Biochimie 96 (2014) 8–13

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

# $Ca^{2+}$ signaling in taste bud cells and spontaneous preference for fat: Unresolved roles of CD36 and GPR120

Souleymane Abdoul-Azize [a,b], Subramaniam Selvakumar [a], Hassimi Sadou [b], Philippe Besnard [a], Naim Akhtar Khan [a,*]

[a] Physiologie de la Nutrition & Toxicologie, UMR U866 INSERM/Université de Bourgogne/Agro-Sup, 6 Boulevard Gabriel, Dijon 21000, France
[b] Laboratoire de Nutrition, Université Abdou Moumouni, Niamey, Niger

ARTICLE INFO

*Article history:*
Received 2 April 2013
Accepted 6 June 2013
Available online 15 June 2013

*Keywords:*
Lipids
Taste receptor cells
CD36
GPR120
Stim1
$Ca^{2+}$ influx factor
Fatty acids

ABSTRACT

Recent compelling evidences from rodent and human studies raise the possibility for an additional sixth taste modality devoted to oro-gustatory perception of dietary lipids. Understanding the mechanisms underlying oro-gustatory detection of dietary fat is critical for the prevention and treatment of obesity. A number of studies have suggested that lingual CD36, a glycoprotein, highly expressed by circumvallate papillae of the tongue, is implicated in the perception of dietary fat taste. G protein-coupled receptors (GPCRs) are important signaling molecules for many aspects of cellular functions. It has been shown that these receptors, particularly GPR120, are also involved in lipid taste perception. We have shown that dietary long-chain fatty acids (LCFAs), in CD36-positive taste bud cells (TBC), induce increases in free intracellular $Ca^{2+}$ concentrations, $[Ca^{2+}]i$, by recruiting $Ca^{2+}$ from endoplasmic reticulum (ER) pool via inositol 1,4,5-triphosphate production, followed by $Ca^{2+}$ influx via opening of store-operated $Ca^{2+}$ (SOC) channels. GPR120 is also coupled to increases in $[Ca^{2+}]i$ by dietary fatty acids. We observed that stromal interaction molecule 1 (STIM1), a sensor of $Ca^{2+}$ depletion in the ER, mediated fatty acid-induced $Ca^{2+}$ signaling and spontaneous preference for fat in the mouse. In this review article, we discuss the recent advances and unresolved roles of CD36 and GPR120 in lipid taste signaling in taste bud cells.


## 1. Introduction

The sense of taste informs the brain about the quality of ingested food. Five basic taste modalities, i.e., sweet, sour, bitter, salty, and umami, have been identified so far. Umami taste allows the recognition of amino acids, particularly of glutamate, and is associated to a savory sensation [1,2]. Salty taste ensures the proper dietary electrolyte balance, and sour and bitter warn against the intake of potentially toxic chemicals. Sweet taste allows the identification of energy-rich nutrients [2]. The detection of nutritionally relevant food components in tongue is facilitated by specialized taste receptor molecules expressed in sensory cells in taste buds [3]. Tastant-mediated signals are generated by a rise in free intracellular calcium concentrations, $[Ca^{2+}]i$, in taste bud cells (TBC) and then are transferred to the gustatory area of brain via connections between the gustatory nerves (chorda tympani and glossopharyngeal nerves) and the nucleus of solitary tract (NST) in the brain stem [1,3,4].

## 2. Oral fat detection

In Western diet, about 40% of daily caloric intake is composed of lipids. This high-fat supply greatly contributes to the prevalence of obesity and associated diseases, for instance, type II diabetes, atherosclerosis and hypertension. Besides, some recent studies have shown that obese subjects exhibit a high preference for dietary lipids as compared to lean subjects [5,6], suggesting that an inappropriate lipid perception might influence obesity risk by impacting feeding behavior. The recognition of fat stimuli was believed to rely mostly on textural, olfactory, and postingestive cues. During the recent years, however, research conducted mainly on rodent models revealed an additional gustatory component for the detection of long-chain fatty acids, LCFAs (7–15). In brief access

*Abbreviations used:* AA, arachidonic acid; $[Ca^{2+}]i$, free intracellular calcium concentration; CaM, calmodulin; CIF, calcium influx factor; $cPLA_2$, cytosolic phospholipase $A_2$; GL, glossopharyngeal nerve; GPCR, G protein-coupled receptors; $iPLA_2$, $Ca^{2+}$-independent phospholipase $A_2$; LCFA, long-chain fatty acid; Lyso-PC, lyso-phosphatidylcholine; PLC, phospholipase C; PTK, protein tyrosine kinase; SFK, Src family kinases; SOC, store-operated $Ca^{2+}$ channel; SOCE, store-operated $Ca^{2+}$ entry; STIM1, stromal interaction molecule 1; TBC, taste bud cells; TRC, taste receptor cells; TRPM5, transient receptor potential melastatin-5.

* Corresponding author. Tel.: +33 380396312; fax: +33 380396330.
*E-mail address:* Naim.Khan@u-bourgogne.fr (N.A. Khan).

0300-9084/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2013.06.005

tests, anosmic and intact rodents prefer oils to xantham gum, which mimics the texture of oil [7,8]. Recent data demonstrate that low concentrations of LCFAs can be specifically detected in oral cavity by laboratory rodents [9]. In rats and mice with an esophageal ligation, deposition of unsaturated LCFAs onto the tongue led to a rapid and sustained rise in flux and protein contents of pancreatobiliary secretions [10]. Bilateral sectioning of the glossopharyngeal and chorda tympani nerves that innervate gustatory papillae diminished the preference for a solution of linoleic acid in mice [4]. Though fatty acids play an important role in lipid taste perception, most of the dietary fat is present in the form of triglycerides. Interestingly, lingual lipases, secreted by Von Ebner glands, present in the cleft of papillae, are sufficient to hydrolyze triacylglycerides even during short exposure times. Kawai and Fushiki [11] have noticed that in 5-min two-bottle preference tests, the addition of orlistat, a lipase inhibitor, diminished the preference for triacylglycerides as they could not be hydrolyzed into free fatty acids which are initially responsible for fat taste. Radioactive triolein applied on rat circumvallate papilla revealed that lingual lipase was released continuously to generate significant amounts of fatty acids and other lipolytic products within 1–5 s, which was enough time to taste fat. These findings suggest that lingual lipase is released to perceive the taste of triacylglycerides and to find nutritive lipids in food.

Psychophysical studies indicate that humans can detect LCFAs with chain lengths ranging from 6 to 18 carbons. Most of the experiments on humans were conducted by using a nose clip in order to avoid the implication of olfaction and by diluting fatty acids in viscous solutions in order to mask specific texture of these agents [12,13]. Hence, the visual cues were easily eliminated by tasting rounds under red light or with blindfolds. The inter-individual variability in LCFAs thresholds spans about 4 orders of magnitude and some investigators suggest that there are fatty acid tasters and non-tasters [14,15]. An account on the advances on fat taste perception in human beings can be seen in a recent review article [16]. Taken together, these observations demonstrate that a gustatory pathway is involved in the attraction for fatty foods [17].

### 2.1. Implication of CD36 and GPR120

The CD36 is an integral membrane ditopic glycoprotein, and its amino acid sequence predicts a large extracellular hydrophobic pocket between two short cytoplasmic tails [18,19]. This receptor-like glycoprotein, which belongs to the scavenger receptor family [20], binds to saturated and unsaturated long-chain fatty acids (LCFAs) with an affinity in the nanomolar range [21]. Fukuwatari et al. [22] and Laugerette et al. [10] documented the expression of CD36 in rat and mouse TBC, respectively. It is highly expressed in circumvallate papillae, to a lesser extend in foliates, and scarcely in fungiform papillae. The physiological importance of CD36 in oral lipid detection is further demonstrated by the fact that *CD36* gene disruption fully suppressed qualitative and quantitative changes in pancreatobiliary secretions, triggered by lingual linoleic acid deposition in intact mice [10].

G protein-coupled receptors (GPCRs) are integral membrane proteins with seven transmembrane α-helices [23]. Sweet, bitter and umami taste sensation is initiated by binding of tastants to specific GPCRs, for instance, T1R2/T1R3 heterodimers for sweet taste [24]. Recently, knockout mice for two GPCRs, i.e., GPR120 and GPR40, have been generated and characterized for their ability to respond to LCFAs [25]. Intriguingly, both the mouse lines exhibited diminished oro-gustatory perception of LCFAs, underscoring the proposed role of these receptors. GPR120 was first detected in gustatory tissues of mice [26]. Taste receptor cells (TRC) are mainly type I (glial-like undifferentiated or immature), type II (receptor) and type III (presynaptic) cells. As far as the fat taste is concerned, the type II cells, expressing PLCβ, are the cells implicated in tastant-induced signal transduction [17]. GPR120 was shown to be predominantly expressed in type II cells of foliate and circumvallate papillae, based on co-expression of transient receptor potential melastatin-5 (TRPM5) channel subunits [25–27]. In this article, we will mainly focus on GPR120 as human fungiform and circumvallate papillae express only GPR120, but not GPR40, at protein and mRNA levels [28]. GPR40 was not detected in rat gustatory papillae [26]. Besides, in the mouse, GPR40 was mainly found in type I cells [25].

### 2.2. Fat taste signaling

The tastant-induced release of neurotransmitters toward afferent nerve fibers triggers the orosensory perception of sapid molecules. This event is known to be mediated by changes in $[Ca^{2+}]i$ in taste bud cells (TBC) [29]. Saturated and unsaturated LCFAs selectively trigger a rapid and huge increase in $[Ca^{2+}]i$ in CD36-positive TBC isolated from mouse circumvallate papillae [4,30]. We further observed that LCFAs-mediated activation of CD36 recruited $Ca^{2+}$ from endoplasmic reticulum (ER) via a phospholipase C (PLC)-dependent mechanism, as evidenced by the production of inositol-tris-phosphate, $IP_3$ [30]. Membrane ion channels that replenish cellular $Ca^{2+}$ after ER $Ca^{2+}$ depletion are regulated by phosphorylation, mediated by Src family kinases (SFKs) [31–33], which are known to directly interact with CD36 [34,35]. In mouse CD36-positive TBC, the LCFAs induced the phosphorylation of Src-PTKs, particularly of Fyn and Yes. Moreover, these SFKs were found to regulate the opening of store-operated $Ca^{2+}$ (SOC) channels, as per capacitative model of $Ca^{2+}$ homeostasis (Fig. 1). Indeed, inhibitors of PTKs and SFKs significantly diminished the LCFAs-induced increases in $[Ca^{2+}]i$ in CD36-positive TBC. Further, in these cells, addition of a fatty acid-induced membrane depolarisation as well as the release of neurotransmitters, 5-hydroxytryptamine and noradrenalin, to the extracellular environment [30]. However, it is unclear whether the release of neurotransmitters occurs within a single cell in the taste bud or if there is a requirement for cell-to-cell communication (Type II to Type III) within the taste bud prior to afferent nerve activation.

Another key participant in chemosensory transduction is the nonselective monovalent cation channel TRPM5. TRPM5 is expressed in a number of neuronal and epithelial cells, including TBC and chemosensitive cells in the gut [36]. Taste cells and some gut cells that express TRPM5 also express taste receptors and other elements of the PLCβ2-signaling stream [37]. TRPM5 channels are transiently and directly opened by small and rapid increases in $[Ca^{2+}]i$, generated during taste stimulation [37]. Liu and Liman [38] have shown that an initial increase in $[Ca^{2+}]i$ activates TRPM5 channels, favoring $Na^+$ influx responsible for the depolarisation of the cell. TRPM5 channels may be implicated in LCFAs-induced plasma membrane depolarisation. Sclafani et al. [39] have reported that TRPM5 played a key role in lipid taste perception as the invalidation of *TRPM5* gene abolished preference for fat in mice subjected to a two-bottle preference test.

The GPR120-initiated cell signaling mechanisms have not yet been elucidated and need to be characterized in TBC. However, we can cite few studies that have dealt with downstream signaling cascade via GPR120 activation in different cells types. GPCRs share common structural motifs to activate heterotrimeric G proteins, such as Gas, Gai, and Gaq. Ligands bind specifically to GPCRs to stimulate and induce a variety of cellular responses via several second messenger pathways, e.g., phospholipases, ion channels, and mitogen-activated protein (MAP) kinases [40]. G proteins are heterotrimeric proteins composed of α, β, and γ subunits localized