Do we taste fat?

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Abbreviations used : LCFA, long chain-fatty acid; PUFA, polyunsaturated fatty acids; DRK, delayed rectifying K^+ channels.

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Abstract

Sense of taste informs the body about the quality of ingested foods. Five sub-modalities allowing the perception of sweet, salty, sour, bitter, and umami stimuli are classically depicted. However, the inborn attraction of mammals for fatty foods raises the possibility of an additional oro-sensory modality devoted to fat perception. During a long time, dietary lipids were thought to be detected only by trigeminal (texture perception), retronasal olfactory, and post-ingestive cues. This minireview analyses recent findings showing that the gustation also plays a significant role in dietary lipid perception.

1. Introduction

Lipids represent around 40% of daily caloric intakes in Western diet while the nutritional recommandation is 10% lower. This chronic high-fat supply associated with a qualitative umbalance (*e.i*. excess of saturated fatty acid and cholesterol, high ω6/ω3 ratio) undoubtedtly increased the risk of obesity, and related diseases including type-2 diabetes, atherosclerosis, hypertension, and even cancers. Preference for fatty foods seems to be a common trait in mammals. Rats, and mice spontaneously prefer lipid-enriched foods in a free-choice situation [1]. Such a behavior might also to exist in humans. Indeed, data show that obese patients exhibit a higher preference for fatty foods than lean subjects [2, 3]. This last result raises the possiblity that an inappropriate orosensory perception of dietary lipids might influence obesity risk by impacting feeding behavior.

From an hedonic point of view, a specific appetite for lipids can be explained by positive fat-associated sensory qualities (fattiness, creaminess, flavour reinforcement). Spontaneous preference for lipids can also constitutes a physiological advantage especially when food is scarce by reason of nutritional roles of lipids, as high energy density nutrient, source of essential fatty acids, and carriers for fat soluble vitamins. Regulation of lipid intake is a complex phenomenon involving both early orosensory stimuli (i.e. texture, odor, taste), and delayed post-ingestive signals. During a long time, the role of gustation in the oral fat perception has been negleted, dietary fat being thought to be detected only by trigeminal (texture perception), and retronasal olfactory cues [3]. However, recent evidences strongly suggest that the gustation also plays a significant role in the dietary lipid perception in rodents. Despite limited data, it is thought that a chemosensory system devoted to oral lipid perception can also exist in Humans.

2. Anatomy of the gustatory system

In mammals, the taste system acts in concert with the olfactory, and the trigeminal systems (temperature, and texture detection) to indicate whether food can be ingested. This function is supported by taste receptor cells which are clustered in taste buds found not only in the dorsal surface of tongue, and in the soft palate, but also in the lanrynx, the pharynx, and the upper side of oesophagus. Lingual epithelium contains a high density of taste buds located in three type of gustatory papillae exhibiting distinct spatial distribution. The circumvallate, and foliate papillae are positioned in the central, and lateral regions of the posterior tongue respectively, while the fungiform papillae are found in the anterior part of the tongue (Fig. 1-A). It is noteworthy that the number of taste buds greatly varies in function of the types of

papillae : low in the fungiform, it is especially high in the circumvallate. Taste receptor cells from fungiforms make synapses with the chorda tampani nerves (cranial nerves VII), while foliates, and circumvallates are related to the glossopharyngeal nerve (cranial nerves IX). These two pairs of gustatory nerves transmit the gustatory informations to the brain (feeding behavior), and digestive tract (digestive anticipation) *via* the nucleus of solitary tract located in medulla.

3. Rodents taste fat

Combination of 3 classical behavioral paradigms, the two bottle preference test, the conditioned taste aversion, and the conditioned place preference, has allowed to progressively demonstrate the involvement of gustation in inborn preference for fatty foods both in rats, and mice. By determining volontary fluid intake, the two bottle preference test has shown that vegetable oils are prefered to mineral oil, or xantham gum, providing evidence that texture is not the salient orosensory cue [4]. This assumption is supported by experiments using the conditioned aversion test where a naive animal learns to avoid a newly encountered tastant after suffering adverse post-ingestive effects. Indeed, aversion to a sucrose/corn oil mixture cannot be produced in rats when corn oil is substituted by mineral oil, suggesting that the chemical composition of oil rather than the texture plays a significant role in the perception of this mixture [5]. Interestingly, rats previously conditioned with corn oil exhibited a stronger aversion to the sucrose/corn oil mixture than animals conditioned with sucrose, indicating that corn oil was the salient feature of mixture [5]. The conditioned place preference test is used to explore the putative rewarding effect of a molecule. This test consists to place an animal in a special cage with distinctive environmental cues, and after a conditioning period, to observe its voluntary moving towards the compartment containing a rewarding molecule. By using this paradigm, it has been demonstrated that corn oil was addictive in the mouse [6, 7]. This effect was reproduced in olfactory-blocked (anosmic) mice providing evidence that olfaction was not required for the rewarding effect of oil [7]. Moreover, the fact that a direct oil administration into the stomach was not associated with an fat addiction supports the assumption that an oral stimulation by a lipid is required to produce addiction [6]. Attraction for fatty foods is dependent from both oral, and post-oral stimuli. It is noteworthy that very short-term experiments (0.5-5 min) performed to suppress post-ingestive influences did not abolished the innate fat preference in rats [5, 8, 9]. Finally, using an experimental design excluding olfaction, textural and post-ingestive influences simultaneouly, Tohru Fushiki'team

from Kyoto University (Japan) has brought the strong evidence for a direct implication of the gustation in the oral lipid perception in rodents [7, 9].

3. Towards a progressive cracking of fat taste

Although dietary lipids consist mainly of triglycerides, long-chain fatty acids (LCFA) appear to be responsible for the oral lipid perception. In a free choice situation, rats exhibited a lower preference for triglycerides, and medium-chain fatty acids than for LCFA [8, 9]. Oral lipid perception seems to be highly sensitive to the lipid structure since LCFA derivatives (e.g. methyl-LCFA) were not recognized [8]. Ability of rats to detect specifically LCFA was recently confirmed by using the conditioned taste aversion method [10]. Moreover, the detection threshold for oleic acid, and linoleic acid appears to be very low since it is in a micromolar concentration range [10]. Lingual lipase, which is responsible for an efficient LCFA release from dietary triglycerides in murines, seems to play a significant role in oral fat perception. Indeed, pharmacological inhibition of this enzyme deeply decreases lipid preference [11]. It is noteworthy that in rodents, lingual lipase level is especially high in the vicinity of taste buds found in the posterior part of the tongue. Indeed, this enzyme is directly released in the clefts of foliate, and circumvallate papillae by the von Ebner's glands [11]. Such an anatomical feature may be sufficient to generate a LCFA signal in taste receptor cells, if a specific lipid sensor system exist.

Taste transduction signal for salty, sour, bitter, sweet, and umami takes place at the apical side of taste receptors cells. Althought molecular mechanisms involved in taste transduction remain to be fully understood, recent findings have shown that ion channels, metabotropic, and ionotropic receptors are responsible for the detection of these sapid molecules [12]. Interaction between a tastant, and its specific detection system (e.i. channel, and/or receptor) generally produces a cellular depolarisation leading to a modulation of intracellular Ca^{++} levels. These changes generate the firing of gustatory afferent nerve fibers, and produce the gustatory signal [12].

Compelling evidences indicate that LCFA are considered as sapid molecules by the peripheral gustatory system. Patch clamp recording of fungiform taste buds from rats have shown that fatty acids were able to inhibit the delayed rectifying K^+ (DRK) channels, known to be implicated in the transduction pathway of a variety of taste stimuli [13, 14]. Indeed, such a regulation induces the depolarization of taste cells inducing, *via* a rise in their intracellular Ca^{++} levels, the transmitter release. Lipid action is direct, and specific of polyunsaturated fatty acids (PUFA). Interestingly, PUFA inhibition was only effective when they were applied

extracellularly what is in good accordance with the generation of a dietary fat signal in the physiological context. This finding raises the possiblity that a gustatory fat signal might affect fat intake, and hence body mass. Consistent with this assumption, differences in PUFA-responsiness of taste receptor cells isolated from fungiform papillae were found in obesity-prone (Osborne-Mendel), and obesity-resistant (S5B/P1) rat strains using patch clamp recording [15]. DRK currents in taste cells were more inhibited by PUFA in the obesity-resistant rats than in the obesity-prone animals. According to the role of DRK channels in cell excitability, taste receptor cells from the obese-resistant strain might produce a stronger electrophysiological signal than receptor cells from obesity-prone rats in presence of PUFA. From these *in vitro* data, is might be concluded that difference in DRK responsiveness to lipids may contribute to the phenotypic difference between obesity-resistant, and obesity-prone rats. Interestingly, the former prefer carbohydrates, while the latter are fat preferring [16]. Nevertheless, the relationship between PUFA sensitivity of taste cells, and fat preference remains to be fully determined, these two strains exhibiting numerous other differences in parameters known to interfere with feeding behavior, as hormonal status [17], and sympathetic nervous activity [18].

A fat signal in taste receptor cells might also be generated by interaction of LCFA with a specific receptor. It has been shown that the lipid-binding protein CD36 is expressed on lingual sensory epithelium in rats [19]. Recent data from our laboratory support that this integral membrane protein plays a crucial role as oral lipid sensor [20]. First, CD36 appears to be restricted to the apical side of some neurosensory cells of taste buds in mouse gustatory papillae. It is especially highly expressed in the circumvallate papillae which is located in the back side of the tongue, and in a lesser extend in foliate papillae (Fig. 1-B). CD36 expression appears to be restricted to cells lining the taste pores (Fig. 1-C). This localization of a protein known to exhibit a very high affinity for LCFA [21] is particularly adaptive to generate a LCFA stimuli from dietary lipids. Indeed, CD36-positive taste cells are directly exposed to a microclimate enriched in LCFA as a result of local lingual lipase release [11]. Second, the role of CD36 as an oral lipid sensor is supported by its predicted protein structure. Indeed, CD36 exhibits an hairspin structure with a large extracellular hydrophobic pocket located between 2 short cytoplasmic tails [22, 23]. Moreover, CD36 appears to be implication in intracellular signalling cascades since its C-terminal tail has been shown to be associated with Scr kinases [24]. These receptor-like features seem to be especially adequate for the tranduction into taste receptor cells of an exogenous fat signal. An alternative possibility might be that CD36 serves as a docking site for LCFA, facilitating the closure of DRK

channels in taste receptor cells [13]. Indeed, CD36 can bind LCFA with a stoechiometry of 1 for 3 [21]. Third, CD36 gene inactivation fully abolished the innate preference for LCFA-enriched solutions, or solid diets observed in wild-type mice. This effect is lipid-specific since the sweet preference, and the bitter aversion remained unchanged in wild-type, and CD36-null mice subjected to a two bottle preference test. Fourth, CD36 affects lipid-induced cephalic phase of the digestion which prepare the digestive tract to incoming fat. Indeed, the induction of bile flux, and protein content of pancreatic juice (digestive enzymes) secondary to an oral LCFA deposition is lacking in CD36-null mice. Postingestive contribution of CD36 can be excluded in these experiments, since mice carried an oesophageal ligation to exclude any lipid ingestion. All together, these new findings demonstrate that CD36 is involved in oral LCFA detection in rodents (Fig. 2). Such a system, which favors both the consumption, and the digestion/absorption of lipids (i.e. energy-rich foods) might constitute a physiological advantage when the food is scarce. By contrast, it might contribute to obesity during plethora periods. Indeed, mice to which corn oil was given as an optional supplement to standard laboratory chow for a long-term exhibit a chronic excessive caloric intake due to an oil consumption, and develop an obesity [25].

4. Lingual fat detection in rodent : an unified working model

This rapid analysis of literature brings two main informations in apparent opposition about the orosensory perception of dietary lipids in rodents. While lipid stimulation of lingual CD36 reinforces fat preference, and contributes to the cephalic phase of digestion preparing the body to lipid incoming, lipid-mediated inhibition of DRK channels in taste cells seems to decrease fat avidity in obesity-resistant rats. Ligand, and anatomic specifities might explain these differences. Indeed, consistently with CD36 binding affinity [21], the lingual CD36-dependent system is LCFA-responsive [20], while the DRK system is strictly PUFA-dependent [14]. Moreover, CD36 is barely expressed in anterior papillae (fungiforms) which is a important place for PUFA controls of DRK channels [15]. We propose that two lipid-mediated orosensory systems co-exist in the rodent tongue. The lingual CD36 might be a lipid sensor implicated in fat taste, whereas the PUFA-dependent DRK system might plays role as a taste modulator enhancing the sensitivity to other tastants. The fact that the PUFA-mediated sensitization of taste cells induces the perception of subthreshold concentrations of saccharin in preference test in obesity resistant rats [15] is in good agreement this last assumption. Further expriments are required to validate this working model.

5. A taste for fat in human : myth or reality ?

Data on the existence of a fat taste in humans are limited, and their interpretation uncertain by reason of a great individual variability. However, recent works of the Richard Mattes's team from Purdue University (USA) strongly suggest the existence in humans of an oral lipid perception at the origin of a fat signal contributing to the cephalic phase of digestion. Indeed, subjects submitted to a load with encapsuled oil (to avoid oral fat exposure) exhibited a higher postprandial rise of blood triglycerides after a sham feeding with full-fat food than with a fat-free version [26]. Since this event is independent of texture, and olfactory cues [26-29], it was hypothesized that an orosensory perception system for dietary lipids was involved. In line with this assumption, these authors have recently reported using a protocol excluding both olfactory, and texture stimuli that patients were able to detect 18C fatty acids of varying saturation [30]. Nevertheless, the mechanism by which oral perception of lipids takes place remains to be established in humans.

6. Conclusions

Physiological basis of the fat preference is going to be deciphered in the laboratory rodents. Lingual CD36 appears to play a significant role in this feeding behavior. Unpublished data from our Laboratory show that CD36 "tastes" fatty foods through a classical gustatory pathway involving a Ca^{++}-dependent stimulation of CD36-positive taste receptor cells, the gustarory nerve route (glossopharyngeal, and chorda tampany nerves), and the activation of gustatory area in the nucleus of solitary tract. Although this finding suggests that "fatty" might constitute a basis taste, at least in mice, and rats, further experiments are required to explore the putative health impact of this orosensory system.

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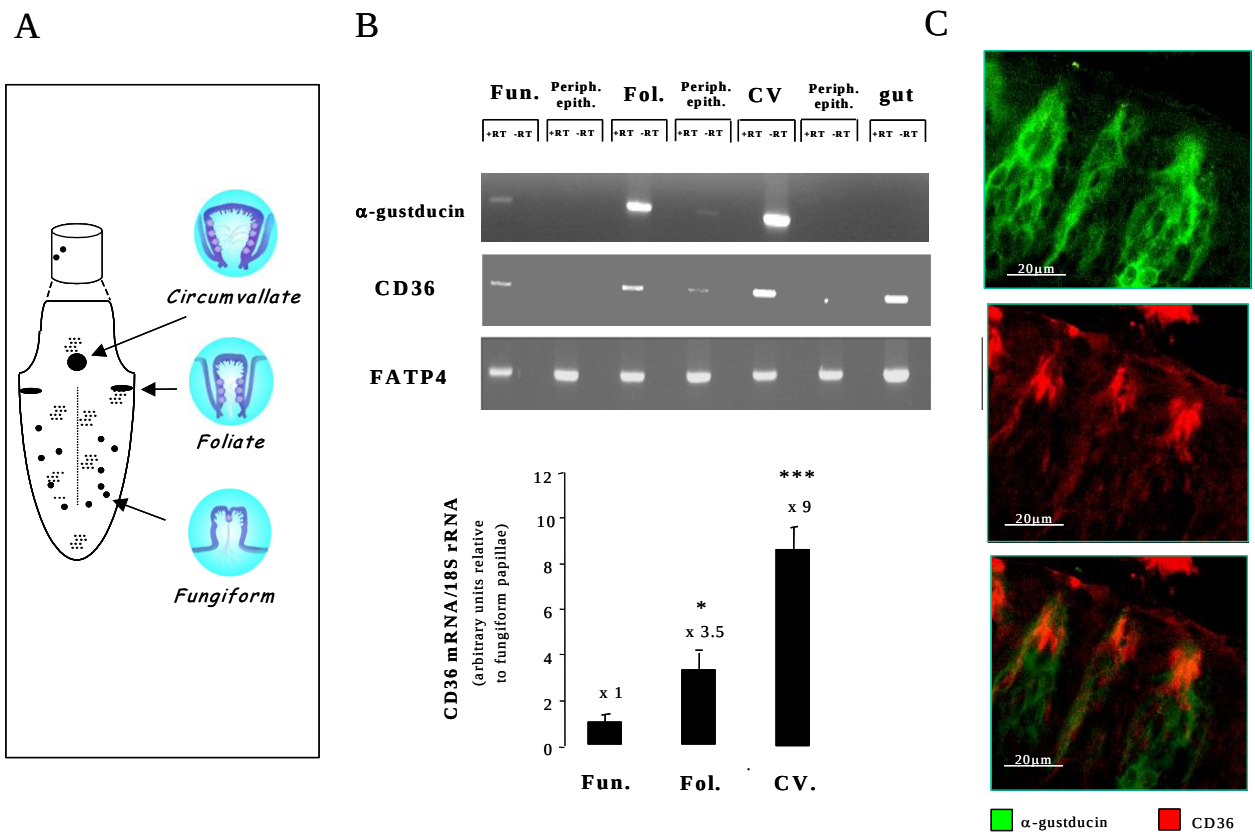

Fig. 1, Laugerette et al.

Fig. 1 : Gustatory papillae in the mouse. Fungiform (Fun.), foliate (Fol.), and circumvallate (CV.) papillae, and their respective nongustatory peripheral epithelium (Periph. epith., negative controls) were isolated under a microscope. Gut RNA was used as positive control for CD36, and FATP-4.

A - localization of gustatory papillae in the lingual epithelium.

B - Expression pattern of CD36 throughout the tongue. Upper side : RT-PCR analysis of CD36, fatty acid transfer protein-4 (FATP-4), and α-gustducin (G protein responsible for the transduction of sweet, and bitter tastes used as a taste bud marker). Lower side : CD36 quantification by the SYBR green method. Each value corresponds to a pool of total RNA from 3-5 mice.

C – Immunolocalization of CD36, and α-gustducin in the circumvallate papillae. CD36 is confined in the apical side of taste bud cells (a), while α-gustducin was found throughout the taste buds in spindle-shaped cells (b). Coexpression of CD36, and α-gustducin was found in some taste receptor cells (c).

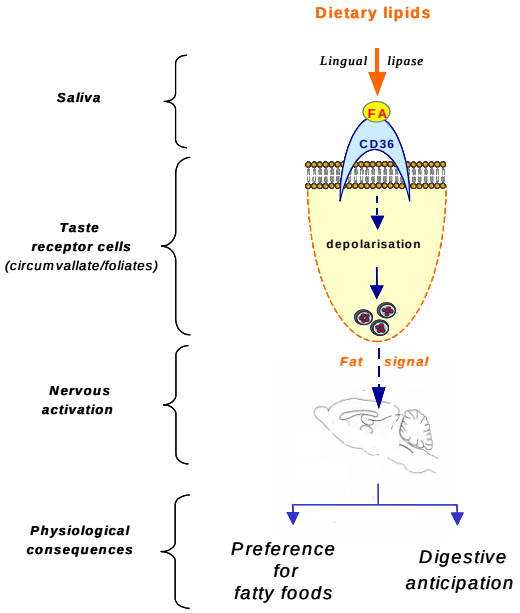

Fig. 2, Laugerette et al.

Fig. 2 : Involvement of CD36 in the orosensory perception of dietary lipids in the mouse.