ca^{2+}]_i$ induced by LA in HTC-8 cells after a 30-minute preincubation (Figure 63A). Similarly,

$[Ca^{2+}]_i$ response, in presence of 2-APB, after Sinigrin, Salicin and PTC stimulation was decreased (Figure 63B, C and D).

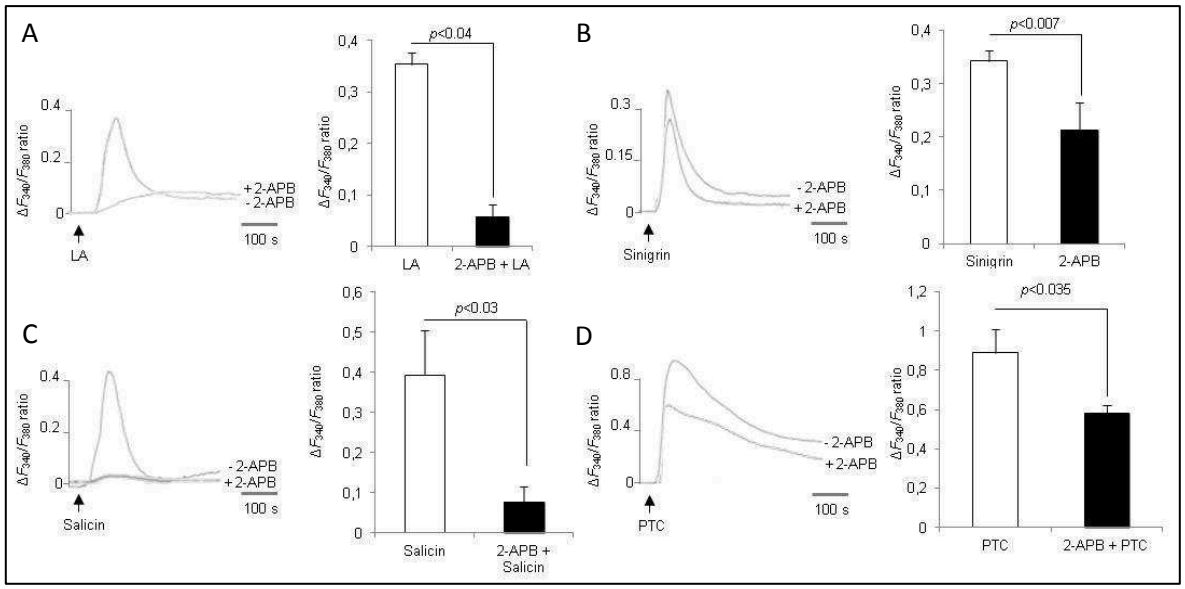

Figure 63. **2-APB induces reduced $[Ca^{2+}]_i$ responses in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 µM) after a 20 minutes preincubation with 2-APB (5 µM) or not and the corresponding graphs beside. B- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) after a 30 minutes preincubation with 2-APB (1 µM) or not and the corresponding graphs beside. C- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) after a 30 minutes preincubation with 2-APB (1 µM) or not and the corresponding graphs beside. D- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) after a 30 minutes preincubation with 2-APB (1 µM) or not and the corresponding graphs beside.

2.20 Ca^{2+}/calmodulin-dependent protein kinase and Stim1 inhibitor decreases fat and bitter Ca^{2+} signalling in HTC-8 cells

Then, we investigated the role of Ca^{2+}/calmodulin-dependent protein kinase and Stim1 using a selective inhibitor of CaMK and Stim1, ML-9. We observed, in the four conditions, that the rise in $[Ca^{2+}]_i$ induced by LA, Sinigrin, Salicin and PTC in HTC-8 cells was significantly decreased after a 30-minute preincubation with ML-9 (Figure 64A, B, C and D respectively).

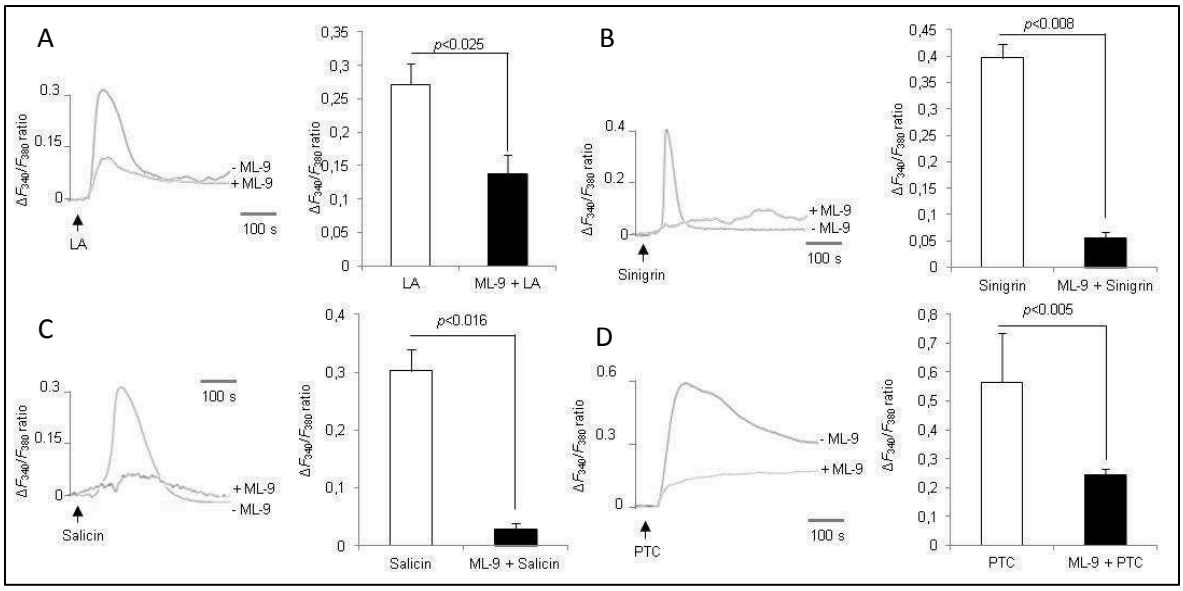

Figure 64. **ML-9 induces reduced $[Ca^{2+}]_i$ responses in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 µM) after a 20 minutes preincubation with ML-9 (10 µM) or not and the corresponding graphs beside. B- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) after a 30 minutes preincubation with ML-9 (1 µM) or not and the corresponding graphs beside. C- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) after a 30 minutes preincubation with ML-9 (1 µM) or not and the corresponding graphs beside. D- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) after a 30 minutes preincubation with ML-9 (1 µM) or not and the corresponding graphs beside.

2.21 Orai1 and 3 siRNA decrease fat and bitter Ca^{2+} signalling in HTC-8 cells

Finally, we treated HTC-8 cells for 72 h with siRNA directed against Orai1, Orai3 and Orai1/3 in the same time. We observed that Orai1 seems to be more involved in fat and bitter downstream signalling as the absence of Orai1 reduced significantly the rise in $[Ca^{2+}]_i$ induced by LA, Sinigrin, Salicin and PTC in HTC-8 cells (Figure 65). Conversely, Orai3 seems to be involved in a variable way as a treatment with siRNA against Orai3 does not seem to impact the Ca^{2+} response induced by LA and Sinigrin (Figure 64A and B). However, the rise in $[Ca^{2+}]_i$ induced by Salicin and PTC in HTC-8 cells was decreased after treatment with Orai3 siRNA (Figure 65C and D). Nevertheless, the treatment with siRNA against Orai1 and 3 induced, in the four conditions, induced a significant decreased in the rise in $[Ca^{2+}]_i$ elicited by LA, Sinigrin, Salicin and PTC in HTC-8 cells (Figure 65).

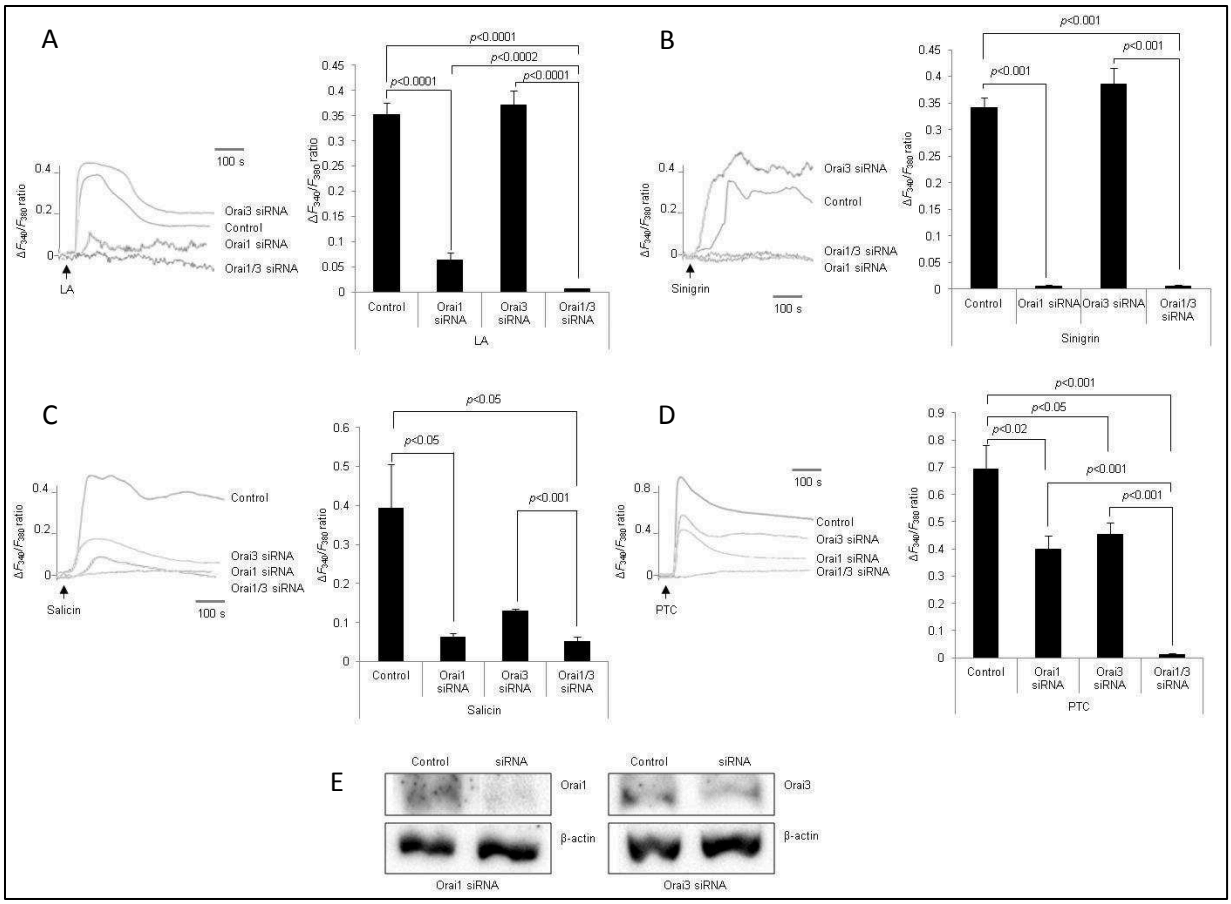

Figure 65. **Orai1 and Orai3 impact differently $[Ca^{2+}]_i$ responses in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of LA (100 μM) after or not a siRNA treatment directed against *Orai1*, *Orai3* or both and the corresponding graphs beside. B- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of PTC (10 mM) after or not a siRNA treatment directed against *Orai1*, *Orai3* or both and the corresponding graphs beside. C- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Salicin (25 mM) after or not a siRNA treatment directed against *Orai1*, *Orai3* or both and the corresponding graphs beside. D- shows the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of Sinigrin (1 mM) after or not a siRNA treatment directed against *Orai1*, *Orai3* or both and the corresponding graphs beside. E- Detection of Orai1, or Orai3 proteins by Western blotting after transfection with either scrambled RNA (control) or siRNA of Orai1, or Orai3

2.22 Oxytocin and its cognate receptor is expressed by human taste bud cell lines

The gene and protein expression profile of HTC-8 cells revealed that the cells express oxytocin and the oxytocin receptor (Figure 50 and 66).

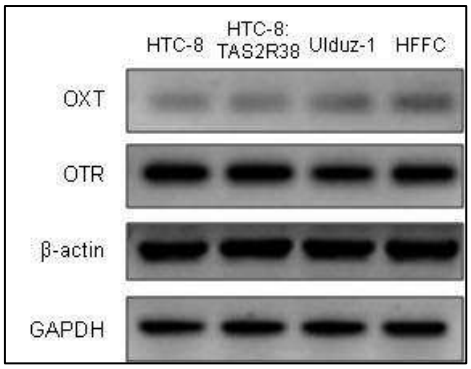

Figure 66. **Oxytocin (OXT) and its cognate receptor (OTR) expression.** OXT and OTR mRNA levels assayed by real-time PCR in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells. B- OXT and OTR protein levels were measured by Western blotting in HTC-8, HTC-8:TAS2R38, Ulduz-1 and HFFC cells (n=5). Values are expressed as mean ± SEM (n=5).

2.23 Oxytocin triggered Ca^{2+} signalling in HTC-8 cells

HTC-8 cells were stained with the Ca^{2+} dye Fura-2/AM and stimulated with increasing concentration of oxytocin. Oxytocin evoked increases in Ca^{2+} signalling (Figure 67A). Concentration eliciting half-maximal responses (EC_{50}) were determined at 15.28 mM (Figure 67B).

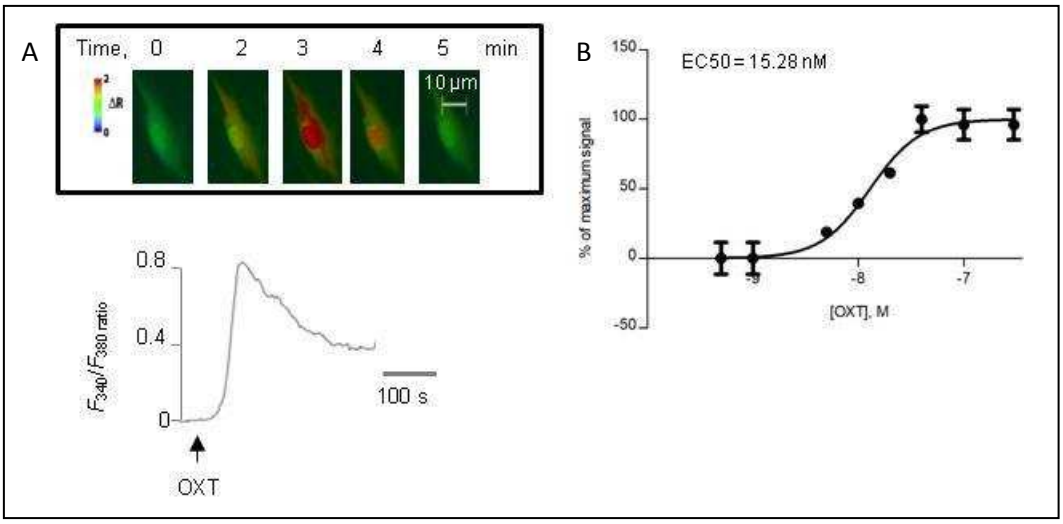

Figure 67. **OXT induces increases in $[Ca^{2+}]_i$ in HTC-8 cells**. Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. A-Colored time-lapse changes show the kinetics of the rise in $[Ca^{2+}]_i$ levels in HTC-8 cells following addition of OXT (40 nM) and the corresponding graphs below. B-Graphs represent concentration-response curve obtained with HTC-8 cells and the corresponding EC_{50} value. Values are expressed as mean ± SEM (n=5).

2.24 GPR120 and CD36 are involved in the lipid-mediated release of oxytocin by HTC-8 cells

We investigated the link between fat taste modulation and oxytocin release in HTC-8 cells. To do so, we subjected HTC-8 cells to five different conditions: LA; LA and AH7614; LA and SSO; LA, AH7614 and SSO; and the control condition and we measured the oxytocin release in the supernantant. We observed that, LA induced a significant decrease in oxytocin release whereas treatment with AH7614 or SSO restored the oxytocin release that occurred in the control condition. Furthermore, the combination of AH7614 and SSO treatment led to an increase in oxytocin release compared to what is observed in the control condition (Figure 68).

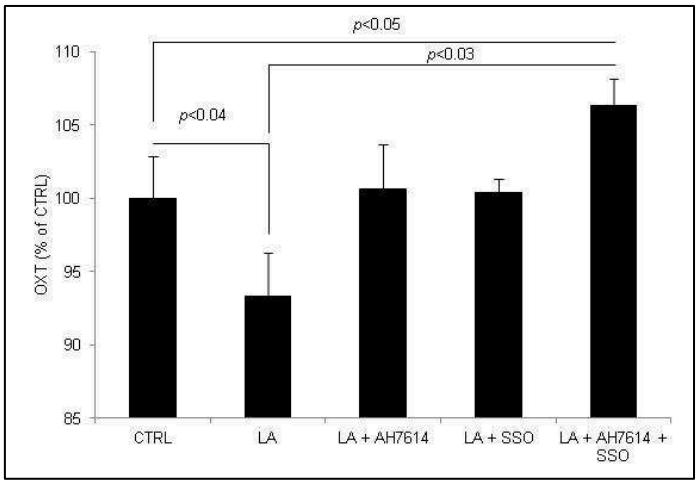

Figure 68. **AH7614, a GPR120 inhibitor and SSO, a CD36 inhibitor increase active OXT release by HTC-8 cells**. OXT release by HTC-8 cells incubated in the presence of HTC-Medium alone (CTRL, control), with 100 µM LA (LA), with 100 µM LA and 30 µM AH7614 (LA + AH7614), with 100 µM LA and 1 µM SSO (LA + SSO) or with 100 µM LA and 30 µM AH7614 in combinaison with 1 µM SSO (LA + AH7614 + SSO). Each value corresponds to the OXT released by $0.5x10^6$ HTC-8 cells. Mean ± SEM (n = 4).

2.25 GPR120 and CD36 mediate the oxytocin mRNA expression in HTC-8 cells

We next studied the role played by these different compounds on oxytocin at the mRNA level. Interestingly, LA induced a decreased in OXT mRNA expression in HTC-8 cells corroboring the results observed with the ELISA experiment. We also observed that treatment with AH7614 and SSO, in the same way, re-established the OXT mRNA expression. However, at the mRNA level, we did not notice the OXT increase that occurred with an AH7614 and SSO simultaneous treatment (Figure 69).

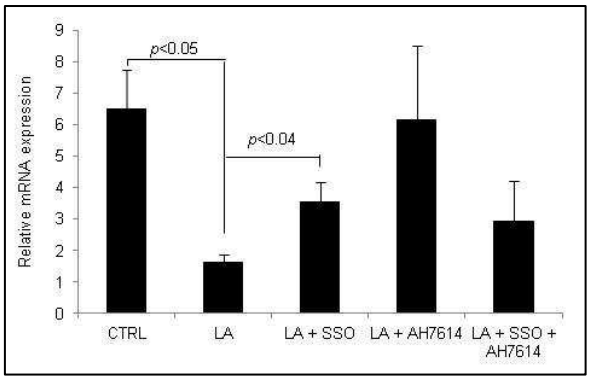

Figure 69. **LA decreases OXT mRNA expression in HTC-8 cells**. *OXT* mRNA expression in HTC-8 cells incubated in the presence of HTC-Medium alone (CTRL, control), with 100 μM LA (LA), with 100 μM LA and 30 μM AH7614 (LA + AH7614), with 100 μM LA and 1 μM SSO (LA + SSO) or with 100 μM LA and 30 μM AH7614 in combinaison with 1 μM SSO (LA + AH7614 + SSO). Each value corresponds to the OXT released by $0.5x10^6$ HTC-8 cells. Mean ± SEM (n = 4).

Discussion

3 Discussion

Within our collaboration with BRAIN institute, we proposed to investigate the potential cross-talk that occurs between fat and bitter taste modalities in HTC-8 cells. First, we characterized the HTC-8 cell profile. Hochheimer et al. (2014) did not detect GNAT3/α-gustducin and PLCβ2 in HTC-8 cells. Thus, they concluded that GNAT2/transducin are also expressed in TBC and could partially complement GNAT3/α-gustducin and may participate to the taste signal transduction pathway (Kusakabe et al., 2000; McLaughlin et al., 1992). Thus, GNAT2/transducin could replace canonical gustatory signalling factors in HTC-8 cells. Regarding the absence of PLCβ2 detection, it is likely that PLδ4, a paralog of the PLCβ2 gene, contributes to the signal transduction as PLCδ4 has been found to be expressed in primates TBC (Hevezi et al., 2009). Interestingly, we observed a positive labeling for α-gustducin, a key factor of the canonical gustatory taste signalling pathway (Kinnamon, 2012) as well as PLCβ2. Thus, HTC-8 cells seem to exhibit a type II receptor profile (Chaudhari and Roper, 2010). Nevertheless, it is possible that GNAT2/transducin and PLCδ4 contribute to fat taste signal transduction. Further studies are needed to explore this assumption.

Karmous et al. (2018) showed a direct association between orosensory perception of fatty acid and PROP. The study sheds light on a possible cross-talk between fat and bitter taste involving cellular and molecular interactions.

Here, we proposed to investigate the likely cross-talk that occurs between fat and bitter tastes. To do so, I spent nearly two years to characterize the HTC-8 cell line. We observed that HTC-8 cells expressed PLCβ2 and α-gustducin among other cell markers and, co-expressed bitter and fat taste receptors.

- **Cross-talk between fat and bitter taste modalities**

It is likely that both bitter and fat tastes share a common downstream Ca^{2+} signalling. We proposed to elucidate the underlying mechanisms that occur in taste bud cells. We first assessed the expression of bitter and fat taste receptors. We observed that fat taste receptors (CD36 and GPR120) and bitter taste receptors (TAS2R38 and TAS2R16) were expressed by HTC-8 cells. Moreover, CD36 was co-expressed with TAS2R38 and TAS2R16 in a homogenous manner. Therefore, we explored the downstream signal transduction. To do so, we selected three bitter agents, PTC known to be a TAS2R38 agonist (Behrens et al., 2013; Bufe et al., 2005; Meyerhof et al., 2010), salicin, a TAS2R16 agonist (Maehashi et al., 2008;

Meyerhof et al., 2010; Soranzo et al., 2005; Thomas et al., 2017) and sinigrin, a glucosinolate to which both TAS2R38 and TAS2R16 respond (Behrens et al., 2013; Meyerhof et al., 2010; Thomas et al., 2017). First of all, calcium assays revealed that HTC-8 cells responded to endogenous Ca^{2+} signalling in a dose-dependent manner to bitter and fat tastants (Hochheimer et al., 2014; Meyerhof et al., 2010). Then, the diminished Ca^{2+} signalling responses in absence of Ca^{2+} in the buffer indicated that the signal transduction mobilized Ca^{2+} from an extra- and intacellular pool (Ozdener et al., 2014). It appeared that fatty and bitter taste signalling depend on the presence of extracellular Ca^{2+}. The addition of LA and the different bitter taste agonists, one after the other, triggered additive responses, suggesting that LA interacted with CD36 while bitter agonists activated their cognate receptor, i.e. TAS2R16 and/or TAS2R38. Then, we focused on the downstream mechanism. Interestingly, it revealed that downstream Ca^{2+} signalling inhibitors known to be involved in fat taste signal transduction via CD36 also inhibited bitter fat taste signalling. Indeed, all of the inhibitors used in the study altered Ca^{2+} signalling after their addition in the medium, strongly supporting the existence of a cross-talk bitter the fat taste and the bitter taste modalities.

The Ca^{2+} influx that occurs through the opening of stored operated calcium channels (SOC channels), such as Orai1 and Orai3, is modulated by phosphorylation, mediated by Src family kinases (SFK) (Babnigg et al., 1997). Furthermore, El-Yassimi et al. (2008) showed that SFK96365, a selective inhibitor of receptor-mediated Ca^{2+} entry, significantly reduced the LCFA-induced increases in $[Ca^{2+}]_i$ in CD36-expressing taste bud cells (TBC) from mice. Besides, Stromal interaction molecule 1 (STIM1) is an endoplasmic reticulum (ER) sensor whose stimulation results in SOC channels activation. STIM1 has been found to be involved in fat taste perception as spontaneous fat preference in $Stim1^{-/-}$ mice was abolished (Dramane et al., 2012). Moreover, STIM1 has been shown to regulate LCFA-induced opening of SOC channels in CD36-expressing mice TBC (Dramane et al., 2012). Indeed, blockers of SOC channels (i.e. SKF96365 and econazole) significantly diminished LA- and bitter agonists-induced increases in $[Ca^{2+}]_i$. As reported (Ozdener et al., 2014), our study corroborates the involvement of STIM1 and SOC channels in Ca^{2+} responses to LCFA and sheds light on the implication of these channels in Ca^{2+} responses elicited by bitter agents.

Albeit Hochheimer et al. (2014) failed to detect PLCβ2 in HTC-8 cells, we obtained a PLCβ2-positive labeling and also found *PLCβ2* mRNA. The use of U73122, a PLC inhibitor, triggering significant reduced calcium response, indicated that LA as well as bitter agonists mobilized Ca^{2+} via a PLC-dependent mechanism. The concentration used is deliberately low

as, it has been shown that U73122 dose-dependently decreases $[Ca^{2+}]_i$ responses (Jan et al., 1998).

We wonder if fat taste receptors antagonist could disrupt bitter taste signal transduction. As expected, Ca^{2+} signalling evoked by LA was significantly curtailed in presence of sulfo-N-succinimidyl (SSO) and AH7614 (a GPR120 antagonist) (Ozdener et al., 2014). Surprisingly, we observed the same phenomenon with PTC, salicin and, to a lesser extent, sinigrin after a 20-minute precincubation with SSO and AH7614 suggesting that bitter agonists mobilized Ca^{2+} by interfering with Ca^{2+} signalling indirectly coupled to fat taste receptors.

Taken together, these findings suggest that fat and bitter taste share a common pathway. We can hypothesize that bitter and fat taste agonists bind to their cognate receptors and activate PLCβ2 that triggers IP_3 production that would lead to intracellular Ca^{2+} release, resulting ultimately in neurotransmitter release. It also emerged that bitter taste signalling interferes with CD36 and GPR120 signalling. Thereby, it appeared that only one pathway seems to exist in HTC-8 cells. These findings are contradictory with Hochheimer's data that indicated two signalling pathways coexist, a PLC-dependent and a PLC-independent Ca^{2+} signalling.

Anyway, these results highlight the cross-talk between fat and bitter taste signalling supporting the concept of "bitter-like fat taste". Nevertheless, this concept needs to be elucidated as it might involve other components.

HTC-8 cells express the PAV (Proline-Alanine-Valine) haplotype corresponding to the taster variant of TAS2R38 (Hochheimer et al., 2014) which has been shown to be associated with highest sensitivity to oleic acid and the highest responsiveness to PROP and generally found in PROP super-tasters (Melis et al., 2015a). In future, it would be interesting to test and compare fat and bitter taste signalling transduction in cells expressing the AVI variant of TAS2R38 linked to the lowest capability to orally detect oleic acid and with the lowest responsiveness to PROP and corresponding to the PROP non-taste status (Melis et al., 2015a).

Taken together, these results open new prospects. Indeed, polymorphism has been shown to be important in taste perception. Appropriate genetic screening for obesity including TAS2R38 SNP, could highlight different options to ameliorate chemosensory alterations including addition of simulated flavors to food to compensate for hyposensitization for fat taste.

- **Oxytocin involvement in fat taste modulation**

Due to the recent findings of the physiological role of oxytocin in feeding regulation and the benefits it presents as a use for the treatment of obesity, we chose to investigate the involvement of fat taste stimulation on oxytocin secretion.

This study also highlighted the important role of oxytocin in food behaviour. Oxytocin is not only delivered to taste buds via blood circulation (Sinclair et al., 2010) but is also produced locally as revealed by oxytocin released assay in HTC-8 cells. This is supported by the fact that oxytocin has been measured in the supernatant of HTC-8 cells. Moreover, we noticed that interestingly oxytocin released is more important when linoleic acid is incubated with SSO and AH7614 In this way, this phenomen could reveal desensitization to fatty acids at the TBC level and that would be responsible for initiating satiety. We can propose the following working model based on our results obtained with oxytocin studies (Figure 70).

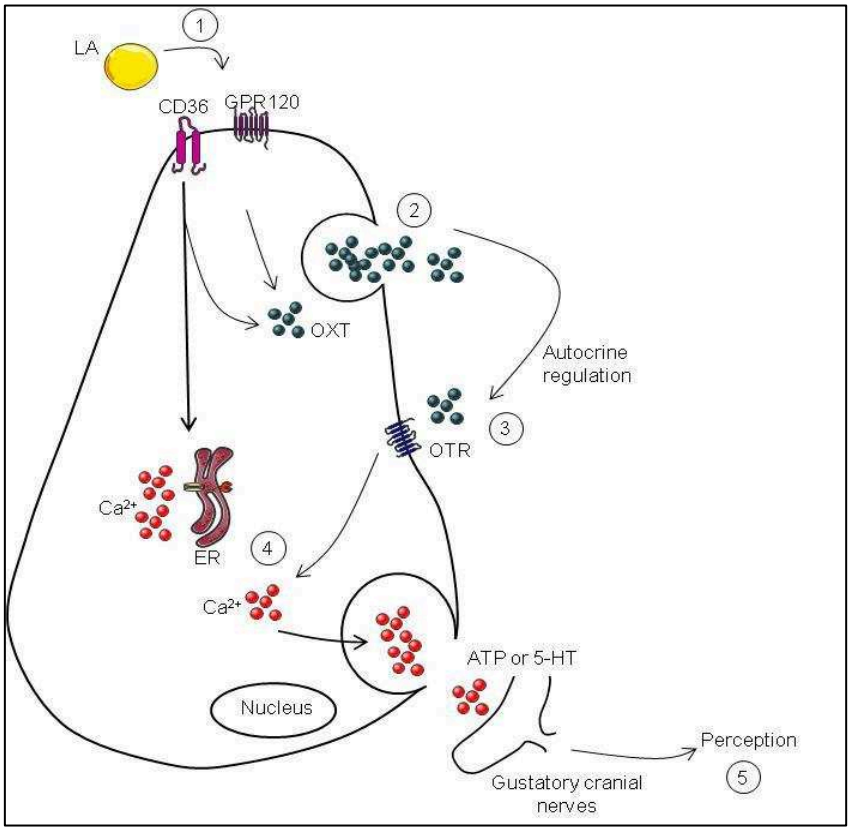

Figure 70. **Proposed model of the involvement of the oxytocin in TBC**. 1) LA bind to the lipid receptors inducing the downregulation of CD36 via the lipid rafts, 2) this desensitization leads to oxytocin release from the TBC in the extracellular environment, 3) the oxytocin released binds to the oxytocin receptors located at the cell membrane level of the TBC, 4) the binding induces the augmentation of the $[Ca^{2+}]_i$, 5) leading to the satiety perception. LCFA, long-chain fatty-acid; ER, endoplasmic reticulum; OXT, oxytocin receptor; OTR, oxytocin. Modified from Gilbertson and Khan (2014) including the obtained results on the oxytocin involvement in fat taste perception.

These findings identify OXT as an important central and peripheral regulator of energy homeostasis linked to lipid-rich food intake.

Furthermore, bariatric surgery triggers drastic changes in body weight and also drastic restoration of postprandial gut peptide release such as GLP-1, PYY and ghrelin (Barja-Fernández et al., 2015), parameters that are dysregulated in obese subjects (Meyer-Gerspach et al., 2014). It, therefore, appeared that influencing the release of hormones might provide a pharmacological alternative to invasive surgery. Avau et al. (2015) recently highlighted the therapeutic potential of targeting TAS2Rs to reduce body weight. They showed that α-gustducin-deficient mice (α-gust$^{-/-}$) mice exhibited lower weights than WT mice when fed a high fat diet (HFD). Moreover, white adipose tissue (WAT) mass was lower in α-gust$^{-/-}$ mice and correlated to an increase in brown adipose tissue (BAT) thermogenic activity. Interestingly, treatment with bitter agonists (denatonium benzoate or quinine) induced an α-gustducin-dependent decrease in body weight associated with decreased food intake. Treatment of pre-adipocytes with TAS2R agonists also decreased differenciation into mature adipocytes. Therefore, interfering with the gustatory signalling pathway using TAS2R agonists seems to protect against the development of HFD-induced obesity by directly affecting adipocyte metabolism.

Obesity is one major public health issues, and its prevalence is steadily increasing. Over the last decades, the role of dietary fatty acids has been largely studied especially their involvement as regulators of energy and lipid metabolism and their implication in human health. Hence the ability to detect fatty acids and the potential cross-talk between fat and bitter tastes remain an important issue to explore as it could constitute new anti-obesity strategies. Taken together, these results suggest the following integrative model including the the involvement triggered by TAS2R16 and TAS2R38, obtained from our studies on the potential cross-talk between fat and bitter taste modalities (Figure 71).

It could have application in the pharmaceutical industry (Schiffman, 2018) where numerous medicines present a stong bitter taste and are aversive to subjects mostly children and many adults. The elucidation of the cross-talk between the bitter and the fat taste could enhance drug acceptance and compliance in pediatric populations.

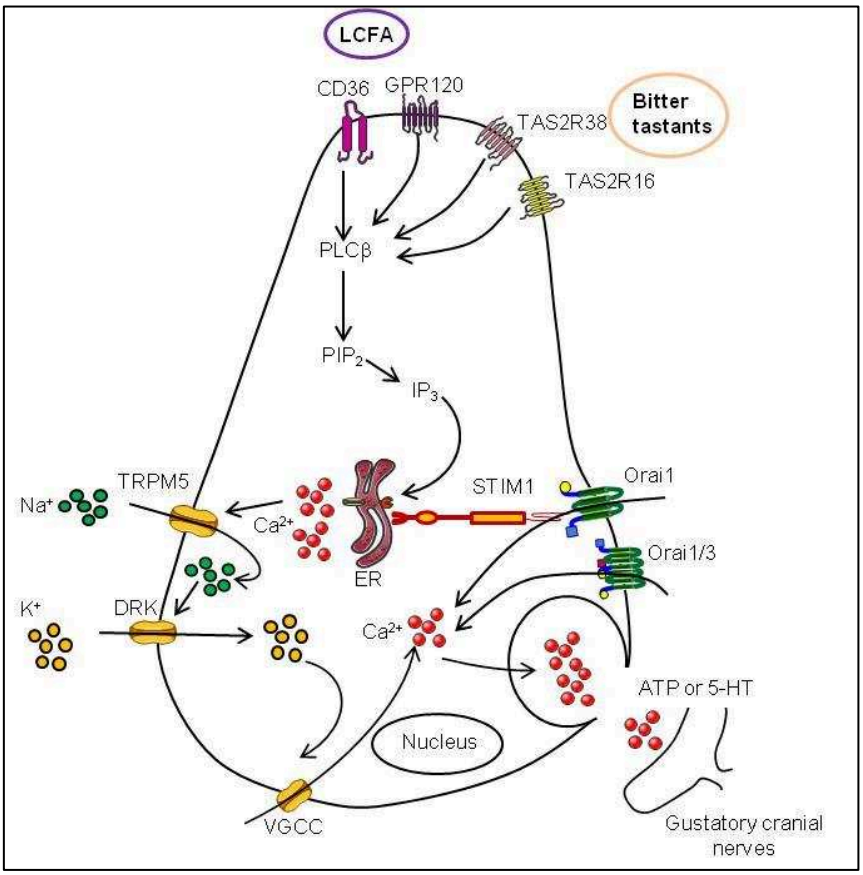

Figure 71. **Proposed model of the cross-talk between fat and bitter taste modalities in TBC.** LCFA and bitter agonists bind to their cognate receptors that triggers the activation of PLCβ leading to the production of IP3. The IP3 triggers the release of intracellular Ca^{2+} from the ER. The Ca^{2+} induces the release of ATP and 5-HT that constitute the feeding information and transit via the gustatory cranial nerves to the brain. LCFA, long-chain fatty-acid; PLCβ2, phospholipase Cβ2; PIP_2, Phosphatidylinositol 4,5-bisphosphate; IP_3, inositol trisphophate; ER,endoplasmic reticulum. Modified from Gilbertson and Khan (2014) including the obtained results on TAS2R16 and TAS2R38 and their potential interaction with CD36 and GPR120 and the Ca^{2+} signalling mechanism.

General discussion and perspectives

4 General discussion and perspectives

Taken together, these results suggest the following integrative model (Figure 72).

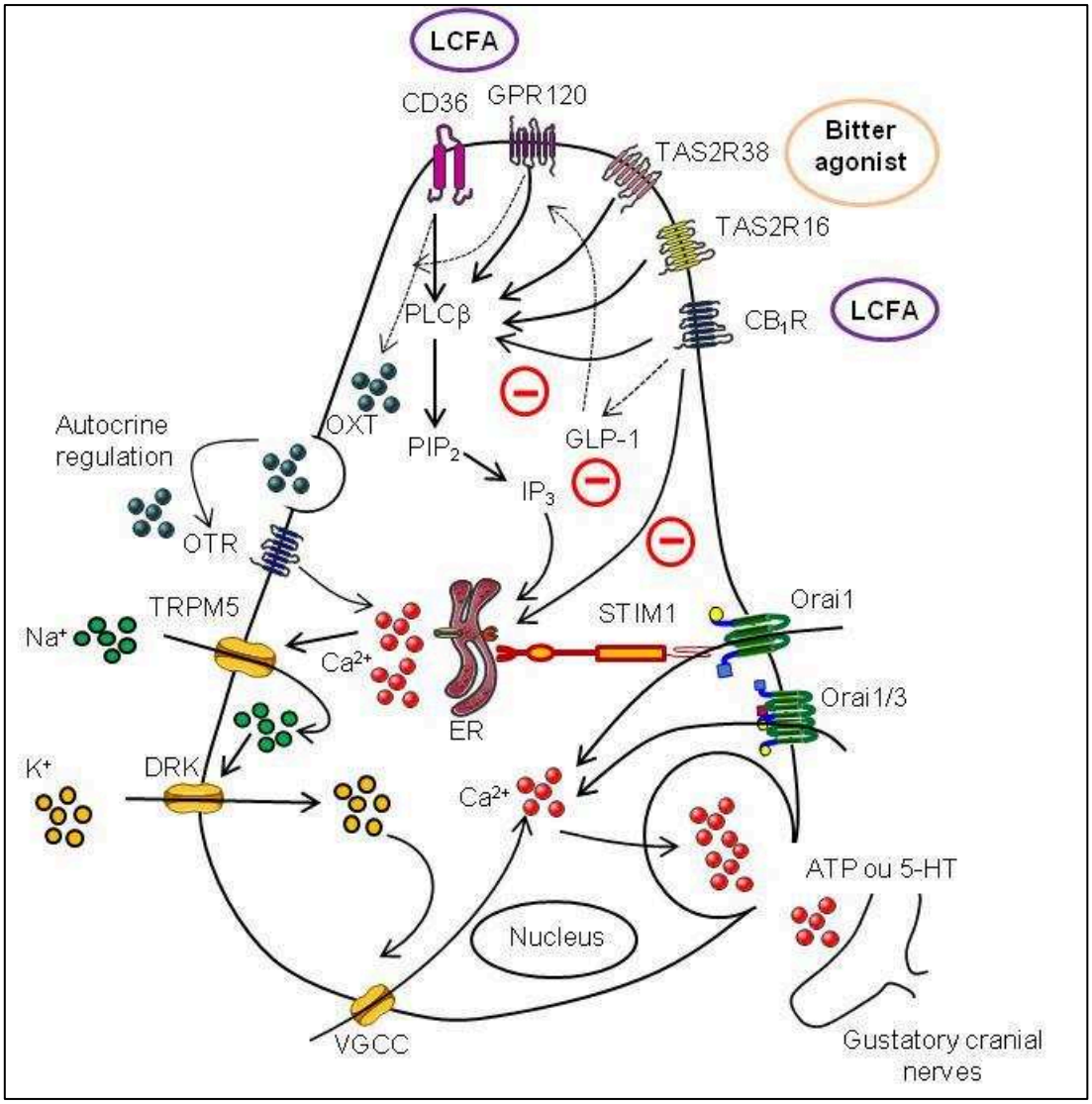

Figure 72. **Proposed general hypothesis of the signalling pathway evoked by LCFA and bitter agonists in TBC**. LCFA, long-chain fatty-acid; PLCβ2, phospholipase Cβ2; PIP_2, Phosphatidylinositol 4,5-bisphosphate; IP_3, Inositol trisphosphate; OTR, oxytocin receptor; OXT, oxytocin; ER, endoplasmic reticulum. The red signs show the inhibitory effect of CB_1R on fat taste signalling. Modified from Gilbertson and Khan (2014) including TAS2R16, TAS2R38 and CB_1R and their interaction with Ca^{2+} signalling mechanism.

Thus, we can propose the following hypothesis: an activation of the ECS coupled to an inhibition of the oxytocin could induce a CD36 upregulating at the cell membrane inducing appetite and activating the food intake. Then, LA triggers the downregulation of CD36 and the upregulation of GPR120 (Ozdener et al., 2014) that induce the release of GLP-1 and

oxytocin from the TBC. The activation of GPR120 and the inhibition of the ECS, i.e., the potential internalisation of CB_1R via the lipid rafts would lead to the satiaty state.

In conclusion, both the ECS and the bitter taste status modify the fat taste signalling pathway modulating the Ca^{2+} response that triggers information to the brain and finally leads to food intake behaviour. To confirm our results, the same methodology could be performed in mouse and human TBC as mouse TBC also respond to linoleic acid, sinigrin, PTC and salicin (Figure 73) and HTC-8 cells respond to AEA and ACEA (Figure 74).

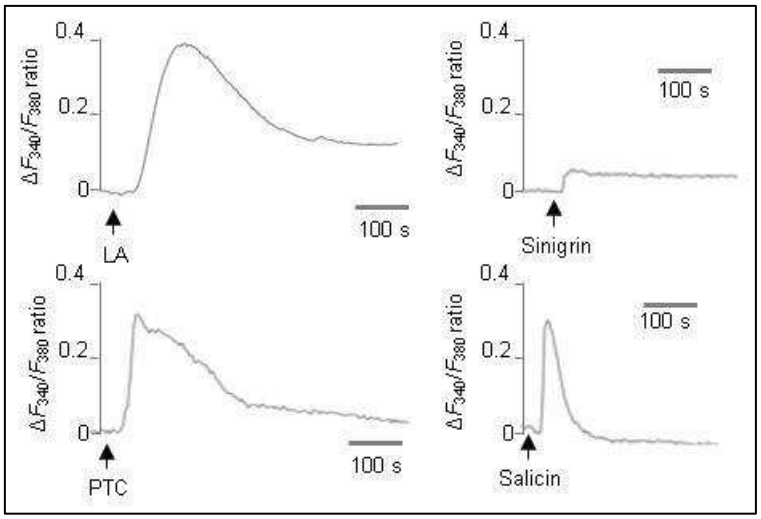

Figure 73. **LA, sinigrin, PTC and salicin induce increases in $[Ca^{2+}]_i$ in mouse fungiform cell line.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. The kinetics of the rise in $[Ca^{2+}]_i$ levels in mouse fungiform cell line following addition of LA (100 µM), sinigrin (1 mM), PTC (10 mM) and salicin (25 mM). Values are expressed as mean ± SEM (n=5).

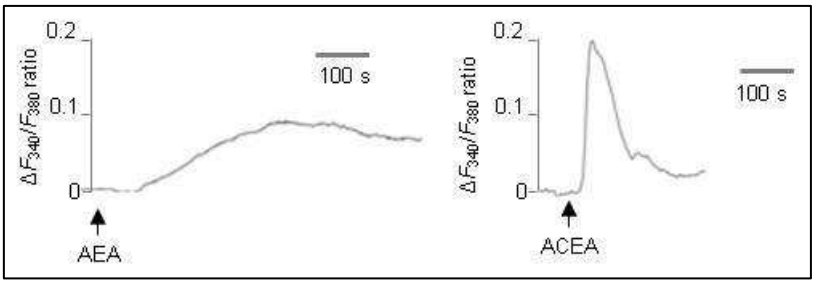

Figure 74. **AEA and ACEA induce increases in $[Ca^{2+}]_i$ in HTC-8 cells.** Ca^{2+} imaging studies were performed in calcium-containing (100% Ca^{2+}) buffer. The changes in free intracellular Ca^{2+} concentrations ($\Delta F_{340}/F_{380}$) were monitored under the Nikon microscope (TiU) by using S-fluor 40x oil immersion objectives. The kinetics of the rise in $[Ca^{2+}]_i$ levels in mouse fungiform cell line following addition of AEA (5 µM) and ACEA (1.5 µM). Values are expressed as mean ± SEM (n=5).

However, these studies have some limitations. Indeed, regarding the CB_1R study, the experiment should be repeated on a greater population including female mice and on human

subjects. About the cross-talk study, behavioral testes should be assessed on mice to ensure the validity of the obtained results.

Bari et al. (2008) reported that CB_1R functions in the context of lipid rafts. Thus, it would be interesting to explore the CB_1R trafficking during the different phases of the eating behaviour considering in the same time the GLP-1 and the oxytocin release variations. Finally, as a cross-talk is likely to occur between fat and bitter taste modalities, it would be relevant to study the existence of a similar cross-talk between sweet and bitter or salty and bitter as suggested by (Khan et al., 2018b).

These results also clearly strengthen the existence of a cross-talk between two taste modalities, here between fat and bitter tastes, that seems to occur at the Ca^{2+} signalling level. It opens the perspective of new anti-obesity strategies such as personalized considering sensitivity threshold of overweigh or cancer patients.

Thus, we could imagine new therapeutic care using molecules modulating the CB_1R activity and also bitter and fat taste receptors resulting in enhancing or weakening food intake effects on the reward circuitry. This therapy could, consequently, trigger the hormone release allowing the integration of anorexigenic affects of GLP-1 and the oxytocin.

Résumé en français

La gustation est une des composantes permettant la perception orosensorielle des aliments et de leur composition. Celle-ci joue un rôle crucial dans les choix alimentaires. L'homme possède un système du goût très performant lui permettant de distinguer les aliments nutritifs des aliments potentiellement toxiques (Jyotaki et al., 2010).

La perception du goût permet l'apport d'informations sur la qualité de la nourriture ingérée par l'organisme. Les signaux générés dans la cavité buccale vont transmettre l'information gustative à l'organisme via le système nerveux central. La cavité orale joue donc un rôle significatif dans le comportement alimentaire.

Cinq goûts ont été identifiés : sucré, amer, acide, salé et umami. Pour autant, depuis peu, la perception du gras est proposée comme une saveur à part entière (Laugerette et al., 2005 ; Khan et Besnard, 2009). Par ailleurs, selon l'Organisation Mondiale de la Santé (OMS), la prévalence de l'obésité a pris des proportions telles qu'elle est, désormais, considérée comme une épidémie mondiale. Or, il a été démontré un lien entre le risque d'obésité et la consommation chronique des graisses alimentaires (Alvheim et al., 2012). C'est donc dans ce contexte que comprendre les mécanismes en jeu dans la perception du gras relève d'une importance capitale.

1. Perception gustative

1.1. Généralités

La perception gustative relaie l'information déclenchée par les aliments ingérés de la cavité buccale au reste de l'organisme. Elle a lieu dans les bourgeons du goût inclus dans les papilles et situés, en grande majorité, dans l'épithélium dorsal de la langue. On dénombre 3 grands types de papilles : les papilles fongiformes, foliées et circumvallates (ou caliciformes) (Figure 70). Le bourgeon du goût possède de 30 à 100 cellules gustatives. Il existe principalement 3 types cellulaires dans les bourgeons du goût : les **cellules de type I** qui sont qualifiées, de par leurs propriétés, de « glial-like cells » ; les **cellules de type II**, correspondant aux cellules réceptrices du goût, qui expriment PLCβ2 (Phospholipase C β2) et les canaux TRPM5 (Transient receptor potential cation channel subfamily M member 5), elles sont responsables de la libération des neurotransmetteurs, et les **cellules de type III** qui sont des cellules pré-synaptiques (Chaudhari et Roper, 2010).

La liaison d'une molécule sapide avec son récepteur conduit à des changements du potentiel membranaire et à une augmentation intracellulaire de la concentration en calcium libre, $[Ca^{2+}]_i$. Cette dépolarisation de la cellule gustative déclenche alors la libération de neurotransmetteurs (El Yassimi et al., 2008). Les signaux gustatifs de la cavité orale sont alors

transmis vers la branche du nerf crânien VII (la chorde du tympan du nerf crânien) et IX (la branche linguale du glossopharyngien) jusqu'au noyau du tractus solitaire (NTS) (Gaillard et al., 2008). Le NTS, quant à lui, est connecté à différentes zones du cerveau impliquées dans la prise alimentaire, la récompense, la mémorisation et le tractus digestif (Besnard et al., 2010). Ce mécanisme permet une intégration nerveuse et déclenche une réponse comportementale et métabolique (Figure 75).

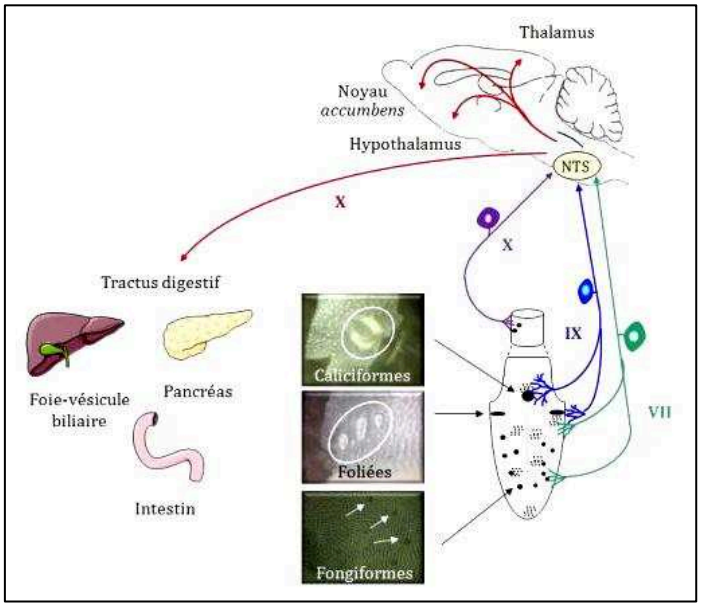

Figure 75 : **Voies nerveuses impliquées dans la perception gustative**. D'après Martin et al., 2010.
VII chorde du tympan, IX nerf glossopharyngien, NTS Noyau du tractus solitaire.

1.2. Perception orosensorielle des lipides alimentaires

Des travaux récents ont proposé qu'il existe un 6^{e} goût consacré à la perception des lipides (Laugerette et al., 2005 ; Khan et Besnard, 2009). Pour définir une saveur comme un goût primaire, il faut que celle-ci réponde à 5 critères : une classe unique de molécules sapides, un système de réception spécifique, une implication des voies gustatives, une perception gustative d'une sensation unique et une réponse physiologique. Le système de perception des lipides semble ainsi répondre à ces conditions (Gilbertson et Khan, 2014).

1.2.1 Récepteur CD36

CD36, aussi connu sous le nom de fatty acid translocase (FAT), est une glycoprotéine membranaire appartenant à la famille des récepteurs scavengers. Elle est capable de lier différents ligands dont les acides gras (Gilbertson et Khan, 2014). En outre, CD36 possède une haute affinité, de l'ordre du nanomolaire, pour les acides gras.

Récemment, Laugerette et al. (2005) ont démontré l'implication de CD36 dans la perception des lipides chez la souris. En effet, l'inactivation du gène CD36 abolit complètement la

préférence aux acides gras à longue chaîne (AGLC). Cette observation a été confirmée depuis par d'autres équipes (Sclafani et al., 2007). De plus, cet effet serait lipide-spécifique, la préférence au sucré et l'aversion à l'amer n'étant pas affectées chez les souris transgéniques n'exprimant pas le gène CD36 (Laugerette et al., 2005).

Le récepteur CD36 est fortement exprimé dans les papilles circumvallates des rongeurs (Laugerette et al., 2005). Sa distribution apicale au niveau de l'épithélium lingual est adéquate pour la génération d'un signal lipidique par les bourgeons du goût. En effet, CD36 étant situé au niveau de ces derniers, il est directement exposé à un microclimat potentiellement riche en AGLC (Khan et Besnard, 2009) : les lipases linguales, libérées par les glandes de Von Ebner dans la fente des papilles circumvallates, dégradent les triglycérides (qui constituent la majorité des lipides d'origine alimentaire) en AGLC, les ligands de CD36 (Figure 76).

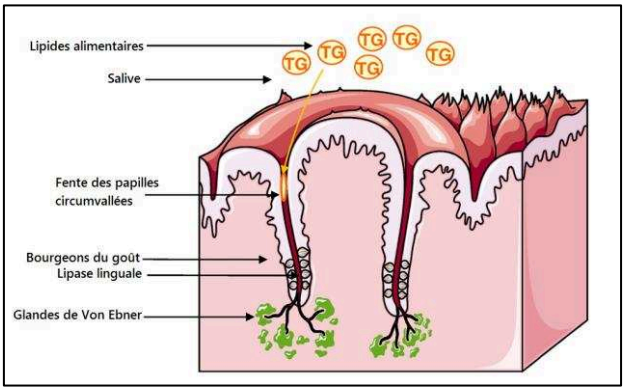

Figure 76 : **Localisation des glandes de Von Ebner**. D'après Khan et Besnard, 2009.

1.2.2 Récepteur GPR120

Les G protein-coupled receptors (GPCR) sont une famille de récepteurs qui répondent à des ligands variés tels que les photons, les odeurs, les phéromones ou les acides aminés. GPR120, un récepteur de la famille GPCR, est capable d'être activé par les AGLC (Gilbertson et Khan, 2014).

GPR120, exprimé dans les cellules entéroendocrines, est aussi présent au niveau de l'épithélium lingual où il jouerait un rôle dans la détection orale des lipides chez la souris. Pour autant, son rôle en tant que récepteur des lipides alimentaires est moins clair. Il a été démontré que GPR120 et CD36 possèdent des rôles bien distincts dans la perception orogustative des lipides alimentaires et qu'ils sont régulés différemment par l'obésité (Ozdener et al., 2014). En effet, CD36 est impliqué dans la perception précoce des lipides alimentaires alors que GPR120 serait impliqué dans la perception et la régulation du rassasiement.

1.3. Mécanisme de signalisation

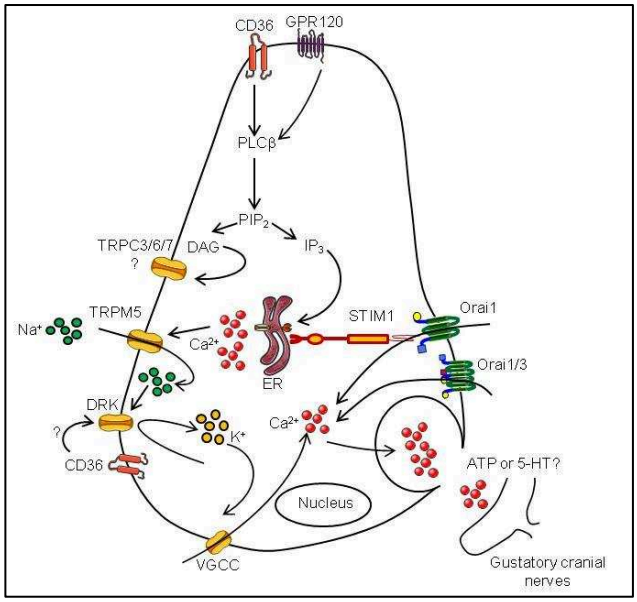

Figure 77 : **Mécanismes d'action des AGLC via CD36 et GPR120 dans une cellule du bourgeon du goût.** D'après Gilbertson et Khan (2014).

La liaison d'AGLC au CD36 linguale et/ou au GPR120 déclenche une cascade de signalisation. L'activation du récepteur conduit à l'activation de la cascade PLCβ-IP_3 (phospholipase Cβ- inositol triphosphate) qui aboutit à la libération de Ca^{2+} par le réticulum endoplasmique (ER). L'augmentation de $[Ca^{2+}]_i$ va alors activer les canaux TRPM5 (Transient receptor potential cation channel subfamily M member 5), responsables de l'influx sodique Na^+. Cet influx conduit à l'activation des canaux DRK (delayed-rectifying potassium channel), responsables de la repolarisation de la cellule via l'efflux de K^+. Ceux-ci seront bloqués par la liaison des AGLC à CD36 lors de l'activation soutenue du bourgeon du goût. L'augmentation massive de $[Ca^{2+}]$ dans la cellule, via les canaux calciques Orai1 et Orai1/3 dits SOC (store operated calcium channels) et les canaux calciques voltage-dépendants (VGCC) activés suite au blocage des canaux DRK, va déclencher l'exocytose de neurotransmetteurs (sérotonine) qui est responsable de la transmission du signal au cerveau via les fibres nerveuses afférentes (Dramane et al., 2012 ; Gilbertson et Khan, 2014) (Figure 77).

2. Détection orosensorielle des composés amers

2.1. Agonistes du goût amer

La capacité de détecter l'amertume est liée à une fonction critique de survie permettant aux animaux de distinguer des aliments potentiellement nocifs. Grâce à sa valeur hédonique négative innée (Steiner et al., 2001), la perception de l'amertume décourage l'alimentation et les différences de sensibilité au goût amer influencent le comportement alimentaire humain. La perception du goût amer est contrôlée par la famille de gènes du récepteur du goût 2 (TAS2R). Il existe 25 gènes TAS2R humains (Drayna, 2005) avec de nombreux polymorphismes associés et un large éventail de différences individuelles dans la sensibilité au goût amer (Chandrashekar et al., 2000). Cela explique en partie pourquoi des milliers

d'agonistes chimiquement dissemblables peuvent déclencher une qualité gustative unique. Les récepteurs les plus étudiés sont TAS2R16 et TAS2R38.

Fox (1932) a découvert fortuitement que la concentration de phénylthiocarbamide (PTC) avait un goût extrêmement amer pour certaines personnes et était pratiquement sans goût pour les autres. En effet, la sensibilité à la PTC, composé contenant un groupe -N-C = S, est utilisée comme marqueur phénotypique pour les sensations orales (Tepper, 2008).

2.2. Transduction du signal via l'amer dans les cellules des bourgeons du goût

Parmi la famille de gènes TAS2R, le gène TAS2R38 est l'un des plus largement étudiés. TAS2R38 code pour un récepteur de sept protéines transmembranaires G (Drayna, 2005). Le récepteur est impliqué dans la liaison PTC et 6-n-propylthiouracile (PROP) (Kim et Drayna, 2005).

La génération du goût amer commence lorsqu'un composé amer pénètre dans la cavité buccale. Le ligand se lie à un récepteur TAS2R exprimé dans la membrane apicale des cellules réceptrices des papilles gustatives. Elle déclenche une cascade d'événements de signalisation impliquant l'activation de l'α-gustducine, du PLCβ2 et de l'inositol trisphosphate (Zhang et al., 2003b), conduisant finalement à la libération de neurotransmetteurs activant une fibre nerveuse afférente transmettant le signal via le nerf crânien. au cerveau. Les signaux gustatifs traversent le cerveau et transmettent les informations aux circuits impliqués dans diverses fonctions, telles que les réflexes physiologiques, la perception discriminatoire et le traitement affectif. Ce système illustre la complexité des mécanismes intervenant entre l'application du stimulus amer et la génération de la réponse comportementale.

3. Comportement alimentaire et système endocannabinoïde

3.1. Système endocannabinoïde

Le système endocannabinoïde comprend des agonistes endogènes (les endocannabinoïdes) et des récepteurs responsables de leurs actions : CB_1R et CB_2R, ainsi que les systèmes enzymatiques qui catalysent leur biosynthèse et dégradation (DiPatrizio et al., 2013).

L'établissement de ce système fait suite à la découverte du Δ-9-tétrahydrocannabinol (Δ^9THC) par Raphael Mechoulam en 1964 (Gaoni et Mechoulam, 1964). Le Δ^9THC est le composé le plus actif produit par la plante Cannabis sativa. Ainsi, les récepteurs cannabinoïdes ont été identifiés : CB_1R et CB_2R. CB_1R est largement exprimé dans le système

nerveux central et périphérique surtout au niveau des structures limbiques (hypothalamus) et du noyau accumbens (Moldrich et Wenger, 2000). Ils ont aussi été détectés dans les bourgeons du goût (Yoshida et al., 2010). CB_2R, quant à lui, est essentiellement exprimé dans les cellules du système immunitaire.

L'anandamide et le 2-arachidonylglycerol (2-AG) ont été identifiés comme les deux endocannabinoïdes endogènes majeurs agissant sur les récepteurs CB_1 et CB_2.

3.2. Cannabinoïdes et prise alimentaire

Il est connu depuis les années 70 que le cannabis augmente l'appétit et la consommation alimentaire, particulièrement des aliments palatables, chez l'homme (Abel, 1975). Plusieurs études se sont alors penchées sur les endocannabinoïdes. Il en résulte que l'administration exogène de cannabinoïdes (anandamide) induit une hyperphagie et une préférence pour les substances palatables (Williams et Kirkham, 1999). Les endocannabinoïdes sont donc des médiateurs orexigènes. De plus, l'utilisation d'antagonistes des récepteurs CB_1 (tel que le rimonabant SR-14716A) réduit la prise alimentaire (Arnone et al., 1997). Les récepteurs CB_1 semblent donc être impliqués dans la prise alimentaire. Les antagonistes des endocannabinoïdes ont alors été proposés pour le traitement de l'obésité (Berry et Mechoulam, 2002).

Ces résultats ont mené à l'hypothèse suivant laquelle le système endocannabinoïde endogène jouerait un rôle dans la régulation de l'appétit et de la prise alimentaire. De plus, il serait aussi impliqué dans le processus de récompense qui provoque la motivation et la valeur hédonique des aliments (Kirkham, 2003). En effet, les récepteurs CB_1 sont exprimés dans le noyau accumbens et dans l'hypothalamus, régions appartenant au circuit de la récompense.

Le système endocannabinoïde est donc un régulateur clé de la prise alimentaire palatable (DiPatrizio et Piomelli, 2012). En effet, les endocannabinoïdes induiraient des modifications du comportement alimentaire via la sensation de la palatabilité des composants alimentaires (Yoshida et al., 2010). Ainsi, le système endocannabinoïde pourrait être un composant critique du mécanisme de feed-back positif requis pour maintenir la prise alimentaire grasse (DiPatrizio et al., 2013).

4. Comportement alimentaire et SNP

Les modalités gustatives pourraient interagir entre elles et avec d'autres sens, et ces interactions pourraient être critiques dans l'obésité (Khan et al., 2018). Une relation entre le goût gras et amer a été récemment proposée. En effet, certains rapports suggèrent qu'il existe

une préférence plus élevée pour les graisses alimentaires chez les perconnes qui ne détectent pas PROP (non-tasters) au cours du test PROP (Karmous et al., 2018; Tepper et Nurse, 1997; Tepper et al., 2008). De plus, Melis et al. (2015a) ont montré qu'il existe une association entre la perception orosensorielle de l'acide oléique, le SNP rs1761667 dans le gène CD36 et le statut de « taster » de PROP. Ces études ont également montré que les sujets non-tasters de l'amer présentaient également une perception réduite des graisses orales, induisant une consommation d'aliments riches en graisses et, par conséquent, un risque élevé d'obésité (Keller, 2012). Notre équipe a montré que les génotypes CD36 rs1761667 et TAS2R38 rs1726866 et rs10246939 étaient associés à des seuils de détection de PROP plus élevés et à un IMC plus élevé chez les sujets obèses que les participants de poids normal. Ces observations ont révélé l'existence d'une association entre le seuil de détection de PROP et les génotypes CD36 et TAS2R38 (Khan et al., 2018).

Dans le présent rapport, nous avons étudié la relation entre le goût gras et amer en étudiant les mécanismes de signalisation en aval dans les cellules fongiformes immortalisées par l'homme, HTC-8. Des individus non tasters ont été signalés comme ayant une capacité réduite à distinguer la teneur en matières grasses dans les aliments (Hayes et Duffy, 2007). En effet, les non-tasters pour l'amer semblaient incapables de distinguer la teneur en graisse des vinaigrettes italiennes riches en matières grasses et des acides gras dans les aliments riches en graisses (Nasser et al., 2001; Tepper et Nurse, 1998). Par conséquent, ces sujets consomment plus de graisses alimentaires et d'aliments riches en énergie que les tasters (Keller et Tepper, 2004; Tepper et al., 2011). Considérant que les « supertasters » de PROP peuvent éviter les aliments riches en graisses en raison de leurs sensations intenses (Duffy et Bartoshuk, 2000), Melis et al. (2015a) ont montré pour la première fois une corrélation entre le SNP CD36 et la diminution de la perception du goût gras observée chez les sujets non dégustateurs de PROP. Il est apparu que les sujets présentaient le phénotype AVI / AVI TAS2R38 homozygote pour l'allèle G du rs171667 pour le CD36, la méthode de détection de l'acide oléique étant 11 fois plus faible que pour les sujets du génotype AVI / AVI TAS2R38 mais avec un génotype AA homozygote polymorphisme. Il est également apparu que les non-dégustateurs de PROP avaient la même résistance à l'acide oléique que les super-dégustateurs. Ils ont observé que le génotype AA de rs1761667 présentait un IMC accru et une sensibilité réduite à la perception des graisses et du goût amer.

Plusieurs études ont montré que les non-tasters de PROP présentent une densité de papilles fongiformes inférieure à celle des tasters de PROP (Melis et al., 2013b; Shahbake et al., 2005;

Yeomans et al., 2009). Ebba et al. (2012) ont émis l'hypothèse que la capacité accrue de dégustation de l'acide linoléique chez les tasters de PROP, par rapport aux non-tasters, pourrait être attribuée à la différence de densité des papilles fongiformes entre ces deux groupes. En effet, nos données ont montré une densité plus faible de papilles fongiformes sur la partie antérieure de la langue des non-dégustateurs PROP, qui présentent également une capacité plus faible à détecter l'acide oléique que les tasters PROP. Cette découverte suggère qu'un taux élevé d'expression de CD36 dans les cellules gustatives semble être un facteur déterminant pour la détection des graisses alimentaires uniquement chez les sujets ayant une faible densité de papilles gustatives (Melis et al., 2015a). Récemment, des enquêtes sur l'association du seuil de détection de PROP aux génotypes CD36 et TAS2R38 dans l'obésité dans une population tunisienne (Karmous et al., 2018), il est apparu que la méthode de détection de PROP était associée à l'IMC.

Aux niveaux cellulaire et moléculaire, le goût gras, à savoir CD36 et GPR120, et le goût amer, à savoir TAS2R16 et TAS2R38, sont exprimés dans les cellules de type II. Ainsi, les deux voies pourraient activer l'α-gustducine pour les récepteurs de signalisation en aval (Roper et Chaudhari, 2017), puis l'activation de PLCβ2 impliquée dans l'hydrolyse du phosphatidylinositol4,5-bisphosphate (PIP_2) en inositol trisphosphate (IP_3). Ensuite, IP_3 se lie à son récepteur IP_3R sur le RE et déclenche la libération de Ca^{2+}. Ainsi, Khan et al. (2018) ont déclaré que le mécanisme commun est la «signalisation du Ca^{2+}» entre la perception du goût amer et le goût de la graisse. L'élucidation d'un dialogue entre les modalités du gras et du goût amer pourrait trouver des applications dans l'industrie alimentaire, le traitement de l'obésité et l'industrie pharmaceutique pour réduire l'amertume des médicaments chez les enfants en particulier (Mennella et al., 2013). Keller (2012) a suggéré que les récepteurs de graisse et de goût amer pourraient jouer des rôles différents. En effet, TAS2R38 serait impliqué dans la perception de la texture des graisses alimentaires en fonction de l'état de du test au PROP et le CD36 influencerait la perception chimiosensorielle des AGLC.

Hypothèses de travail :
Implication de CB_1R dans la perception des acides gras alimentaires

Les proportions d'acide linoléique (18: 2) ont considérablement augmenté dans le régime alimentaire occidental depuis le vingtième siècle, passant de 1 à 8% de l'apport énergétique total. Cette augmentation est corrélée à l'augmentation de la prévalence de l'obésité au cours de cette même période (Alvheim et al., 2012). Récemment, l'hypothèse d'une préférence spontanée pour les lipides associés au goût des graisses a émergé et pourrait expliquer l'aspect comportemental lié à l'obésité. Yoshida et al. (2010) ont testé les préférences alimentaires des souris de type sauvage et $CB_1R^{-/-}$ traitées avec un agoniste de l'endocannabinoïde, 2-AG. En conséquence, seule la consommation de solutions de sucre a été augmentée chez les souris sauvages traitées avec 2-AG. La consommation de solutions amères, salées, acides et umami n'a pas été affectée par le traitement. Aucun changement de consommation n'a été observé chez les souris $CB_1R^{-/-}$ (Yoshida et al., 2010). Cette étude démontre une modification de la perception de l'appétence par les endocannabinoïdes via les récepteurs CB_1R. Étant donné que l'excès de prise alimentaire en lipides est associé à l'obésité et que le système endocannabinoïde via les récepteurs CB_1R améliore la prise alimentaire (perception de la douceur en particulier), nous proposons d'étudier le rôle du récepteur CB_1 dans la préférence des lipides alimentaires. L'implication du système endocannabinoïde dans la perception gustative des lipides alimentaires via le CB_1R lingual n'a pas encore encore été étudiée. Nous proposons d'étudier ce rôle potentiel chez la souris via des tests de comportements, des études biochimiques et du signal calcique. Nous émettons l'hypothèse que le système endocannabinoïde, via le CB_1R lingual, affecte la détection du goût du gras dès la cavité buccale.

Le projet vise à élucider les tâches suivantes:

1) Etudier les conséquences de la délétion de Cb1r chez les souris invalidées pour le gène ($CB_1R^{-/-}$) sur leur perception des lipides alimentaires.
2) Etudier la conséquence de la délétion du gène sur la régulation des protéines CD36 et GPR120.
3) Etudier les mécanismes de signalisation cellulaire, via les lipides, des récepteurs CD36 et GPR120 dans les cellules gustatives des souris $CB_1R^{-/-}$.
4) Etudier les conséquences de la délétion du gène sur la sécrétion de GLP-1.

Ceci permettrait la synthèse « d'agonistes inverses », agonistes pour CD36 et GPR120 et antagoniste de CB_1R pouvant alors intervenir dans le traitement de l'obésité.

Résultats et Discussion

Williams et Kirkham (1999) ont démontré que CB_1R est responsable de l'augmentation de l'apport alimentaire, induite par un agoniste des endocannabinoïdes. Plus tard, Yoshida et al. (2010) ont révélé que les endocannabinoïdes amélioraient les réponses gustatives aux goûts sucrés via CB_1R. En effet, l'activation du système endocannabinoïde (ECS) semble être associée à une hyperphagie et à une préférence pour les aliments palatables. Fait intéressant, CB_1R est également exprimé dans un sous-ensemble de cellules de papilles gustatives (Yoshida et al., 2010). Nous rapportons ici que les souris $CB_1R^{-/-}$ ne présentaient aucune préférence pour les solutions lipidiques par rapport aux souris WT. Le même comportement a également été observé lorsque les souris WT ont été traitées avec le rimonabant, un bloqueur CB_1R, confirmant le rôle de ce récepteur dans la détection des lipides alimentaires. Nous avons employé l'acide linoléique (AL) comme candidat pour les acides gras à longue chaîne (AGLC), car cet acide gras est abondamment présent dans les aliments occidentaux; cependant, il est possible que les acides gras saturés tels que l'acide palmitique (AP) puissent également déclencher la même réponse gustative. En effet, nous avons montré précédemment que AL et AP provoquaient les mêmes augmentations de $[Ca^{2+}]_i$ dans les cellules des papilles chez la souris (Gaillard et al., 2008).

Dans la présente étude, pour les expériences comportementales, nous avons utilisé des souris knock-out pour CB_1R et il est possible que le système cannabinoïde hypothalamique, via la zone dopaminergique, soit impliqué dans la préférence gustative (Melis et al., 2013). Néanmoins, nous avons cherché à élucider les mécanismes cellulaires dans la modulation de la préférence en matière de graisse. Nous avons tout d'abord testé l'hypothèse d'une altération des protéines CD36 et GPR120 dans les TBC de souris $CB_1R^{-/-}$. Dans notre étude, les expressions des protéines CD36 et GPR120 n'ont pas été altérées par l'absence de CB_1R, ce qui suggère que l'absence de préférence pour les solutions lipidiques peut être due à une signalisation en aval altérée. De plus, nous avons vérifié la délivrance de l'acide linoléique dans les deux conditions et nous avons observé une absorption identique des acides gras exogènes.

Des études antérieures ont indiqué que l'activation de CD36 et GPR120 par un AGLC a déclenché la mobilisation de $[Ca^{2+}]_i$ à partir du pool intracellulaire de réticulum endoplasmique Ca^{2+} pendant la perception du goût gras (Galindo et al., 2012; Ozdener et al., 2014). Dans notre étude, nous montrons, pour la première fois, que l'augmentation induite par

AL dans $[Ca^{2+}]_i$ a été altérée lorsque CB_1R a été inactivé par le rimonabant ou par l'absence de CB_1R. En outre, l'ACEA agoniste CB_1R a également augmenté le flux de calcium dans les TBC, mais avec une puissance inférieure à celle d'AL. Cependant, l'effet de l'ACEA a été maintenu dans les TBC des souris $CB_1R^{-/-}$, augmentant la possibilité que l'augmentation de $[Ca^{2+}]_i$ puisse être médiée par d'autres récepteurs que CB_1R, par exemple TRPV1. En effet, il a été montré que l'activation de TRPV1 par les endocannabinoïdes induit la signalisation du calcium (Kentish et Page, 2015; Ryskamp et al., 2014). En outre, le blocage de TRPV1 avec A784168 a totalement supprimé la réponse $[Ca^{2+}]_i$ induite par les ACEA, indiquant que le signal calcique résiduel observé dans $CB_1R^{-/-}$ TBC avec ACEA pourrait être dû à l'activité TRPV1. En outre, il semble que la signalisation en aval couplée à CB_1R soit dépendante du PLC, conformément aux observations de De Petrocellis et al. (2007). Cependant, il reste à élucider si l'anandamide, utilisé dans la présente étude, active la sous-unité $G_{\beta\delta}$ de CB_1R et active le PLC via la voie PI-3-kinase. Dans l'ensemble, nos données indiquent que CB_1R pourrait jouer un rôle crucial dans la perception du goût du gras en modulant la signalisation du calcium.

Comme décrit précédemment, les souris GLP-$1^{-/-}$ ont des réponses gustatives réduites aux graisses alimentaires, ce qui suggère que la détection orosensorielle des AGLC pourrait être associée à la sécrétion de GLP-1 lingual (Martin et al., 2012). Les données rapportées ici ont montré que la sécrétion de GLP-1 active induite par AL est fortement diminuée chez les souris $CB_1R^{-/-}$, ce qui suggère l'existence d'un lien entre la signalisation CB_1R et la production de GLP-1. Par conséquent, l'activation de CB_1R peut stimuler la production de proglucagon et de GLP-1r et, ainsi, moduler le seuil de perception des AGLC. Des investigations supplémentaires sont nécessaires pour explorer la possibilité que la sécrétion de GLP-1 soit stimulée par la signalisation $[Ca^{2+}]_i$ dans les TBC ou par d'autres mécanismes (Takai et al., 2015).

En conclusion, le présent rapport montre que CB_1R influence la perception du goût des graisses via la régulation de la signalisation calcique dans les TBC. Il est proposé que l'activation de CB_1R induise une réponse $[Ca^{2+}]_i$ qui renforce la perception des lipides, médiée par le CD36. L'activation de l'ECS pourrait, de ce fait, augmenter les stimuli sensoriels relayant l'appétence des aliments et, en fin de compte, stimuler la prise alimentaire. La pertinence physiopathologique d'une telle voie de régulation est étayée par le fait que le tonus de l'ECS augmente dans l'obésité. Par conséquent, l'ECS semble émerger comme un modulateur clé de la détection orale des sucres et des graisses et pourrait représenter une cible

potentielle pour développer de nouvelles stratégies anti-obésité ou, inversement, augmenter la prise alimentaire en cas de perte d'appétit dans la cachexie.

Hypothèse de travail :

Communication bidirectionnelle entre les modalités gustatives amères et lipidiques dans les cellules gustatives humaines

L'obésité constitue l'un des principaux problèmes de santé publique en ce début du 21ème siècle. Sa prévalence augmente régulièrement, en particulier chez les enfants. Ce constat n'est pas anodin car l'obésité est généralement associée à diverses pathologies graves (diabète de type 2, hypertension et cancer,…). L'abondance alimentaire contribue grandement à ce phénomène. En effet, dans les pays occidentaux, les lipides alimentaires représentent près de 40% de l'apport calorique quotidien alors que les recommandations nutritionnelles sont inférieures de 5 à 10%. Le goût amer est détecté par le récepteur lingual T2R38. Il a été proposé que les non-dégustateurs d'acides gras ne soient pas non-tasters du goût amer. En outre, le polymorphisme du gène CD36 entraîne une diminution de l'expression des protéines et, par conséquent, une diminution de la détection oro-sensorielle du goût des graisses. Certains sujets qui expriment le polymorphisme du CD36 présentent également une augmentation du seuil de détection de l'amertume. Ainsi, il pourrait exister une interaction entre les deux modalités gustatives, à savoir les goûts gras et amers. Par conséquent, la capacité à détecter les acides gras et le dialogue possible entre les goûts gras et amer restent un sujet important à explorer car cela pourrait constituer de nouvelles stratégies anti-obésité. Nous avons émis l'hypothèse que le goût amer et le goût gras partagent un mécanisme de signalisation en aval commun. Nous avons étudié la relation entre le goût amer et le gras et le gras en étudiant les mécanismes de signalisation en aval dans les cellules fongiformes.

Le projet vise à élucider les tâches suivantes:

1) Etude de l'expression des récepteurs gustatifs des lipides alimentaires (CD36 et GPR120) et du récepteur de goût amer (TAS2R38) pour déterminer si les 2 récepteurs sont co-exprimés par des cellules fongiformes humaines.
2) Etude de la signalisation cellulaire, en particulier le signal Ca^{2+}, via CD36 / GPR120 et T2R38 pour étudier la communication bidirectionnelle entre ces 2 familles de récepteurs.
3) Etude du rôle joué par les agents autocrines / paracrines comme le GLP-1, le CCK… qui sont libérés du TBC dans la modulation de deux modalités gustatives.

Ces tâches seront effectuées sur des cellules fongiformes humaines (HTC-8) immortalisées par l'institut BRAIN (Zwingenberg, Allemagne).

La mise en évidence du cross-talk entre ces deux modalités gustatives pourrait permettre d'établir de nouvelles stratégies alimentaires personnalisées tenant compte des polymorphismes des récepteurs gustatifs amers et gras (seuil de sensibilité) pour les patients en surpoids ou atteints de cancer.

Résultats et discussion

Dans le cadre de notre collaboration avec l'institut BRAIN, nous avons proposé d'étudier l'interaction potentielle entre les modalités du goût du gras et de l'amer dans les cellules HTC-8. Tout d'abord, nous avons caractérisé le profil des cellules HTC-8. Hochheimer et al. (2014) n'ont pas détecté GNAT3/α-gustducine et PLCβ2 dans les cellules HTC-8. Ils ont donc conclu que GNAT2/transducine qui est également exprimée dans les cellules des bourgeons du goût, pourrait compléter partiellement l'action de GNAT3/α-gustducin et pourrait participer à la voie de transduction du signal gustatif (Kusakabe et al., 2000; McLaughlin et al., 1992). Ainsi, GNAT2/transducine pourrait remplacer les facteurs de signalisation gustatifs canoniques dans les cellules HTC-8. En ce qui concerne l'absence de détection de PLCβ2, il est probable que PLδ4, un paralogue du gène PLCβ2, contribue à la transduction du signal, car PLCδ4 est exprimé chez les cellules des bourgeons du goût des primates (Hevezi et al., 2009). Il est intéressant de noter que, dans notre étude, nous avons observé un marquage positif pour l'α-gustducine, facteur clé de la voie de signalisation canonique du goût gustatif (Kinnamon, 2012), ainsi que pour PLCβ2. Ainsi, les cellules HTC-8 semblent présenter un profil de récepteur de type II (Chaudhari et Roper, 2010). Néanmoins, il est possible que GNAT2/transducine et PLCδ4 contribuent à la transduction du signal du goût du gras. D'autres études sont nécessaires pour explorer cette hypothèse.

Ici, nous avons proposé d'étudier l'interaction probable entre les goûts gras et amers. Pour ce faire, j'ai passé près de deux ans à caractériser la lignée cellulaire HTC-8. Nous avons observé que les cellules HTC-8 expriment PLCβ2 et l'α-gustducine parmi d'autres marqueurs cellulaires et, principalement, co-expriment les récepteurs du goût amer et du goût du gras.

Karmous et al. (2018) ont montré une association directe entre l'IMC, la perception orosensorielle des acides gras et PROP. L'étude met en lumière un possible croisement entre goût gras et amer impliquant des interactions cellulaires et moléculaires.

Il est probable que les goûts amer et gras partagent une signalisation en aval commune de Ca^{2+}. Nous avons proposé d'élucider les mécanismes sous-jacents qui se produisent dans les cellules des papilles gustatives. Nous avons d'abord évalué l'expression des récepteurs amers et du goût gras. Nous avons observé que les récepteurs du goût gras (CD36 et GPR120) et les récepteurs du goût amer (TAS2R38 et TAS2R16) étaient exprimés par les cellules HTC-8. De plus, CD36 est co-exprimé avec TAS2R38 et TAS2R16 d'une manière homogène. Pour ce

faire, nous avons sélectionné trois agents amers, PTC (phenylthiocarbamide) connu pour être un agoniste du TAS2R38 (Behrens et al., 2013; Bufe et al., 2005; Meyerhof et al., 2010), la salicine, un agoniste du TAS2R16 (Maehashi et al., 2008; Meyerhof et al., 2010; Soranzo et al., 2005; Thomas et al., 2017) et la sinigrine, un glucosinolate auquel TAS2R38 et TAS2R16 répondent tous deux (Behrens et al., 2013; Meyerhof et al., 2010 ; Thomas et al., 2017). Tout d'abord, nous avons montré que cellules HTC-8 réagissaient de manière dose-dépendante à la signalisation endogène du Ca^{2+} pour les saveurs amères et lipidiques (Hochheimer et al., 2014; Meyerhof et al., 2010). Ensuite, les réponses diminuées du signal en l'absence de Ca^{2+} dans le tampon indiquent que la transduction du signal a mobilisé le calcium à partir d'un pool extracellulaire (Ozdener et al., 2014). Il est apparu que la signalisation du goût du gras et amer dépend de la présence de Ca^{2+} extracellulaire. L'addition d'AL et des différents agonistes du goût amer, l'un après l'autre, a déclenché des réponses additives, suggérant que l'AL interagissait avec le CD36 tandis que les agonistes amers activaient seulement leur récepteur apparenté, à savoir TAS2R16 et / ou TAS2R38. Ensuite, nous nous sommes concentrés sur le mécanisme en aval. Il a révélé que les inhibiteurs de la signalisation du Ca^{2+} en aval, connus pour être impliqués dans la transduction du signal du goût du gras via CD36, inhibaient également la signalisation de l'amer. En effet, tous les inhibiteurs utilisés dans l'étude ont altéré la signalisation du Ca^{2+} après leur adjonction dans le milieu, ce qui a fortement soutenu l'existence d'un croisement amer du goût gras et des modalités de goût amer.

L'influx de Ca^{2+} qui se produit par l'ouverture des canaux SOC (store operated calcium channels), tels que Orai1 et Orai3, est modulé par la phosphorylation, médiée par les kinases de la famille Src (Babnigg et al., 1997). El-Yassimi et al. (2008) ont montré que les inhibiteurs de SFK réduisaient de manière significative les augmentations de $[Ca^{2+}]_i$ induites par les AGLC dans les cellules de papilles gustatives (TBC) exprimant CD36 chez la souris. En outre, STIM1 (stromal interaction molecule 1) est un capteur de réticulum endoplasmique (RE) dont la stimulation entraîne l'activation des canaux SOC. STIM1 s'est avéré impliqué dans la perception du goût du gras, car la préférence spontanée vis-à-vis des AGLC chez les souris $STIM1^{-/-}$ a été supprimée (Dramane et al., 2012). De plus, il a été démontré que STIM1 régule l'ouverture des canaux SOC induite par les AGLC chez des souris exprimant CD36 (Dramane et al., 2012). En effet, les bloqueurs des canaux SOC (c.-à-d. SKF96365 et econazole) ont diminué de manière significative les augmentations induites par les agonistes amers et amers de $[Ca^{2+}]_i$. Comme indiqué (Ozdener et al., 2014), notre étude corrobore

l'implication des canaux SOC dans les réponses au Ca^{2+} au LCFA et met en évidence l'implication de ces canaux dans les réponses au Ca^{2+} provoquées par les agents amers.

Bien que Hochheimer et al. (2014) n'aient pas réussi à détecter PLCβ2 dans les cellules HTC-8, nous avons obtenu un marquage PLCβ2 positif et avons également trouvé l'ARNm de PLC*β*2. L'utilisation de l'U73122, un inhibiteur des PLC, déclenchant une réponse calcique réduite significative, a indiqué que les agonistes du goût du gras et les agonistes amers ont mobilisé le Ca^{2+} via un mécanisme dépendant du PLC. La concentration utilisée est délibérément faible, car il a été démontré que le U73122 diminuait de manière dose-dépendante les réponses $[Ca^{2+}]_i$ (Jan et al., 1998).

Nous nous demandons si un antagoniste des récepteurs du goût gras pourrait perturber la transduction du signal de goût amer. Comme prévu, la signalisation de Ca^{2+} évoquée par AL a été significativement réduite en présence de sulfo-N-succinimidyle (SSO) et AH7614 (un antagoniste de GPR120) (Ozdener et al., 2014). Fait intéressant, nous avons observé le même phénomène avec PTC, la salicine et, dans une moindre mesure, la sinigrine après 20 minutes de pré-incubation avec SSO et AH7614, suggérant que les agonistes amers ont mobilisé le Ca^{2+} en interférant avec la signalisation indirecte.

Pris ensemble, ces résultats suggèrent que le goût du gras et de l'amer partagent une voie commune. Nous pouvons supposer que les agonistes de goût amer et gras se lient à leurs récepteurs apparentés et activent PLCβ2 qui déclenche la production d'IP_3 qui conduirait à la libération intracellulaire de Ca^{2+}, aboutissant finalement à la libération de neurotransmetteurs. Il est également apparu que la signalisation de goût amer interfère avec la signalisation CD36 et GPR120. Ainsi, il semble qu'une seule voie existe dans les cellules HTC-8. Ces résultats sont contradictoires avec les données d'Hochheimer et al. (2014), qui indiquent que deux voies de signalisation coexistent, une signalisation dépendante du PLC et une signalisation du Ca^{2+} indépendante du PLC. Cela signifie que l'influx de Ca^{2+} extracellulaire et intracellulaire est impliqué dans la signalisation en aval, à la fois amère et lipidique. Quoi qu'il en soit, ces résultats mettent en évidence le croisement entre le goût gras et le goût amer qui confirme le concept de «goût gras amer». Néanmoins, ce concept doit être élucidé car il pourrait impliquer d'autres composants.

Les cellules HTC-8 expriment l'haplotype PAV (Proline-Alanine-Valine) correspondant à la variante « taster » de TAS2R38 (Hochheimer et al., 2014) et associée à la plus haute sensibilité à l'acide oléique et PROP (Melis et al., 2015). À l'avenir, il serait intéressant de

tester et de comparer la transduction de signalisation du gras et du goût amer dans les cellules exprimant la variante de test AVI du TAS2R38 liée à la capacité la plus faible de détecter l'acide oléique (Melis et al., 2015).

En raison des découvertes récentes sur le rôle physiologique de l'ocytocine dans la régulation de l'alimentation et des avantages qu'elle présente pour le traitement de l'obésité, nous avons étudié l'implication de la stimulation du goût du gras dans la sécrétion de l'ocytocine. L'ocytocine n'est pas seulement administrée aux papilles via la circulation sanguine contrairement à Sinclair et al. (2010), mais est également produite localement, comme l'a révélé le dosage libéré par l'ocytocine dans les cellules HTC-8. Ceci est étayé par le fait que l'ocytocine a été mesurée dans le surnageant des cellules HTC-8. De plus, il est intéressant de noter que la libération d'ocytocine est plus importante lorsque l'acide linoléique est incubé avec la SSO et l'AH7614. Ce phénomène pourrait révéler une désensibilisation aux acides gras au niveau des TBC et initier la satiété.

Ces résultats renforcent l'importance du rôle anorexigène de l'ocytocine et révèlent que sa sécrétion est modulée par les récepteurs du goût du gras, i.e., CD36 et GPR120. Ainsi, il semble que l'ocytocine influence la satiété liée à la détection du goût du gras.

La chirurgie bariatrique entraîne des modifications drastiques du poids corporel et une restauration drastique de la libération peptidique intestinale postprandiale, telles que GLP-1, PYY et la ghréline (Barja-Fernández et al., 2015), des paramètres qui sont dérégulés chez les sujets obèses (Meyer-Gerspach et al., 2014). Il est donc apparu qu'influencer la libération d'hormones pouvait constituer une alternative pharmacologique à la chirurgie invasive. Avau et al. (2015) ont récemment souligné le potentiel thérapeutique du ciblage des TAS2R pour réduire le poids corporel. Ils ont montré que les souris déficientes en α-gustducine (les souris α-gust$^{-/-}$) présentaient un poids inférieur à celui des souris WT lorsqu'elles recevaient un régime riche en graisses (HFD). De plus, la masse du tissu adipeux blanc (WAT) était plus faible chez les souris α-gust$^{-/-}$ et était corrélée à une augmentation de l'activité thermogénique du tissu adipeux brun (BAT). Fait intéressant, le traitement avec des agonistes amers (benzoate de dénatonium ou quinine) induit une diminution du poids corporel dépendante de l'α-gustducine, associée à une diminution de la prise alimentaire. Le traitement des pré-adipocytes avec des agonistes de TAS2R a également diminué la différenciation en adipocytes matures. Par conséquent, interférer avec la voie de signalisation gustative avec les

agonistes de TAS2R semble protéger contre le développement de l'obésité induite par un régime riche en graisses en affectant directement le métabolisme des adipocytes.

L'obésité est l'un des principaux problèmes de santé publique et sa prévalence ne cesse d'augmenter partout dans le monde. Au cours de la dernière décennie, le rôle des acides gras alimentaires a été largement étudié, en particulier leur implication en tant que régulateurs du métabolisme énergétique et lipidique et leur implication dans la santé humaine. Par conséquent, la capacité à détecter les acides gras et l'interaction potentielle entre les saveurs de gras et les goûts amers reste une question importante à explorer car elle pourrait constituer de nouvelles stratégies anti-obésité.

Un dépistage génétique approprié de l'obésité, y compris le SNP TAS2R38, pourrait mettre en évidence différentes options pour améliorer les altérations chimiosensorielles, notamment l'ajout d'arômes simulés aux aliments pour compenser l'hyposensibilisation au goût gras.

Il pourrait également avoir une application dans l'industrie pharmaceutique (Schiffman, 2018) où de nombreux médicaments présentent un goût amer important et sont aversifs pour la plupart des enfants et de nombreux adultes. L’élucidation de l’interaction entre le goût amer et le goût de graisse pourrait améliorer l’acceptation et l’observance du médicament chez les populations pédiatriques

Discussion générale

Pris ensemble, ces résultats suggèrent le modèle intégratif suivant (Figure 78).

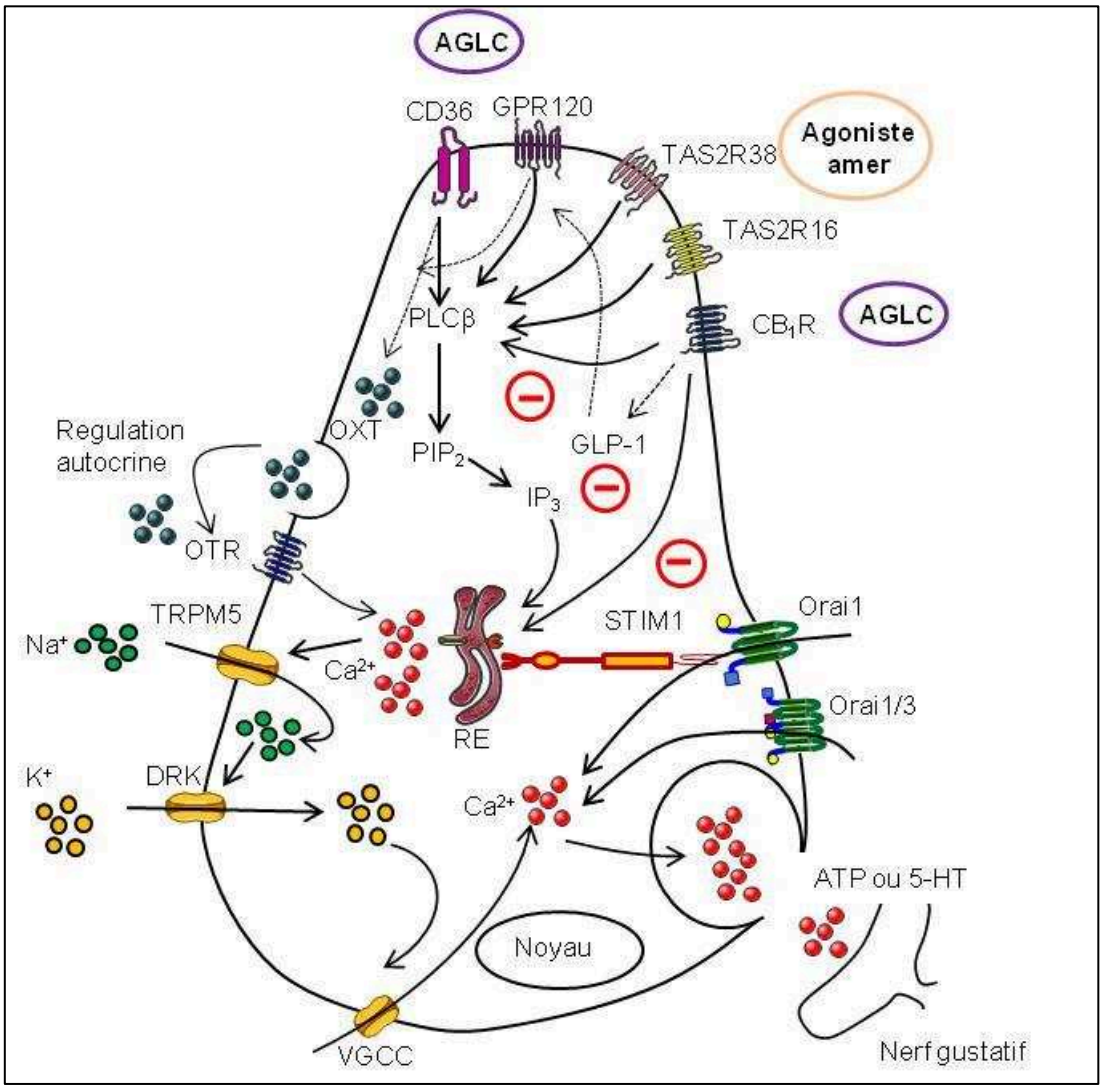

Figure 78. **Hypothèse proposée de la voie de signalisation induite par les AGLC et par les agonistes amers dans les cellules des bourgeons du goût**. AGLC, acide gras à longue chaîne; PLCβ2, phospholipase Cβ2; PIP_2, Phosphatidylinositol4,5-bisphosphate; IP_3, Inositol trisphosphate; OTR, récepteur de l'ocytocine ; OXT, ocytocine ; RE, reticulum endosplasmique. D'après Gilbertson et Khan (2014).

Ainsi, nous pouvons proposer l'hypothèse suivante: une levée d'inhibition de l'ECS couplée à une inhibition de l'ocytocine pourrait induire une régulation positive de la CD36 au niveau de la membrane cellulaire en induisant l'appétit et en activant la prise alimentaire. Ensuite, LA déclenche la régulation négative de CD36 et la régulation positive de GPR120 (Ozdener et al., 2014) qui induisent la libération de GLP-1 et d'ocytocine à partir des TBC. L'activation de

GPR120 et l'inhibition de l'ECS, c'est-à-dire l'internalisation potentielle de CB_1R via les radeaux lipidiques conduiraient à l'état de satiété.

En conclusion, l'ECS et le statut amer du goût modifient la voie de signalisation du goût gras modulant la réponse au Ca^{2+} qui déclenche l'information au cerveau et conduit finalement au comportement alimentaire. Pour confirmer nos résultats, la même méthodologie pourrait être utilisée chez les souris et les humains, le TBC chez la souris réagissant également à l'acide linoléique, à la sinigrine, au PTC et à la salicine. Les cellules HTC-8 répondent à l'AEA et à l'ACEA.

Enfin, Bari et al. (2008) ont signalé que CB_1R fonctionne dans le contexte des radeaux lipidiques. Ainsi, il serait intéressant d'explorer le trafic de CB_1R au cours des différentes phases du comportement prandial en tenant compte en même temps des variations de la libération du GLP-1 et de l'ocytocine. Enfin, étant donné qu'un cross-talk est susceptible de se produire entre les modalités du goût du gras et de l'amer, il serait pertinent d'étudier l'existence d'un dialogue similaire entre le sucré et l'amer ou le salé et l'amer, comme le suggère (Khan et al., 2018b).

References

References

Abel, E.L. (1975). Cannabis: effects on hunger and thirst. Behav Biol 15, 255–281.

Abumrad, N.A., and Davidson, N.O. (2012). Role of the gut in lipid homeostasis. Physiol. Rev. 92, 1061–1085.

Abumrad, N.A., el-Maghrabi, M.R., Amri, E.Z., Lopez, E., and Grimaldi, P.A. (1993). Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J. Biol. Chem. 268, 17665–17668.

Adamantidis, A.R., Tsai, H.-C., Boutrel, B., Zhang, F., Stuber, G.D., Budygin, E.A., Touriño, C., Bonci, A., Deisseroth, K., and de Lecea, L. (2011). Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. J. Neurosci. 31, 10829–10835.

Affleck, V.S., Coote, J.H., and Pyner, S. (2012). The projection and synaptic organisation of NTS afferent connections with presympathetic neurons, GABA and nNOS neurons in the paraventricular nucleus of the hypothalamus. Neuroscience 219, 48–61.

Altabas, V., and Zjačić-Rotkvić, V. (2015). Anti-ghrelin antibodies in appetite suppression: recent advances in obesity pharmacotherapy.

Altirriba, J., Poher, A.-L., Caillon, A., Arsenijevic, D., Veyrat-Durebex, C., Lyautey, J., Dulloo, A., and Rohner-Jeanrenaud, F. (2014). Divergent effects of oxytocin treatment of obese diabetic mice on adiposity and diabetes. Endocrinology 155, 4189–4201.

Altirriba, J., Poher, A.-L., and Rohner-Jeanrenaud, F. (2015a). Chronic Oxytocin Administration as a Treatment Against Impaired Leptin Signaling or Leptin Resistance in Obesity. Front Endocrinol (Lausanne) 6, 119.

Altirriba, J., Poher, A.-L., and Rohner-Jeanrenaud, F. (2015b). Chronic Oxytocin Administration as a Treatment Against Impaired Leptin Signaling or Leptin Resistance in Obesity. Front Endocrinol (Lausanne) 6, 119.

Alvheim, A.R., Malde, M.K., Osei-Hyiaman, D., Lin, Y.H., Pawlosky, R.J., Madsen, L., Kristiansen, K., Frøyland, L., and Hibbeln, J.R. (2012). Dietary linoleic acid elevates

endogenous 2-AG and anandamide and induces obesity. Obesity (Silver Spring, Md.) 20, 1984–1994.

Ancel, D., Bernard, A., Subramaniam, S., Hirasawa, A., Tsujimoto, G., Hashimoto, T., Passilly-Degrace, P., Khan, N.-A., and Besnard, P. (2015). The oral lipid sensor GPR120 is not indispensable for the orosensory detection of dietary lipids in mice. J. Lipid Res. 56, 369–378.

Aoki, Y., Yahata, N., Watanabe, T., Takano, Y., Kawakubo, Y., Kuwabara, H., Iwashiro, N., Natsubori, T., Inoue, H., Suga, M., et al. (2014). Oxytocin improves behavioural and neural deficits in inferring others' social emotions in autism. Brain 137, 3073–3086.

Aristotle (350AD). De Anima.

Arletti, R., Benelli, A., and Bertolini, A. (1989). Influence of oxytocin on feeding behavior in the rat. Peptides 10, 89–93.

Arnone, M., Maruani, J., Chaperon, F., Thiébot, M.H., Poncelet, M., Soubrié, P., and Le Fur, G. (1997). Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology (Berl.) 132, 104–106.

Avau, B., Bauters, D., Steensels, S., Vancleef, L., Laermans, J., Lesuisse, J., Buyse, J., Lijnen, H.R., Tack, J., and Depoortere, I. (2015). The Gustatory Signaling Pathway and Bitter Taste Receptors Affect the Development of Obesity and Adipocyte Metabolism in Mice. PLoS ONE 10, e0145538.

Avila, M., Ojcius, D.M., and Yilmaz, Ö. (2009). The Oral Microbiota: Living with a Permanent Guest. DNA and Cell Biology 28, 405–411.

Babnigg, G., Bowersox, S.R., and Villereal, M.L. (1997). The role of pp60c-src in the regulation of calcium entry via store-operated calcium channels. J. Biol. Chem. 272, 29434–29437.

Baggio, L.L., and Drucker, D.J. (2007). Biology of incretins: GLP-1 and GIP. Gastroenterology 132, 2131–2157.

Baillie, A.G., Coburn, C.T., and Abumrad, N.A. (1996). Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog. The Journal of Membrane Biology 153, 75–81.

Balazova, L., Krskova, K., Suski, M., Sisovsky, V., Hlavacova, N., Olszanecki, R., Jezova, D., and Zorad, S. (2016). Metabolic effects of subchronic peripheral oxytocin administration in lean and obese zucker rats. J. Physiol. Pharmacol. 67, 531–541.

Bargmann, W., and Scharrer, E. (1951). The site of origin of the hormones of the posterior pituitary. Am. Sci. 39, 255–259.

Bari, M., Oddi, S., De Simone, C., Spagnolo, P., Gasperi, V., Battista, N., Centonze, D., and Maccarrone, M. (2008). Type-1 cannabinoid receptors colocalize with caveolin-1 in neuronal cells. Neuropharmacology 54, 45–50.

Barja-Fernández, S., Folgueira, C., Castelao, C., Leis, R., Casanueva, F.F., and Seoane, L.M. (2015). Peripheral signals mediate the beneficial effects of gastric surgery in obesity. Gastroenterol Res Pract 2015, 560938.

Bartoshuk, L.M., Duffy, V.B., and Miller, I.J. (1994). PTC/PROP tasting: anatomy, psychophysics, and sex effects. Physiology & Behavior 56, 1165–1171.

Baskin, D.G., Kim, F., Gelling, R.W., Russell, B.J., Schwartz, M.W., Morton, G.J., Simhan, H.N., Moralejo, D.H., and Blevins, J.E. (2010). A new oxytocin-saporin cytotoxin for lesioning oxytocin-receptive neurons in the rat hindbrain. Endocrinology 151, 4207–4213.

Batterham, R.L., Cohen, M.A., Ellis, S.M., Le Roux, C.W., Withers, D.J., Frost, G.S., Ghatei, M.A., and Bloom, S.R. (2003). Inhibition of Food Intake in Obese Subjects by Peptide YY3–36. New England Journal of Medicine 349, 941–948.

Beck, B., Burlet, A., Max, J.P., and Stricker-Krongrad, A. (2002). Effects of long-term ingestion of aspartame on hypothalamic neuropeptide Y, plasma leptin and body weight gain and composition. Physiol. Behav. 75, 41–47.

Behrens, M., Gunn, H.C., Ramos, P.C.M., Meyerhof, W., and Wooding, S.P. (2013). Genetic, functional, and phenotypic diversity in TAS2R38-mediated bitter taste perception. Chem. Senses 38, 475–484.

Bell, K.I., and Tepper, B.J. (2006). Short-term vegetable intake by young children classified by 6-n-propylthoiuracil bitter-taste phenotype. Am. J. Clin. Nutr. 84, 245–251.

Berridge, K.C. (1996). Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 20, 1–25.

Berry, E.M., and Mechoulam, R. (2002). Tetrahydrocannabinol and endocannabinoids in feeding and appetite. Pharmacol. Ther. 95, 185–190.

Besnard, P. (2016). Lipids and obesity: Also a matter of taste? Reviews in Endocrine & Metabolic Disorders.

Besnard, P., Passilly-Degrace, P., and Khan, N.A. (2016). Taste of Fat: A Sixth Taste Modality? Physiol. Rev. 96, 151–176.

Besnard, P., Christensen, J.E., Brignot, H., Bernard, A., Passilly-Degrace, P., Nicklaus, S., Pais de Barros, J.-P., Collet, X., Lelouvier, B., Servant, F., et al. (2018). Obese Subjects With Specific Gustatory Papillae Microbiota and Salivary Cues Display an Impairment to Sense Lipids. Sci Rep 8, 6742.

Bläker, M., Kock, K., Ahlers, C., Buck, F., and Schmale, H. (1993). Molecular cloning of human von Ebner's gland protein, a member of the lipocalin superfamily highly expressed in lingual salivary glands. Biochim. Biophys. Acta 1172, 131–137.

Blevins, J.E., and Baskin, D.G. (2015). Translational and therapeutic potential of oxytocin as an anti-obesity strategy: Insights from rodents, nonhuman primates and humans. Physiol. Behav. 152, 438–449.

Blevins, J.E., Graham, J.L., Morton, G.J., Bales, K.L., Schwartz, M.W., Baskin, D.G., and Havel, P.J. (2015). Chronic oxytocin administration inhibits food intake, increases energy expenditure, and produces weight loss in fructose-fed obese rhesus monkeys. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308, R431-438.

Blouet, C., and Schwartz, G.J. (2010). Hypothalamic nutrient sensing in the control of energy homeostasis. Behav. Brain Res. 209, 1–12.

Boesveldt, S., and Lundström, J.N. (2014). Detecting fat content of food from a distance: olfactory-based fat discrimination in humans. PLoS ONE 9, e85977.

Bolton, B., and Halpern, B.P. (2010). Orthonasal and retronasal but not oral-cavity-only discrimination of vapor-phase fatty acids. Chem. Senses 35, 229–238.

Bouret, S.G., Draper, S.J., and Simerly, R.B. (2004). Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304, 108–110.

Bradley, E.R., and Woolley, J.D. (2017). Oxytocin effects in schizophrenia: Reconciling mixed findings and moving forward. Neurosci Biobehav Rev 80, 36–56.

Briand, L., Eloit, C., Nespoulous, C., Bézirard, V., Huet, J.-C., Henry, C., Blon, F., Trotier, D., and Pernollet, J.-C. (2002). Evidence of an Odorant-Binding Protein in the Human Olfactory Mucus: Location, Structural Characterization, and Odorant-Binding Properties. Biochemistry 41, 7241–7252.

Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J.-D., and Meyerhof, W. (2002). The human TAS2R16 receptor mediates bitter taste in response to beta-glucopyranosides. Nat. Genet. 32, 397–401.

Bufe, B., Breslin, P.A.S., Kuhn, C., Reed, D.R., Tharp, C.D., Slack, J.P., Kim, U.-K., Drayna, D., and Meyerhof, W. (2005). The Molecular Basis of Individual Differences in Phenylthiocarbamide and Propylthiouracil Bitterness Perception. Curr Biol 15, 322–327.

Burd, C., Senerat, A., Chambers, E., and Keller, K.L. (2013). PROP taster status interacts with the built environment to influence children's food acceptance and body weight status. Obesity (Silver Spring) 21, 786–794.

Burdyga, G., Lal, S., Varro, A., Dimaline, R., Thompson, D.G., and Dockray, G.J. (2004). Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. J. Neurosci. 24, 2708–2715.

Burkett, J.P., Andari, E., Johnson, Z.V., Curry, D.C., de Waal, F.B.M., and Young, L.J. (2016). Oxytocin-dependent consolation behavior in rodents. Science 351, 375–378.

Cabras, T., Melis, M., Castagnola, M., Padiglia, A., Tepper, B.J., Messana, I., and Tomassini Barbarossa, I. (2012). Responsiveness to 6-n-propylthiouracil (PROP) is associated with salivary levels of two specific basic proline-rich proteins in humans. PLoS ONE 7, e30962.

Calvo, S.S.-C., and Egan, J.M. (2015). The endocrinology of taste receptors. Nature Reviews Endocrinology 11, 213–227.

Campa, D., De Rango, F., Carrai, M., Crocco, P., Montesanto, A., Canzian, F., Rose, G., Rizzato, C., Passarino, G., and Barale, R. (2012). Bitter taste receptor polymorphisms and human aging. PLoS ONE 7, e45232.

Cartoni, C., Yasumatsu, K., Ohkuri, T., Shigemura, N., Yoshida, R., Godinot, N., le Coutre, J., Ninomiya, Y., and Damak, S. (2010). Taste preference for fatty acids is mediated by GPR40 and GPR120. J. Neurosci. 30, 8376–8382.

Cejka, D., Losert, D., and Wacheck, V. (2006). Short interfering RNA (siRNA): tool or therapeutic? Clin. Sci. 110, 47–58.

Chalé-Rush, A., Burgess, J.R., and Mattes, R.D. (2007). Multiple routes of chemosensitivity to free fatty acids in humans. Am. J. Physiol. Gastrointest. Liver Physiol. 292, G1206-1212.

Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S., and Ryba, N.J. (2000). T2Rs function as bitter taste receptors. Cell 100, 703–711.

Chaudhari, N., and Roper, S.D. (2010). The cell biology of taste. J. Cell Biol. 190, 285–296.

Chevrot, M., Bernard, A., Ancel, D., Buttet, M., Martin, C., Abdoul-Azize, S., Merlin, J.-F., Poirier, H., Niot, I., Khan, N.A., et al. (2013). Obesity alters the gustatory perception of lipids in the mouse: plausible involvement of lingual CD36. J Lipid Res 54, 2485–2494.

Cohn, Z.J., Kim, A., Huang, L., Brand, J., and Wang, H. (2010). Lipopolysaccharide-induced inflammation attenuates taste progenitor cell proliferation and shortens the life span of taste bud cells. BMC Neurosci 11, 72.

Colon-Gonzalez, F., Kim, G.W., Lin, J.E., Valentino, M.A., and Waldman, S.A. (2013). Obesity pharmacotherapy: What is next? Molecular Aspects of Medicine 34, 71–83.

Connor, W.E., Neuringer, M., and Reisbick, S. (1992). Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. Nutr. Rev. 50, 21–29.

Cota, D., Marsicano, G., Tschöp, M., Grübler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thöne-Reineke, C., Ortmann, S., et al. (2003). The endogenous cannabinoid

system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112, 423–431.

Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., and Gilula, N.B. (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384, 83–87.

Dale, H.H. (1906). On some physiological actions of ergot. J. Physiol. (Lond.) 34, 163–206.

Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K., and Nakazato, M. (2000). Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 141, 4255–4261.

De Petrocellis, L., Marini, P., Matias, I., Moriello, A.S., Starowicz, K., Cristino, L., Nigam, S., and Di Marzo, V. (2007). Mechanisms for the coupling of cannabinoid receptors to intracellular calcium mobilization in rat insulinoma beta-cells. Exp. Cell Res. 313, 2993–3004.

Deblon, N., Veyrat-Durebex, C., Bourgoin, L., Caillon, A., Bussier, A.-L., Petrosino, S., Piscitelli, F., Legros, J.-J., Geenen, V., Foti, M., et al. (2011). Mechanisms of the anti-obesity effects of oxytocin in diet-induced obese rats. PLoS ONE 6, e25565.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., and Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Di Marzo, V., and Matias, I. (2005). Endocannabinoid control of food intake and energy balance. Nat. Neurosci. 8, 585–589.

Di Marzo, V., Goparaju, S.K., Wang, L., Liu, J., Bátkai, S., Járai, Z., Fezza, F., Miura, G.I., Palmiter, R.D., Sugiura, T., et al. (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410, 822–825.

DiBaise, J.K., Zhang, H., Crowell, M.D., Krajmalnik-Brown, R., Decker, G.A., and Rittmann, B.E. (2008). Gut microbiota and its possible relationship with obesity. Mayo Clin. Proc. 83, 460–469.

Dinh, T.P., Carpenter, D., Leslie, F.M., Freund, T.F., Katona, I., Sensi, S.L., Kathuria, S., and Piomelli, D. (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc. Natl. Acad. Sci. U.S.A. 99, 10819–10824.

DiPatrizio, N.V., and Piomelli, D. (2012). The thrifty lipids: endocannabinoids and the neural control of energy conservation. Trends Neurosci. 35, 403–411.

DiPatrizio, N.V., Joslin, A., Jung, K.-M., and Piomelli, D. (2013). Endocannabinoid signaling in the gut mediates preference for dietary unsaturated fats. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 27, 2513–2520.

Domes, G., Heinrichs, M., Michel, A., Berger, C., and Herpertz, S.C. (2007). Oxytocin improves “mind-reading” in humans. Biol. Psychiatry 61, 731–733.

Donaldson, Z.R., and Young, L.J. (2008). Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322, 900–904.

Dramane, G., Abdoul-Azize, S., Hichami, A., Vögtle, T., Akpona, S., Chouabe, C., Sadou, H., Nieswandt, B., Besnard, P., and Khan, N.A. (2012a). STIM1 regulates calcium signaling in taste bud cells and preference for fat in mice. J. Clin. Invest. 122, 2267–2282.

Dramane, G., Abdoul-Azize, S., Hichami, A., Vögtle, T., Akpona, S., Chouabe, C., Sadou, H., Nieswandt, B., Besnard, P., and Khan, N.A. (2012b). STIM1 regulates calcium signaling in taste bud cells and preference for fat in mice. J. Clin. Invest. 122, 2267–2282.

Drayna, D. (2005). Human taste genetics. Annu Rev Genomics Hum Genet 6, 217–235.

Drewnowski, A., and Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. Am. J. Clin. Nutr. 72, 1424–1435.

Drewnowski, A., Brunzell, J.D., Sande, K., Iverius, P.H., and Greenwood, M.R. (1985). Sweet tooth reconsidered: taste responsiveness in human obesity. Physiol. Behav. 35, 617–622.

Drover, V.A., Ajmal, M., Nassir, F., Davidson, N.O., Nauli, A.M., Sahoo, D., Tso, P., and Abumrad, N.A. (2005). CD36 deficiency impairs intestinal lipid secretion and clearance of chylomicrons from the blood. J. Clin. Invest. 115, 1290–1297.

Duffy, V.B., and Bartoshuk, L.M. (2000). Food Acceptance and Genetic Variation in Taste. Journal of the American Dietetic Association 100, 647–655.

Duffy, V.B., Davidson, A.C., Kidd, J.R., Kidd, K.K., Speed, W.C., Pakstis, A.J., Reed, D.R., Snyder, D.J., and Bartoshuk, L.M. (2004). Bitter Receptor Gene (TAS2R38), 6-n-Propylthiouracil (PROP) Bitterness and Alcohol Intake. Alcoholism: Clinical and Experimental Research 28, 1629–1637.

Ebba, S., Abarintos, R.A., Kim, D.G., Tiyouh, M., Stull, J.C., Movalia, A., and Smutzer, G. (2012). The examination of fatty acid taste with edible strips. Physiol. Behav. 106, 579–586.

Eckertova, M., Ondrejcakova, M., Krskova, K., Zorad, S., and Jezova, D. (2011). Subchronic treatment of rats with oxytocin results in improved adipocyte differentiation and increased gene expression of factors involved in adipogenesis. Br. J. Pharmacol. 162, 452–463.

Elabd, S.K., Sabry, I., Mohasseb, M., and Algendy, A. (2014). Oxytocin as a novel therapeutic option for type I diabetes and diabetic osteopathy. Endocr Regul 48, 87–102.

El-Yassimi, A., Hichami, A., Besnard, P., and Khan, N.A. (2008). Linoleic acid induces calcium signaling, Src kinase phosphorylation, and neurotransmitter release in mouse CD36-positive gustatory cells. J. Biol. Chem. 283, 12949–12959.

Endemann, G., Stanton, L.W., Madden, K.S., Bryant, C.M., White, R.T., and Protter, A.A. (1993). CD36 is a receptor for oxidized low density lipoprotein. J. Biol. Chem. 268, 11811–11816.

Erlanson-Albertsson, C. (2005). Appetite regulation and energy balance. Acta Paediatr Suppl 94, 40–41.

Feeney, E.L., and Hayes, J.E. (2014). Exploring associations between taste perception, oral anatomy and polymorphisms in the carbonic anhydrase (gustin) gene CA6. Physiol. Behav. 128, 148–154.

Feeney, E., O'Brien, S., Scannell, A., Markey, A., and Gibney, E.R. (2011). Genetic variation in taste perception: does it have a role in healthy eating? Proceedings of the Nutrition Society 70, 135–143.

Feinle, C., O'Donovan, D., Doran, S., Andrews, J.M., Wishart, J., Chapman, I., and Horowitz, M. (2003). Effects of fat digestion on appetite, APD motility, and gut hormones in response to duodenal fat infusion in humans. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G798-807.

Feldman, R., Monakhov, M., Pratt, M., and Ebstein, R.P. (2016). Oxytocin Pathway Genes: Evolutionary Ancient System Impacting on Human Affiliation, Sociality, and Psychopathology. Biol. Psychiatry 79, 174–184.

Feng, P., Chai, J., Zhou, M., Simon, N., Huang, L., and Wang, H. (2014). Interleukin-10 is produced by a specific subset of taste receptor cells and critical for maintaining structural integrity of mouse taste buds. J. Neurosci. 34, 2689–2701.

Feng, P., Jyotaki, M., Kim, A., Chai, J., Simon, N., Zhou, M., Bachmanov, A.A., Huang, L., and Wang, H. (2015). Regulation of bitter taste responses by tumor necrosis factor. Brain, Behavior, and Immunity 49, 32–42.

Fenselau, H., Campbell, J.N., Verstegen, A.M.J., Madara, J.C., Xu, J., Shah, B.P., Resch, J.M., Yang, Z., Mandelblat-Cerf, Y., Livneh, Y., et al. (2017). A rapidly acting glutamatergic ARC→PVH satiety circuit postsynaptically regulated by α-MSH. Nat. Neurosci. 20, 42–51.

Feron, G., and Poette, J. (2013). In-mouth mechanism leading to the perception of fat in humans: from detection to preferences. The particular role of saliva. OCL 20, 102–107.

Fox, A.L. (1932a). The Relationship between Chemical Constitution and Taste. Proc. Natl. Acad. Sci. U.S.A. 18, 115–120.

Fox, A.L. (1932b). The Relationship between Chemical Constitution and Taste. Proceedings of the National Academy of Sciences of the United States of America 18, 115–120.

Fredriksson, R., Höglund, P.J., Gloriam, D.E.I., Lagerström, M.C., and Schiöth, H.B. (2003). Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. FEBS Lett. 554, 381–388.

Gaillard, D., Laugerette, F., Darcel, N., El-Yassimi, A., Passilly-Degrace, P., Hichami, A., Khan, N.A., Montmayeur, J.-P., and Besnard, P. (2008). The gustatory pathway is involved in CD36-mediated orosensory perception of long-chain fatty acids in the mouse. FASEB J. 22, 1458–1468.

Gajdosechova, L., Krskova, K., Segarra, A.B., Spolcova, A., Suski, M., Olszanecki, R., and Zorad, S. (2014). Hypooxytocinaemia in obese Zucker rats relates to oxytocin degradation in liver and adipose tissue. J. Endocrinol. 220, 333–343.

Galindo, M.M., Voigt, N., Stein, J., van Lengerich, J., Raguse, J.-D., Hofmann, T., Meyerhof, W., and Behrens, M. (2012). G Protein–Coupled Receptors in Human Fat Taste Perception. Chem Senses 37, 123–139.

Gamber, K.M., Macarthur, H., and Westfall, T.C. (2005). Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. Neuropharmacology 49, 646–652.

Gaoni, Y., and Mechoulam, R. (1964). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. J. Am. Chem. Soc. 86, 1646–1647.

Garneau, N.L., Nuessle, T.M., Sloan, M.M., Santorico, S.A., Coughlin, B.C., and Hayes, J.E. (2014). Crowdsourcing taste research: genetic and phenotypic predictors of bitter taste perception as a model. Front Integr Neurosci 8.

Gibbons, C., Caudwell, P., Finlayson, G., Webb, D.-L., Hellström, P.M., Näslund, E., and Blundell, J.E. (2013). Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. J. Clin. Endocrinol. Metab. 98, E847-855.

Gilbertson, T.A., and Khan, N.A. (2014). Cell signaling mechanisms of oro-gustatory detection of dietary fat: advances and challenges. Prog. Lipid Res. 53, 82–92.

Gilbertson, T.A., Yu, T., and Shah, B.P. (2010). Gustatory Mechanisms for Fat Detection. In Fat Detection: Taste, Texture, and Post Ingestive Effects, J.-P. Montmayeur, and J. le Coutre, eds. (Boca Raton (FL): CRC Press/Taylor & Francis), p.

Giovannucci, E., Rimm, E.B., Colditz, G.A., Stampfer, M.J., Ascherio, A., Chute, C.C., and Willett, W.C. (1993). A Prospective Study of Dietary Fat and Risk of Prostate Cancer. J Natl Cancer Inst 85, 1571–1579.

Glendinning, J. (1992). Effect of salivary proline-rich proteins on ingestive responses to tannic acid in mice. Chemical Senses 17, 1–12.

Godinot, N., Yasumatsu, K., Barcos, M.E., Pineau, N., Ledda, M., Viton, F., Ninomiya, Y., le Coutre, J., and Damak, S. (2013). Activation of tongue-expressed GPR40 and GPR120 by non caloric agonists is not sufficient to drive preference in mice. Neuroscience 250, 20–30.

Gómez, R., Navarro, M., Ferrer, B., Trigo, J.M., Bilbao, A., Del Arco, I., Cippitelli, A., Nava, F., Piomelli, D., and Rodríguez de Fonseca, F. (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J. Neurosci. 22, 9612–9617.

Goodson, J.M., Groppo, D., Halem, S., and Carpino, E. (2009). Is Obesity an Oral Bacterial Disease? Journal of Dental Research 88, 519–523.

Greenberg, D., McCaffery, J., Potack, J.Z., Bray, G.A., and York, D.A. (1999). Differential satiating effects of fats in the small intestine of obesity-resistant and obesity-prone rats. Physiol. Behav. 66, 621–626.

Greene, T.A., Alarcon, S., Thomas, A., Berdougo, E., Doranz, B.J., Breslin, P.A.S., and Rucker, J.B. (2011). Probenecid inhibits the human bitter taste receptor TAS2R16 and suppresses bitter perception of salicin. PLoS ONE 6, e20123.

Greenwalt, D.E., Lipsky, R.H., Ockenhouse, C.F., Ikeda, H., Tandon, N.N., and Jamieson, G.A. (1992). Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. Blood 80, 1105–1115.

Hamosh, M., and Scow, R.O. (1973). Lingual lipase and its role in the digestion of dietary lipid. J. Clin. Invest. 52, 88–95.

Hayes, J.E., and Duffy, V.B. (2007). Revisiting Sugar–Fat Mixtures: Sweetness and Creaminess Vary with Phenotypic Markers of Oral Sensation. Chem Senses 32, 225–236.

Hayes, J.E., Bartoshuk, L.M., Kidd, J.R., and Duffy, V.B. (2008). Supertasting and PROP Bitterness Depends on More Than the TAS2R38 Gene. Chem Senses 33, 255–265.

Hayes, J.E., Wallace, M.R., Knopik, V.S., Herbstman, D.M., Bartoshuk, L.M., and Duffy, V.B. (2011). Allelic variation in TAS2R bitter receptor genes associates with variation in sensations from and ingestive behaviors toward common bitter beverages in adults. Chem. Senses 36, 311–319.

Heit, B., Kim, H., Cosío, G., Castaño, D., Collins, R., Lowell, C.A., Kain, K.C., Trimble, W.S., and Grinstein, S. (2013). Multimolecular signaling complexes enable Syk-mediated signaling of CD36 internalization. Dev. Cell 24, 372–383.

Henkin, R.I., Martin, B.M., and Agarwal, R.P. (1999a). Decreased parotid saliva gustin/carbonic anhydrase VI secretion: an enzyme disorder manifested by gustatory and olfactory dysfunction. Am. J. Med. Sci. 318, 380–391.

Henkin, R.I., Martin, B.M., and Agarwal, R.P. (1999b). Efficacy of exogenous oral zinc in treatment of patients with carbonic anhydrase VI deficiency. Am. J. Med. Sci. 318, 392–405.

Herisson, F.M., Waas, J.R., Fredriksson, R., Schiöth, H.B., Levine, A.S., and Olszewski, P.K. (2016). Oxytocin Acting in the Nucleus Accumbens Core Decreases Food Intake. J. Neuroendocrinol. 28.

Hevezi, P., Moyer, B.D., Lu, M., Gao, N., White, E., Echeverri, F., Kalabat, D., Soto, H., Laita, B., Li, C., et al. (2009). Genome-Wide Analysis of Gene Expression in Primate Taste Buds Reveals Links to Diverse Processes. PLOS ONE 4, e6395.

Higashida, H., Furuhara, K., Yamauchi, A.-M., Deguchi, K., Harashima, A., Munesue, S., Lopatina, O., Gerasimenko, M., Salmina, A.B., Zhang, J.-S., et al. (2017). Intestinal transepithelial permeability of oxytocin into the blood is dependent on the receptor for advanced glycation end products in mice. Sci Rep 7, 7883.

Hilairet, S., Bouaboula, M., Carrière, D., Le Fur, G., and Casellas, P. (2003). Hypersensitization of the Orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. J. Biol. Chem. 278, 23731–23737.

Hinrichs, A.L., Wang, J.C., Bufe, B., Kwon, J.M., Budde, J., Allen, R., Bertelsen, S., Evans, W., Dick, D., Rice, J., et al. (2006). Functional variant in a bitter-taste receptor (hTAS2R16) influences risk of alcohol dependence. Am. J. Hum. Genet. 78, 103–111.

Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S., and Tsujimoto, G. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nature Medicine 11, 90–94.

Hirasawa, A., Hara, T., Katsuma, S., Adachi, T., and Tsujimoto, G. (2008). Free Fatty Acid Receptors and Drug Discovery. Biological and Pharmaceutical Bulletin 31, 1847–1851.

Hochheimer, A., Krohn, M., Rudert, K., Riedel, K., Becker, S., Thirion, C., and Zinke, H. (2014). Endogenous gustatory responses and gene expression profile of stably proliferating human taste cells isolated from fungiform papillae. Chem. Senses 39, 359–377.

Hoebe, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., Sovath, S., Shamel, L., Hartung, T., Zähringer, U., et al. (2005). CD36 is a sensor of diacylglycerides. Nature 433, 523–527.

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders.

Howard, A.D., Feighner, S.D., Cully, D.F., Arena, J.P., Liberator, P.A., Rosenblum, C.I., Hamelin, M., Hreniuk, D.L., Palyha, O.C., Anderson, J., et al. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273, 974–977.

Hume, C., Sabatier, N., and Menzies, J. (2017). High-Sugar, but Not High-Fat, Food Activates Supraoptic Nucleus Neurons in the Male Rat. Endocrinology 158, 2200–2211.

Iwasaki, Y., Maejima, Y., Suyama, S., Yoshida, M., Arai, T., Katsurada, K., Kumari, P., Nakabayashi, H., Kakei, M., and Yada, T. (2015). Peripheral oxytocin activates vagal afferent neurons to suppress feeding in normal and leptin-resistant mice: a route for ameliorating hyperphagia and obesity. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308, R360-369.

Jan, C.-R., Ho, C.-M., Wu, S.-N., and Tseng, C.-J. (1998). The phospholipase C inhibitor U73122 increases cytosolic calcium in MDCK cells by activating calcium influx and releasing stored calcium. Life Sciences 63, 895–908.

Jewett, D.C., Cleary, J., Levine, A.S., Schaal, D.W., and Thompson, T. (1995). Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. Psychopharmacology 120, 267–271.

Johnstone, L.E., Fong, T.M., and Leng, G. (2006). Neuronal activation in the hypothalamus and brainstem during feeding in rats. Cell Metab. 4, 313–321.

Jollès, P., and Jollès, J. (1984). What's new in lysozyme research? Always a model system, today as yesterday. Mol. Cell. Biochem. 63, 165–189.

Jyotaki, M., Shigemura, N., and Ninomiya, Y. (2010). Modulation of sweet taste sensitivity by orexigenic and anorexigenic factors. Endocr. J. 57, 467–475.

Karmous, I., Plesník, J., Khan, A.S., Šerý, O., Abid, A., Mankai, A., Aouidet, A., and Khan, N.A. (2018a). Orosensory detection of bitter in fat-taster healthy and obese participants: Genetic polymorphism of CD36 and TAS2R38. Clin Nutr 37, 313–320.

Karmous, I., Plesník, J., Khan, A.S., Šerý, O., Abid, A., Mankai, A., Aouidet, A., and Khan, N.A. (2018b). Orosensory detection of bitter in fat-taster healthy and obese participants: Genetic polymorphism of CD36 and TAS2R38. Clinical Nutrition 37, 313–320.

Katoh, A., Shoguchi, K., Matsuoka, H., Yoshimura, M., Ohkubo, J.-I., Matsuura, T., Maruyama, T., Ishikura, T., Aritomi, T., Fujihara, H., et al. (2014). Fluorescent visualisation of the hypothalamic oxytocin neurones activated by cholecystokinin-8 in rats expressing c-fos-enhanced green fluorescent protein and oxytocin-monomeric red fluorescent protein 1 fusion transgenes. J. Neuroendocrinol. 26, 341–347.

Kaufman, A., Choo, E., Koh, A., and Dando, R. (2018). Inflammation arising from obesity reduces taste bud abundance and inhibits renewal. PLOS Biology 16, e2001959.

Kawai, T., and Fushiki, T. (2003). Importance of lipolysis in oral cavity for orosensory detection of fat. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 285, R447–454.

Keast, R.S., and Costanzo, A. (2015). Is fat the sixth taste primary? Evidence and implications. Flavour 4, 5.

Keast, R.S.J., and Roper, J. (2007). A Complex Relationship among Chemical Concentration, Detection Threshold, and Suprathreshold Intensity of Bitter Compounds. Chem Senses 32, 245–253.

Keast, R.S.J., Bournazel, M.M.E., and Breslin, P.A.S. (2003). A psychophysical investigation of binary bitter-compound interactions. Chem. Senses 28, 301–313.

Keast, R.S.J., Azzopardi, K.M., Newman, L.P., and Haryono, R.Y. (2014). Impaired oral fatty acid chemoreception is associated with acute excess energy consumption. Appetite 80, 1–6.

Keller, K.L. (2012a). Genetic influences on oral fat perception and preference: Presented at the symposium “The Taste for Fat: New Discoveries on the Role of Fat in Sensory Perception, Metabolism, Sensory Pleasure and Beyond” held at the Institute of Food Technologists 2011 Annual Meeting, New Orleans, LA, June 12, 2011. J. Food Sci. 77, S143-147.

Keller, K.L. (2012b). Genetic influences on oral fat perception and preference: Presented at the symposium “The Taste for Fat: New Discoveries on the Role of Fat in Sensory Perception, Metabolism, Sensory Pleasure and Beyond” held at the Institute of Food Technologists 2011 Annual Meeting, New Orleans, LA, June 12, 2011. Journal of Food Science 77, S143–147.

Keller, K.L., and Tepper, B.J. (2004). Inherited Taste Sensitivity to 6-n-Propylthiouracil in Diet and Body Weight in Children. Obesity Research 12, 904–912.

Keller, K.L., Liang, L.C.H., Sakimura, J., May, D., van Belle, C., Breen, C., Driggin, E., Tepper, B.J., Lanzano, P.C., Deng, L., et al. (2012). Common variants in the CD36 gene are associated with oral fat perception, fat preferences, and obesity in African Americans. Obesity (Silver Spring) 20, 1066–1073.

Kentish, S.J., and Page, A.J. (2015). The role of gastrointestinal vagal afferent fibres in obesity. J. Physiol. (Lond.) 593, 775–786.

Khan, N.A., and Besnard, P. (2009). Oro-sensory perception of dietary lipids: new insights into the fat taste transduction. Biochim. Biophys. Acta 1791, 149–155.

Khan, A.S., Murtaza, B., Hichami, A., and Khan, N.A. (2018a). A cross-talk between fat and bitter taste modalities. Biochimie.

Khan, A.S., Hichami, A., and Khan, N.A. (2018b). Taste perception and its effects on oral nutritional supplements in younger life phases. Curr Opin Clin Nutr Metab Care 21, 411–415.

Kim, U.K., and Drayna, D. (2005). Genetics of individual differences in bitter taste perception: lessons from the PTC gene. Clinical Genetics 67, 275–280.

Kim, U., Jorgenson, E., Coon, H., Leppert, M., Risch, N., and Drayna, D. (2003). Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science 299, 1221–1225.

Kinnamon, S.C. (2012). Taste Receptor Signaling-- From Tongues to Lungs. Acta Physiol (Oxf) 204, 158–168.

Kirchner, H., Heppner, K.M., and Tschöp, M.H. (2012). The role of ghrelin in the control of energy balance. Handb Exp Pharmacol 161–184.

Kirkham, T.C. (2003). Endogenous cannabinoids: a new target in the treatment of obesity. Am. J. Physiol. Regul. Integr. Comp. Physiol. 284, R343-344.

Kirkham, T.C., Williams, C.M., Fezza, F., and Di Marzo, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br. J. Pharmacol. 136, 550–557.

Kock, K., Ahlers, C., and Schmale, H. (1994). Structural organization of the genes for rat von Ebner's gland proteins 1 and 2 reveals their close relationship to lipocalins. Eur. J. Biochem. 221, 905–916.

Kojima, M., and Kangawa, K. (2005). Ghrelin: structure and function. Physiol. Rev. 85, 495–522.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660.

Kublaoui, B.M., Gemelli, T., Tolson, K.P., Wang, Y., and Zinn, A.R. (2008). Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice. Mol. Endocrinol. 22, 1723–1734.

Kuda, O., Pietka, T.A., Demianova, Z., Kudova, E., Cvacka, J., Kopecky, J., and Abumrad, N.A. (2013). Sulfo-N-succinimidyl oleate (SSO) inhibits fatty acid uptake and signaling for intracellular calcium via binding CD36 lysine 164: SSO also inhibits oxidized low density lipoprotein uptake by macrophages. J. Biol. Chem. 288, 15547–15555.

Kusakabe, Y., Yasuoka, A., Asano-Miyoshi, M., Iwabuchi, K., Matsumoto, I., Arai, S., Emori, Y., and Abe, K. (2000). Comprehensive study on G protein alpha-subunits in taste bud

cells, with special reference to the occurrence of Galphai2 as a major Galpha species. Chem. Senses 25, 525–531.

Langlet, F., Levin, B.E., Luquet, S., Mazzone, M., Messina, A., Dunn-Meynell, A.A., Balland, E., Lacombe, A., Mazur, D., Carmeliet, P., et al. (2013). Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting. Cell Metab. 17, 607–617.

Lasisi, T.J., Shittu, S.-T.T., and Alada, A.R. (2018). Re-establishing normal diet following high fat-diet-induced obesity reverses the altered salivary composition in Wistar rats. J Basic Clin Physiol Pharmacol.

Lauffer, L.M., Iakoubov, R., and Brubaker, P.L. (2009). GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes 58, 1058–1066.

Laugerette, F., Passilly-Degrace, P., Patris, B., Niot, I., Febbraio, M., Montmayeur, J.-P., and Besnard, P. (2005). CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. J. Clin. Invest. 115, 3177–3184.

Lawson, E.A., Marengi, D.A., DeSanti, R.L., Holmes, T.M., Schoenfeld, D.A., and Tolley, C.J. (2015). Oxytocin reduces caloric intake in men. Obesity (Silver Spring) 23, 950–956.

Lee, R.J., Kofonow, J.M., Rosen, P.L., Siebert, A.P., Chen, B., Doghramji, L., Xiong, G., Adappa, N.D., Palmer, J.N., Kennedy, D.W., et al. (2014). Bitter and sweet taste receptors regulate human upper respiratory innate immunity. J. Clin. Invest. 124, 1393–1405.

Lee, S., Eguchi, A., Tsuzuki, S., Matsumura, S., Inoue, K., Iwanaga, T., Masuda, D., Yamashita, S., and Fushiki, T. (2015). Expression of CD36 by Olfactory Receptor Cells and Its Abundance on the Epithelial Surface in Mice. PLoS ONE 10, e0133412.

Leinonen, J., Parkkila, S., Kaunisto, K., Koivunen, P., and Rajaniemi, H. (2001). Secretion of carbonic anhydrase isoenzyme VI (CA VI) from human and rat lingual serous von Ebner's glands. J. Histochem. Cytochem. 49, 657–662.

Li, J.X., Yoshida, T., Monk, K.J., and Katz, D.B. (2013). Lateral hypothalamus contains two types of palatability-related taste responses with distinct dynamics. J. Neurosci. 33, 9462–9473.

Lieverse, R.J., Jansen, J.B., Masclee, A.M., and Lamers, C.B. (1994). Satiety effects of cholecystokinin in humans. Gastroenterology 106, 1451–1454.

Lipchock, S.V., Mennella, J.A., Spielman, A.I., and Reed, D.R. (2013). Human bitter perception correlates with bitter receptor messenger RNA expression in taste cells. Am. J. Clin. Nutr. 98, 1136–1143.

Little, T.J., and Feinle-Bisset, C. (2010). Oral and Gastrointestinal Sensing of Dietary Fat and Appetite Regulation in Humans: Modification by Diet and Obesity. Front Neurosci 4.

Little, T.J., and Feinle-Bisset, C. (2011). Effects of dietary fat on appetite and energy intake in health and obesity--oral and gastrointestinal sensory contributions. Physiol. Behav. 104, 613–620.

Liu, D., Archer, N., Duesing, K., Hannan, G., and Keast, R. (2016). Mechanism of fat taste perception: Association with diet and obesity. Prog. Lipid Res. 63, 41–49.

Lobo, M.V., Huerta, L., Ruiz-Velasco, N., Teixeiro, E., de la Cueva, P., Celdrán, A., Martín-Hidalgo, A., Vega, M.A., and Bragado, R. (2001). Localization of the lipid receptors CD36 and CLA-1/SR-BI in the human gastrointestinal tract: towards the identification of receptors mediating the intestinal absorption of dietary lipids. J. Histochem. Cytochem. 49, 1253–1260.

Love-Gregory, L., and Abumrad, N.A. (2011). CD36 genetics and the metabolic complications of obesity: Current Opinion in Clinical Nutrition and Metabolic Care 14, 527–534.

Love-Gregory, L., Sherva, R., Schappe, T., Qi, J.-S., McCrea, J., Klein, S., Connelly, M.A., and Abumrad, N.A. (2011). Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile. Hum. Mol. Genet. 20, 193–201.

Maehashi, K., Matano, M., Wang, H., Vo, L.A., Yamamoto, Y., and Huang, L. (2008). Bitter peptides activate hTAS2Rs, the human bitter receptors. Biochem. Biophys. Res. Commun. 365, 851–855.

Maejima, Y., Iwasaki, Y., Yamahara, Y., Kodaira, M., Sedbazar, U., and Yada, T. (2011). Peripheral oxytocin treatment ameliorates obesity by reducing food intake and visceral fat mass. Aging (Albany NY) 3, 1169–1177.

Maejima, Y., Sakuma, K., Santoso, P., Gantulga, D., Katsurada, K., Ueta, Y., Hiraoka, Y., Nishimori, K., Tanaka, S., Shimomura, K., et al. (2014). Oxytocinergic circuit from paraventricular and supraoptic nuclei to arcuate POMC neurons in hypothalamus. FEBS Lett. 588, 4404–4412.

Maejima, Y., Rita, R.S., Santoso, P., Aoyama, M., Hiraoka, Y., Nishimori, K., Gantulga, D., Shimomura, K., and Yada, T. (2015). Nasal oxytocin administration reduces food intake without affecting locomotor activity and glycemia with c-Fos induction in limited brain areas. Neuroendocrinology 101, 35–44.

Maejima, Y., Aoyama, M., Sakamoto, K., Jojima, T., Aso, Y., Takasu, K., Takenosihita, S., and Shimomura, K. (2017). Impact of sex, fat distribution and initial body weight on oxytocin's body weight regulation. Sci Rep 7, 8599.

Maejima, Y., Yokota, S., Nishimori, K., and Shimomura, K. (2018). The Anorexigenic Neural Pathways of Oxytocin and Their Clinical Implication. NEN 107, 91–104.

Makranz, C., Cohen, G., Baron, A., Levidor, L., Kodama, T., Reichert, F., and Rotshenker, S. (2004). Phosphatidylinositol 3-kinase, phosphoinositide-specific phospholipase-Cgamma and protein kinase-C signal myelin phagocytosis mediated by complement receptor-3 alone and combined with scavenger receptor-AI/II in macrophages. Neurobiol. Dis. 15, 279–286.

Malnic, B., Hirono, J., Sato, T., and Buck, L.B. (1999). Combinatorial Receptor Codes for Odors. Cell 96, 713–723.

Mangold, J.E., Payne, T.J., Ma, J.Z., Chen, G., and Li, M.D. (2008). Bitter taste receptor gene polymorphisms are an important factor in the development of nicotine dependence in African Americans. J. Med. Genet. 45, 578–582.

Martin, B., Dotson, C.D., Shin, Y.-K., Ji, S., Drucker, D.J., Maudsley, S., and Munger, S.D. (2009). Modulation of taste sensitivity by GLP-1 signaling in taste buds. Annals of the New York Academy of Sciences 1170, 98–101.

Martin, C., Chevrot, M., Passilly-Degrace, P., and Besnard, P. (2010). Détection Oro-sensorielle Des Lipides Alimentaires : Impacts Sur Le Comportement Alimentaire et La Santé. Innovations Agronomiques 10, 81–93.

Martin, C., Chevrot, M., Poirier, H., Passilly-Degrace, P., Niot, I., and Besnard, P. (2011a). CD36 as a lipid sensor. Physiol. Behav. 105, 36–42.

Martin, C., Passilly-Degrace, P., Gaillard, D., Merlin, J.-F., Chevrot, M., and Besnard, P. (2011b). The lipid-sensor candidates CD36 and GPR120 are differentially regulated by dietary lipids in mouse taste buds: impact on spontaneous fat preference. PloS One 6, e24014.

Martin, C., Passilly-Degrace, P., Chevrot, M., Ancel, D., Sparks, S.M., Drucker, D.J., and Besnard, P. (2012). Lipid-mediated release of GLP-1 by mouse taste buds from circumvallate papillae: putative involvement of GPR120 and impact on taste sensitivity. J. Lipid Res. 53, 2256–2265.

Masuda, D., Hirano, K., Oku, H., Sandoval, J.C., Kawase, R., Yuasa-Kawase, M., Yamashita, Y., Takada, M., Tsubakio-Yamamoto, K., Tochino, Y., et al. (2009). Chylomicron remnants are increased in the postprandial state in CD36 deficiency. J. Lipid Res. 50, 999–1011.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., and Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Mattes, R.D. (2009). Oral detection of short-, medium-, and long-chain free fatty acids in humans. Chem. Senses 34, 145–150.

Mattes, R.D. (2011). Accumulating evidence supports a taste component for free fatty acids in humans. Physiol. Behav. 104, 624–631.

Matzinger, D., Degen, L., Drewe, J., Meuli, J., Duebendorfer, R., Ruckstuhl, N., D'Amato, M., Rovati, L., and Beglinger, C. (2000). The role of long chain fatty acids in regulating food intake and cholecystokinin release in humans. Gut 46, 688–693.

McLaughlin, S.K., McKinnon, P.J., and Margolskee, R.F. (1992). Gustducin is a taste-cell-specific G protein closely related to the transducins. Nature 357, 563–569.

Mela, D.J., and Sacchetti, D.A. (1991). Sensory preferences for fats: relationships with diet and body composition. Am. J. Clin. Nutr. 53, 908–915.

Melis, M., Aragoni, M.C., Arca, M., Cabras, T., Caltagirone, C., Castagnola, M., Crnjar, R., Messana, I., Tepper, B.J., and Barbarossa, I.T. (2013a). Marked Increase in PROP Taste Responsiveness Following Oral Supplementation with Selected Salivary Proteins or Their Related Free Amino Acids. PLoS One 8.

Melis, M., Atzori, E., Cabras, S., Zonza, A., Calò, C., Muroni, P., Nieddu, M., Padiglia, A., Sogos, V., Tepper, B.J., et al. (2013b). The gustin (CA6) gene polymorphism, rs2274333 (A/G), as a mechanistic link between PROP tasting and fungiform taste papilla density and maintenance. PLoS ONE 8, e74151.

Melis, M., Scheggi, S., Carta, G., Madeddu, C., Lecca, S., Luchicchi, A., Cadeddu, F., Frau, R., Fattore, L., Fadda, P., et al. (2013c). PPARα regulates cholinergic-driven activity of midbrain dopamine neurons via a novel mechanism involving α7 nicotinic acetylcholine receptors. J. Neurosci. 33, 6203–6211.

Melis, M., Sollai, G., Muroni, P., Crnjar, R., and Barbarossa, I.T. (2015a). Associations between orosensory perception of oleic acid, the common single nucleotide polymorphisms (rs1761667 and rs1527483) in the CD36 gene, and 6-n-propylthiouracil (PROP) tasting. Nutrients 7, 2068–2084.

Melis, M., Arca, M., Aragoni, M.C., Cabras, T., Caltagirone, C., Castagnola, M., Crnjar, R., Messana, I., Tepper, B.J., and Barbarossa, I.T. (2015b). Dose-Dependent Effects of L-Arginine on PROP Bitterness Intensity and Latency and Characteristics of the Chemical Interaction between PROP and L-Arginine. PLOS ONE 10, e0131104.

Melis, M., Sollai, G., Muroni, P., Crnjar, R., and Barbarossa, I.T. (2015c). Associations between orosensory perception of oleic acid, the common single nucleotide polymorphisms (rs1761667 and rs1527483) in the CD36 gene, and 6-n-propylthiouracil (PROP) tasting. Nutrients 7, 2068–2084.

Melis, M., Carta, G., Pintus, S., Pintus, P., Piras, C.A., Murru, E., Manca, C., Di Marzo, V., Banni, S., and Tomassini Barbarossa, I. (2017). Polymorphism rs1761667 in the CD36 Gene Is Associated to Changes in Fatty Acid Metabolism and Circulating Endocannabinoid Levels Distinctively in Normal Weight and Obese Subjects. Front. Physiol. 8.

Mennella, J.A., and Beauchamp, G.K. (2008). Optimizing oral medications for children. Clin Ther 30, 2120–2132.

Mennella, J.A., Pepino, M.Y., and Reed, D.R. (2005). Genetic and Environmental Determinants of Bitter Perception and Sweet Preferences. Pediatrics 115, e216–e222.

Mennella, J.A., Spector, A.C., Reed, D.R., and Coldwell, S.E. (2013a). The Bad Taste of Medicines: Overview of Basic Research on Bitter Taste. Clin Ther 35, 1225–1246.

Mennella, J.A., Spector, A.C., Reed, D.R., and Coldwell, S.E. (2013b). The bad taste of medicines: overview of basic research on bitter taste. Clin Ther 35, 1225–1246.

Meyer-Gerspach, A.C., Wölnerhanssen, B., Beglinger, B., Nessenius, F., Napitupulu, M., Schulte, F.H., Steinert, R.E., and Beglinger, C. (2014). Gastric and intestinal satiation in obese and normal weight healthy people. Physiol. Behav. 129, 265–271.

Meyerhof, W., Batram, C., Kuhn, C., Brockhoff, A., Chudoba, E., Bufe, B., Appendino, G., and Behrens, M. (2010). The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors. Chem. Senses 35, 157–170.

Miller, I.J. (1986). Variation in human fungiform taste bud densities among regions and subjects. The Anatomical Record 216, 474–482.

Miller, I.J., and Reedy, F.E. (1990). Variations in human taste bud density and taste intensity perception. Physiol. Behav. 47, 1213–1219.

Miyamoto, J., Hasegawa, S., Kasubuchi, M., Ichimura, A., Nakajima, A., and Kimura, I. (2016). Nutritional Signaling via Free Fatty Acid Receptors. Int J Mol Sci 17.

Miyamoto, T., Miyazaki, T., Okada, Y., and Sato, T. (1996). Whole-cell recording from non-dissociated taste cells in mouse taste bud. Journal of Neuroscience Methods 64, 245–252.

Mo, X.-L., Wei, H.-K., Peng, J., and Tao, Y.-X. (2013). Free fatty acid receptor GPR120 and pathogenesis of obesity and type 2 diabetes mellitus. Prog Mol Biol Transl Sci 114, 251–276.

Moldrich, G., and Wenger, T. (2000). Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study. Peptides 21, 1735–1742.

Moran, T.H., Baldessarini, A.R., Salorio, C.F., Lowery, T., and Schwartz, G.J. (1997). Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. Am. J. Physiol. 272, R1245-1251.

Mounayar, R., Septier, C., Chabanet, C., Feron, G., and Neyraud, E. (2013). Oral Fat Sensitivity in Humans: Links to Saliva Composition Before and After Stimulation by Oleic Acid. Chem. Percept. 6, 118–126.

Mounayar, R., Morzel, M., Brignot, H., Tremblay-Franco, M., Canlet, C., Lucchi, G., Ducoroy, P., Feron, G., and Neyraud, E. (2014). Salivary markers of taste sensitivity to oleic acid: a combined proteomics and metabolomics approach. Metabolomics 10, 688–696.

Mrizak, I., Šerý, O., Plesnik, J., Arfa, A., Fekih, M., Bouslema, A., Zaouali, M., Tabka, Z., and Khan, N.A. (2015). The A allele of cluster of differentiation 36 (CD36) SNP 1761667 associates with decreased lipid taste perception in obese Tunisian women. Br. J. Nutr. 113, 1330–1337.

Müller, T.D., Nogueiras, R., Andermann, M.L., Andrews, Z.B., Anker, S.D., Argente, J., Batterham, R.L., Benoit, S.C., Bowers, C.Y., Broglio, F., et al. (2015). Ghrelin. Mol Metab 4, 437–460.

Mullis, K., Kay, K., and Williams, D.L. (2013). Oxytocin action in the ventral tegmental area affects sucrose intake. Brain Res. 1513, 85–91.

Naber, F., van Ijzendoorn, M.H., Deschamps, P., van Engeland, H., and Bakermans-Kranenburg, M.J. (2010). Intranasal oxytocin increases fathers' observed responsiveness during play with their children: a double-blind within-subject experiment. Psychoneuroendocrinology 35, 1583–1586.

Nasser, J.A., Kissileff, H.R., Boozer, C.N., Chou, C.J., and Pi-Sunyer, F.X. (2001). PROP taster status and oral fatty acid perception. Eating Behaviors 2, 237–245.

Newman, L.P., and Keast, R.S.J. (2013). The Test–Retest Reliability of Fatty Acid Taste Thresholds. Chem. Percept. 6, 70–77.

Neyraud, E., Palicki, O., Schwartz, C., Nicklaus, S., and Feron, G. (2012). Variability of human saliva composition: possible relationships with fat perception and liking. Arch. Oral Biol. 57, 556–566.

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. (2012). Host-Gut Microbiota Metabolic Interactions. Science 336, 1262–1267.

Niot, I., and Besnard, P. (2017). Appetite control by the tongue-gut axis and evaluation of the role of CD36/SR-B2. Biochimie 136, 27–32.

Niot, I., Poirier, H., Tran, T.T.T., and Besnard, P. (2009). Intestinal absorption of long-chain fatty acids: evidence and uncertainties. Prog. Lipid Res. 48, 101–115.

Noel, C.A., Cassano, P.A., and Dando, R. (2017). College-Aged Males Experience Attenuated Sweet and Salty Taste with Modest Weight Gain. J Nutr 147, 1885–1891.

Oberland, S., Ackels, T., Gaab, S., Pelz, T., Spehr, J., Spehr, M., and Neuhaus, E.M. (2015). CD36 is involved in oleic acid detection by the murine olfactory system. Front Cell Neurosci 9, 366.

Oh, D.Y., Talukdar, S., Bae, E.J., Imamura, T., Morinaga, H., Fan, W., Li, P., Lu, W.J., Watkins, S.M., and Olefsky, J.M. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 142, 687–698.

OMS (2012). http://www.roche.fr/innovation-recherche-medicale/decouverte-scientifique-medicale/cardio-metabolisme/enquete-nationale-obepi-2012.html

Ong, Z.Y., Bongiorno, D.M., Hernando, M.A., and Grill, H.J. (2017). Effects of Endogenous Oxytocin Receptor Signaling in Nucleus Tractus Solitarius on Satiation-Mediated Feeding and Thermogenic Control in Male Rats. Endocrinology 158, 2826–2836.

Osei-Hyiaman, D., DePetrillo, M., Pacher, P., Liu, J., Radaeva, S., Bátkai, S., Harvey-White, J., Mackie, K., Offertáler, L., Wang, L., et al. (2005). Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 115, 1298–1305.

Ott, V., Finlayson, G., Lehnert, H., Heitmann, B., Heinrichs, M., Born, J., and Hallschmid, M. (2013). Oxytocin reduces reward-driven food intake in humans. Diabetes 62, 3418–3425.

Ozbek, S., Balasubramanian, P.G., Chiquet-Ehrismann, R., Tucker, R.P., and Adams, J.C. (2010). The evolution of extracellular matrix. Mol. Biol. Cell 21, 4300–4305.

Ozdener, M.H., Subramaniam, S., Sundaresan, S., Sery, O., Hashimoto, T., Asakawa, Y., Besnard, P., Abumrad, N.A., and Khan, N.A. (2014). CD36- and GPR120-mediated Ca^{2+} signaling in human taste bud cells mediates differential responses to fatty acids and is altered in obese mice. Gastroenterology 146, 995–1005.

Padiglia, A., Zonza, A., Atzori, E., Chillotti, C., Calò, C., Tepper, B.J., and Barbarossa, I.T. (2010). Sensitivity to 6-n-propylthiouracil is associated with gustin (carbonic anhydrase VI) gene polymorphism, salivary zinc, and body mass index in humans. The American Journal of Clinical Nutrition 92, 539–545.

Park, M.I., Camilleri, M., O'Connor, H., Oenning, L., Burton, D., Stephens, D., and Zinsmeister, A.R. (2007). Effect of different macronutrients in excess on gastric sensory and motor functions and appetite in normal-weight, overweight, and obese humans. Am J Clin Nutr 85, 411–418.

Parker, H.E., Gribble, F.M., and Reimann, F. (2014). The role of gut endocrine cells in control of metabolism and appetite. Exp. Physiol. 99, 1116–1120.

Passilly-Degrace, P., Gaillard, D., and Besnard, P. (2009). Orosensory perception of dietary lipids in mammals. Results Probl Cell Differ 47, 221–238.

Passilly-Degrace, P., Chevrot, M., Bernard, A., Ancel, D., Martin, C., and Besnard, P. (2014). Is the taste of fat regulated? Biochimie 96, 3–7.

Paster, B.J., Boches, S.K., Galvin, J.L., Ericson, R.E., Lau, C.N., Levanos, V.A., Sahasrabudhe, A., and Dewhirst, F.E. (2001). Bacterial diversity in human subgingival plaque. J. Bacteriol. 183, 3770–3783.

Pepino, M.Y., Love-Gregory, L., Klein, S., and Abumrad, N.A. (2012). The fatty acid translocase gene CD36 and lingual lipase influence oral sensitivity to fat in obese subjects. J. Lipid Res. 53, 561–566.

Perea-Martinez, I., Nagai, T., and Chaudhari, N. (2013). Functional Cell Types in Taste Buds Have Distinct Longevities. PLoS ONE 8.

Peres, R.C.R., Camargo, G., Mofatto, L.S., Cortellazzi, K.L., Santos, M.C.L.G., Santos, M.N., Bergamaschi, C.C., and Line, S.R.P. (2010). Association of polymorphisms in the carbonic anhydrase 6 gene with salivary buffer capacity, dental plaque pH, and caries index in children aged 7–9 years. The Pharmacogenomics Journal 10, 114–119.

Peterschmitt, Y., Abdoul-Azize, S., Murtaza, B., Barbier, M., Khan, A.S., Millot, J.-L., and Khan, N.A. (2018). Fatty Acid Lingual Application Activates Gustatory and Reward Brain Circuits in the Mouse. Nutrients 10.

Poette, J., Mekoué, J., Neyraud, E., Berdeaux, O., Renault, A., Guichard, E., Genot, C., and Feron, G. (2014). Fat sensitivity in humans: oleic acid detection threshold is linked to saliva composition and oral volume. Flavour and Fragrance Journal 29, 39–49.

Pohl, J., Ring, A., Korkmaz, U., Ehehalt, R., and Stremmel, W. (2005). FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts. Mol. Biol. Cell 16, 24–31.

Poirier, B., Bidouard, J.-P., Cadrouvele, C., Marniquet, X., Staels, B., O'Connor, S.E., Janiak, P., and Herbert, J.-M. (2005). The anti-obesity effect of rimonabant is associated with an improved serum lipid profile. Diabetes Obes Metab 7, 65–72.

Poirier, H., Degrace, P., Niot, I., Bernard, A., and Besnard, P. (1996). Localization and regulation of the putative membrane fatty-acid transporter (FAT) in the small intestine. Comparison with fatty acid-binding proteins (FABP). Eur. J. Biochem. 238, 368–373.

Power, M.L., and Schulkin, J. (2008). Anticipatory physiological regulation in feeding biology: cephalic phase responses. Appetite 50, 194–206.

Prentice, R.L., and Sheppard, L. (1990). Dietary fat and cancer: consistency of the epidemiologic data, and disease prevention that may follow from a practical reduction in fat consumption. Cancer Causes Control 1, 81–97.

Ramirez, I. (1993). Role of olfaction in starch and oil preference. Am. J. Physiol. 265, R1404-1409.

Ravinet Trillou, C., Arnone, M., Delgorge, C., Gonalons, N., Keane, P., Maffrand, J.-P., and Soubrie, P. (2003). Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-

induced obese mice. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 284, R345–353.

Ravinet Trillou, C., Delgorge, C., Menet, C., Arnone, M., and Soubrié, P. (2004). CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. Int. J. Obes. Relat. Metab. Disord. 28, 640–648.

Ravussin, E., and Smith, S.R. (2002). Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. Ann. N. Y. Acad. Sci. 967, 363–378.

Reidelberger, R.D., and O'Rourke, M.F. (1989). Potent cholecystokinin antagonist L 364718 stimulates food intake in rats. Am. J. Physiol. 257, R1512-1518.

Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Néliat, G., and Caput, D. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Ring, A., Le Lay, S., Pohl, J., Verkade, P., and Stremmel, W. (2006). Caveolin-1 is required for fatty acid translocase (FAT/CD36) localization and function at the plasma membrane of mouse embryonic fibroblasts. Biochim. Biophys. Acta 1761, 416–423.

Ritter, R.C. (2004). Gastrointestinal mechanisms of satiation for food. Physiol. Behav. 81, 249–273.

Roper, S.D., and Chaudhari, N. (2017). Taste buds: cells, signals and synapses. Nat. Rev. Neurosci. 18, 485–497.

le Roux, C.W., Patterson, M., Vincent, R.P., Hunt, C., Ghatei, M.A., and Bloom, S.R. (2005). Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. J. Clin. Endocrinol. Metab. 90, 1068–1071.

Running, C.A., Craig, B.A., and Mattes, R.D. (2015). Oleogustus: The Unique Taste of Fat. Chem. Senses 40, 507–516.

Ryskamp, D.A., Redmon, S., Jo, A.O., and Križaj, D. (2014). TRPV1 and Endocannabinoids: Emerging Molecular Signals that Modulate Mammalian Vision. Cells 3, 914–938.

Sacerdote, C., Guarrera, S., Smith, G.D., Grioni, S., Krogh, V., Masala, G., Mattiello, A., Palli, D., Panico, S., Tumino, R., et al. (2007). Lactase Persistence and Bitter Taste Response: Instrumental Variables and Mendelian Randomization in Epidemiologic Studies of Dietary Factors and Cancer Risk. Am J Epidemiol 166, 576–581.

Sayed, A., Šerý, O., Plesnik, J., Daoudi, H., Rouabah, A., Rouabah, L., and Khan, N.A. (2015). CD36 AA genotype is associated with decreased lipid taste perception in young obese, but not lean, children. Int J Obes (Lond) 39, 920–924.

Sayegh, A.I., and Ritter, R.C. (2003). Cholecystokinin activates specific enteric neurons in the rat small intestine. Peptides 24, 237–244.

Schiffman, S.S. (2018). Influence of medications on taste and smell. World J Otorhinolaryngol Head Neck Surg 4, 84–91.

Schmale, H., Holtgreve-Grez, H., and Christiansen, H. (1990). Possible role for salivary gland protein in taste reception indicated by homology to lipophilic-ligand carrier proteins. Nature 343, 366–369.

Schwartz, G.J. (2006). Integrative capacity of the caudal brainstem in the control of food intake. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 361, 1275–1280.

Schwartz, G.J. (2011). Gut fat sensing in the negative feedback control of energy balance--recent advances. Physiol. Behav. 104, 621–623.

Schwartz, G.J., Fu, J., Astarita, G., Li, X., Gaetani, S., Campolongo, P., Cuomo, V., and Piomelli, D. (2008). The lipid messenger OEA links dietary fat intake to satiety. Cell Metab. 8, 281–288.

Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., and Baskin, D.G. (2000). Central nervous system control of food intake. Nature 404, 661–671.

Sclafani, A., and Ackroff, K. (2012). Role of gut nutrient sensing in stimulating appetite and conditioning food preferences. Am. J. Physiol. Regul. Integr. Comp. Physiol. 302, R1119-1133.

Sclafani, A., Ackroff, K., and Abumrad, N.A. (2007). CD36 gene deletion reduces fat preference and intake but not post-oral fat conditioning in mice. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 293, R1823–R1832.

Sclafani, A., Zukerman, S., and Ackroff, K. (2013). GPR40 and GPR120 fatty acid sensors are critical for postoral but not oral mediation of fat preferences in the mouse. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305, R1490-1497.

Shahbake, M., Hutchinson, I., Laing, D.G., and Jinks, A.L. (2005). Rapid quantitative assessment of fungiform papillae density in the human tongue. Brain Res. 1052, 196–201.

Shepherd, G.M. (2006). Smell images and the flavour system in the human brain. Nature 444, 316–321.

Shepherd, G.M. (2011). Neurogastronomy: How the Brain Creates Flavor and Why It Matters (Columbia University Press).

Shin, Y.-K., Martin, B., Golden, E., Dotson, C.D., Maudsley, S., Kim, W., Jang, H.-J., Mattson, M.P., Drucker, D.J., Egan, J.M., et al. (2008). Modulation of taste sensitivity by GLP-1 signaling. Journal of Neurochemistry 106, 455–463.

Shoelson, S.E., Lee, J., and Goldfine, A.B. (2006). Inflammation and insulin resistance. J Clin Invest 116, 1793–1801.

Shuto, T., Kato, K., Mori, Y., Viriyakosol, S., Oba, M., Furuta, T., Okiyoneda, T., Arima, H., Suico, M.A., and Kai, H. (2005). Membrane-anchored CD14 is required for LPS-induced TLR4 endocytosis in TLR4/MD-2/CD14 overexpressing CHO cells. Biochem. Biophys. Res. Commun. 338, 1402–1409.

Silverstein, R.L., and Febbraio, M. (2000). CD36 and atherosclerosis. Curr. Opin. Lipidol. 11, 483–491.

Silverstein, R.L., and Febbraio, M. CD36, a Scavenger Receptor Involved in Immunity, Metabolism, Angiogenesis, and Behavior. Science Signaling 2, re3.

Simons, P.J., Kummer, J.A., Luiken, J.J.F.P., and Boon, L. (2011). Apical CD36 immunolocalization in human and porcine taste buds from circumvallate and foliate papillae. Acta Histochemica 113, 839–843.

Sinclair, M.S., Perea-Martinez, I., Dvoryanchikov, G., Yoshida, M., Nishimori, K., Roper, S.D., and Chaudhari, N. (2010). Oxytocin signaling in mouse taste buds. PLoS ONE 5, e11980.

Sindelar, D.K., Palmiter, R.D., Woods, S.C., and Schwartz, M.W. (2005). Attenuated feeding responses to circadian and palatability cues in mice lacking neuropeptide Y. Peptides 26, 2597–2602.

Sly, W.S., and Hu, P.Y. (1995). Human carbonic anhydrases and carbonic anhydrase deficiencies. Annu. Rev. Biochem. 64, 375–401.

Socransky, S.S., and Haffajee, A.D. (2005). Periodontal microbial ecology. Periodontol. 2000 38, 135–187.

Soranzo, N., Bufe, B., Sabeti, P.C., Wilson, J.F., Weale, M.E., Marguerie, R., Meyerhof, W., and Goldstein, D.B. (2005). Positive selection on a high-sensitivity allele of the human bitter-taste receptor TAS2R16. Curr. Biol. 15, 1257–1265.

Spetter, M.S., Feld, G.B., Thienel, M., Preissl, H., Hege, M.A., and Hallschmid, M. (2018). Oxytocin curbs calorie intake via food-specific increases in the activity of brain areas that process reward and establish cognitive control. Sci Rep 8, 2736.

Spiegel, K., Tasali, E., Leproult, R., Scherberg, N., and Van Cauter, E. (2011). Twenty-four-hour profiles of acylated and total ghrelin: relationship with glucose levels and impact of time of day and sleep. J. Clin. Endocrinol. Metab. 96, 486–493.

Spielman, A.I., D'Abundo, S., Field, R.B., and Schmale, H. (1993). Protein analysis of human von Ebner saliva and a method for its collection from the foliate papillae. J. Dent. Res. 72, 1331–1335.

Steiner, J.E., Glaser, D., Hawilo, M.E., and Berridge, K.C. (2001). Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. Neuroscience & Biobehavioral Reviews 25, 53–74.

Stewart, J.E., and Keast, R.S.J. (2012). Recent fat intake modulates fat taste sensitivity in lean and overweight subjects. Int J Obes (Lond) 36, 834–842.

Stewart, J.E., Feinle-Bisset, C., Golding, M., Delahunty, C., Clifton, P.M., and Keast, R.S.J. (2010). Oral sensitivity to fatty acids, food consumption and BMI in human subjects. Br. J. Nutr. 104, 145–152.

Stewart, J.E., Newman, L.P., and Keast, R.S.J. (2011a). Oral sensitivity to oleic acid is associated with fat intake and body mass index. Clin Nutr 30, 838–844.

Stewart, J.E., Seimon, R.V., Otto, B., Keast, R.S.J., Clifton, P.M., and Feinle-Bisset, C. (2011b). Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men. Am. J. Clin. Nutr. 93, 703–711.

Stewart, J.E., Feinle-Bisset, C., and Keast, R.S.J. (2011c). Fatty acid detection during food consumption and digestion: Associations with ingestive behavior and obesity. Prog. Lipid Res. 50, 225–233.

Stricker-Krongrad, A., and Beck, B. (2004). Up-regulation of neuropeptide Y receptors in the hypothalamus of monosodium glutamate-lesioned Sprague-Dawley rats. Nutr Neurosci 7, 241–245.

Su, X., and Abumrad, N.A. (2009). Cellular fatty acid uptake: a pathway under construction. Trends Endocrinol. Metab. 20, 72–77.

Subramaniam, S., Ozdener, M.H., Abdoul-Azize, S., Saito, K., Malik, B., Maquart, G., Hashimoto, T., Marambaud, P., Aribi, M., Tordoff, M.G., et al. (2016). ERK1/2 activation in human taste bud cells regulates fatty acid signaling and gustatory perception of fat in mice and humans. FASEB J. 30, 3489–3500.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., and Waku, K. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Sundaresan, S., Shahid, R., Riehl, T.E., Chandra, R., Nassir, F., Stenson, W.F., Liddle, R.A., and Abumrad, N.A. (2013). CD36-dependent signaling mediates fatty acid-induced gut release of secretin and cholecystokinin. FASEB J. 27, 1191–1202.

Swinburn, B.A., Sacks, G., Hall, K.D., McPherson, K., Finegood, D.T., Moodie, M.L., and Gortmaker, S.L. (2011). The global obesity pandemic: shaped by global drivers and local environments. The Lancet 378, 804–814.

Takagi, K., Legrand, R., Asakawa, A., Amitani, H., François, M., Tennoune, N., Coëffier, M., Claeyssens, S., Rego, J.-C. do, Déchelotte, P., et al. (2013). Anti-ghrelin immunoglobulins modulate ghrelin stability and its orexigenic effect in obese mice and humans. Nature Communications 4, 2685.

Takai, S., Yasumatsu, K., Inoue, M., Iwata, S., Yoshida, R., Shigemura, N., Yanagawa, Y., Drucker, D.J., Margolskee, R.F., and Ninomiya, Y. (2015). Glucagon-like peptide-1 is specifically involved in sweet taste transmission. FASEB J 29, 2268–2280.

Takayanagi, Y., Kasahara, Y., Onaka, T., Takahashi, N., Kawada, T., and Nishimori, K. (2008). Oxytocin receptor-deficient mice developed late-onset obesity. Neuroreport 19, 951–955.

Takeda, N., Jain, R., Li, D., Li, L., Lu, M.M., and Epstein, J.A. (2013). Lgr5 Identifies Progenitor Cells Capable of Taste Bud Regeneration after Injury. PLoS ONE 8, e66314.

Tepper, B.J. (2008). Nutritional implications of genetic taste variation: the role of PROP sensitivity and other taste phenotypes. Annu. Rev. Nutr. 28, 367–388.

Tepper, B.J., and Nurse, R.J. (1997). Fat perception is related to PROP taster status. Physiol. Behav. 61, 949–954.

Tepper, B.J., and Nurse, R.J. (1998). PROP Taster Status Is Related to Fat Perception and Preferencea. Annals of the New York Academy of Sciences 855, 802–804.

Tepper, B.J., Koelliker, Y., Zhao, L., Ullrich, N.V., Lanzara, C., d'Adamo, P., Ferrara, A., Ulivi, S., Esposito, L., and Gasparini, P. (2008). Variation in the bitter-taste receptor gene TAS2R38, and adiposity in a genetically isolated population in Southern Italy. Obesity (Silver Spring) 16, 2289–2295.

Tepper, B.J., Neilland, M., Ullrich, N.V., Koelliker, Y., and Belzer, L.M. (2011). Greater energy intake from a buffet meal in lean, young women is associated with the 6-n-propylthiouracil (PROP) non-taster phenotype. Appetite 56, 104–110.

Thanakun, S., Pornprasertsuk-Damrongsri, S., and Izumi, Y. (2017). Increased oral inflammation, leukocytes, and leptin, and lower adiponectin in overweight or obesity. Oral Diseases 23, 956–965.

Thienel, M., Fritsche, A., Heinrichs, M., Peter, A., Ewers, M., Lehnert, H., Born, J., and Hallschmid, M. (2016). Oxytocin's inhibitory effect on food intake is stronger in obese than normal-weight men. Int J Obes (Lond) 40, 1707–1714.

Thomas, A., Sulli, C., Davidson, E., Berdougo, E., Phillips, M., Puffer, B.A., Paes, C., Doranz, B.J., and Rucker, J.B. (2017). The Bitter Taste Receptor TAS2R16 Achieves High Specificity and Accommodates Diverse Glycoside Ligands by using a Two-faced Binding Pocket. Sci Rep 7.

Tomassini Barbarossa, I., Ozdener, M.H., Melis, M., Love-Gregory, L., Mitreva, M., Abumrad, N.A., and Pepino, M.Y. (2017). Variant in a common odorant-binding protein gene is associated with bitter sensitivity in people. Behav. Brain Res. 329, 200–204.

Tran, T.T.T., Poirier, H., Clément, L., Nassir, F., Pelsers, M.M.A.L., Petit, V., Degrace, P., Monnot, M.-C., Glatz, J.F.C., Abumrad, N.A., et al. (2011). Luminal lipid regulates CD36 levels and downstream signaling to stimulate chylomicron synthesis. J. Biol. Chem. 286, 25201–25210.

Tucker, R.M., and Mattes, R.D. (2013). Influences of repeated testing on nonesterified fatty acid taste. Chem. Senses 38, 325–332.

Tucker, R.M., Kaiser, K.A., Parman, M.A., George, B.J., Allison, D.B., and Mattes, R.D. (2017). Comparisons of Fatty Acid Taste Detection Thresholds in People Who Are Lean vs. Overweight or Obese: A Systematic Review and Meta-Analysis. PLOS ONE 12, e0169583.

Ulven, T. (2012). Short-chain free fatty acid receptors FFA2/GPR43 and FFA3/GPR41 as new potential therapeutic targets. Front Endocrinol (Lausanne) 3.

Ulven, T., and Christiansen, E. (2015). Dietary Fatty Acids and Their Potential for Controlling Metabolic Diseases Through Activation of FFA4/GPR120. Annu. Rev. Nutr. 35, 239–263.

Uysal, K.T., Wiesbrock, S.M., Marino, M.W., and Hotamisligil, G.S. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature 389, 610–614.

Valdivia, S., Patrone, A., Reynaldo, M., and Perello, M. (2014). Acute high fat diet consumption activates the mesolimbic circuit and requires orexin signaling in a mouse model. PLoS ONE 9, e87478.

Venkataraman, C., and Kuo, F. (2005). The G-protein coupled receptor, GPR84 regulates IL-4 production by T lymphocytes in response to CD3 crosslinking. Immunol. Lett. 101, 144–153.

Ventre, J., Doebber, T., Wu, M., MacNaul, K., Stevens, K., Pasparakis, M., Kollias, G., and Moller, D.E. (1997). Targeted disruption of the tumor necrosis factor-alpha gene: metabolic consequences in obese and nonobese mice. Diabetes 46, 1526–1531.

Verhulst, P.-J., and Depoortere, I. (2012). Ghrelin's second life: from appetite stimulator to glucose regulator. World J. Gastroenterol. 18, 3183–3195.

Verty, A.N.A., McFarlane, J.R., McGregor, I.S., and Mallet, P.E. (2004). Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. Endocrinology 145, 3224–3231.

Vickers, S.P., Webster, L.J., Wyatt, A., Dourish, C.T., and Kennett, G.A. (2003). Preferential effects of the cannabinoid CB1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. Psychopharmacology (Berl.) 167, 103–111.

Voigt, N., Stein, J., Galindo, M.M., Dunkel, A., Raguse, J.-D., Meyerhof, W., Hofmann, T., and Behrens, M. (2014). The role of lipolysis in human orosensory fat perception. J. Lipid Res. 55, 870–882.

Volkow, N.D., and Morales, M. (2015). The Brain on Drugs: From Reward to Addiction. Cell 162, 712–725.

Vors, C., Drai, J., Gabert, L., Pineau, G., Laville, M., Vidal, H., Guichard, E., Michalski, M.-C., and Feron, G. (2015). Salivary composition in obese vs normal-weight subjects: towards a role in postprandial lipid metabolism? International Journal of Obesity 39, 1425–1428.

Wang, H., Zhou, M., Brand, J., and Huang, L. (2009). Inflammation and taste disorders: mechanisms in taste buds. Annals of the New York Academy of Sciences 1170, 596–603.

Wang, J., Wu, X., Simonavicius, N., Tian, H., and Ling, L. (2006). Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. J. Biol. Chem. 281, 34457–34464.

Webb, J., Bolhuis, D.P., Cicerale, S., Hayes, J.E., and Keast, R. (2015). The Relationships Between Common Measurements of Taste Function. Chem. Percept. 8, 11–18.

Williams, C.M., and Kirkham, T.C. (1999). Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. Psychopharmacology 143, 315–317.

Wood, N., Johnson, R.B., and Streckfus, C.F. (2003). Comparison of body composition and periodontal disease using nutritional assessment techniques: Third National Health and Nutrition Examination Survey (NHANES III). J. Clin. Periodontol. 30, 321–327.

Wu, Z., Xu, Y., Zhu, Y., Sutton, A.K., Zhao, R., Lowell, B.B., Olson, D.P., and Tong, Q. (2012). An obligate role of oxytocin neurons in diet induced energy expenditure. PLoS ONE 7, e45167.

Xiao, L., Priest, M.F., Nasenbeny, J., Lu, T., and Kozorovitskiy, Y. (2017). Biased Oxytocinergic Modulation of Midbrain Dopamine Systems. Neuron 95, 368-384.e5.

Yee, K.K., Li, Y., Redding, K.M., Iwatsuki, K., Margolskee, R.F., and Jiang, P. (2013). Lgr5-EGFP Marks Taste Bud Stem/Progenitor Cells in Posterior Tongue. STEM CELLS 31, 992–1000.

Yeomans, M.R., Prescott, J., and Gould, N.J. (2009). Acquired hedonic and sensory characteristics of odours: influence of sweet liker and propylthiouracil taster status. Q J Exp Psychol (Hove) 62, 1648–1664.

Yi, K.J., So, K.H., Hata, Y., Suzuki, Y., Kato, D., Watanabe, K., Aso, H., Kasahara, Y., Nishimori, K., Chen, C., et al. (2015). The regulation of oxytocin receptor gene expression during adipogenesis. J. Neuroendocrinol. 27, 335–342.

Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., and Nishimori, K. (2009). Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. J. Neurosci. 29, 2259–2271.

Yoshida, R., Ohkuri, T., Jyotaki, M., Yasuo, T., Horio, N., Yasumatsu, K., Sanematsu, K., Shigemura, N., Yamamoto, T., Margolskee, R.F., et al. (2010). Endocannabinoids selectively enhance sweet taste. Proceedings of the National Academy of Sciences 107, 935–939.

Zhang, G., and Cai, D. (2011). Circadian intervention of obesity development via resting-stage feeding manipulation or oxytocin treatment. Am. J. Physiol. Endocrinol. Metab. 301, E1004-1012.

Zhang, G., Bai, H., Zhang, H., Dean, C., Wu, Q., Li, J., Guariglia, S., Meng, Q., and Cai, D. (2011). Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. Neuron 69, 523–535.

Zhang, G.-H., Zhang, H.-Y., Wang, X.-F., Zhan, Y.-H., Deng, S.-P., and Qin, Y.-M. (2009). The Relationship between Fungiform Papillae Density and Detection Threshold for Sucrose in the Young Males. Chem Senses 34, 93–99.

Zhang, X., Fitzsimmons, R.L., Cleland, L.G., Ey, P.L., Zannettino, A.C.W., Farmer, E.-A., Sincock, P., and Mayrhofer, G. (2003a). CD36/fatty acid translocase in rats: distribution, isolation from hepatocytes, and comparison with the scavenger receptor SR-B1. Lab. Invest. 83, 317–332.

Zhang, Y., Hoon, M.A., Chandrashekar, J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S., and Ryba, N.J.P. (2003b). Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112, 293–301.

Zolotukhin, S. (2013). Metabolic hormones in saliva: origins and functions. Oral Diseases 19, 219–229.