Molecular pharmacology of promiscuous 7TM receptors sensing organic nutrients

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Abbreviations: 7TM, seven transmembrane; CaR, calcium-sensing receptor; CCK, cholecystokinin; CRD, cysteine rich domain; FFA, free fatty acid; GABA_B, \(\gamma\)-aminobutyric acid type B; GI, gastrointestinal; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; GPRC6A, G protein-coupled receptor family C, group 6, subtype A; IMP, 5'-inosine monophosphate; L-Asp, L-aspartate; L-Glu, L-glutamate; L-Lys, L-lysine; L-Phe, L-phenylalanine; L-Trp, L-tryptophan; mGlu, metabotropic glutamate; T1R; taste1 receptor; VFT, Venus flytrap
Abstract

A number of highly promiscuous seven transmembrane (7TM) receptors have been cloned and characterized within the last few years. Interestingly, many of these receptors are activated broadly by amino acids, proteolytic degradation products, carbohydrates or free fatty acids and are expressed in taste tissue, the gastro-intestinal tract, endocrine glands, adipose tissue and/or kidney. These receptors thus hold the potential to act as sensors of food intake regulating e.g. release of incretin hormones from the gut, insulin/glucagon from the pancreas and leptin from adipose tissue. The promiscuous tendency in ligand recognition of these receptors is in contrast to the typical specific interaction with one physiological agonist seen for most receptors, which challenges the classic “lock-and-key” concept. We here review the molecular mechanisms of nutrient sensing of the calcium-sensing receptor (CaR), the G protein-coupled receptor family C, group 6, subtype A (GPRC6A) and the taste1 receptor T1R1/T1R3 sensing L-α-amino acids; the carbohydrate-sensing T1R2/T1R3 receptor; the proteolytic degradation product sensor GPR93 (also termed GPR92); and the free fatty acid (FFA) sensing receptors FFA1, FFA2, FFA3, GPR84 and GPR120. The involvement of the individual receptors in sensing of food intake has been validated to different degrees due to limited availability of specific pharmacological tools and/or receptor knockout mice. However, as a group, the receptors represent potential drug targets, to treat e.g. type II diabetes by mimicking food intake by potent agonists or positive allosteric modulators. The ligand-receptor interactions of the promiscuous receptors of organic nutrients thus remain an interesting subject of emerging functional importance.
**Introduction**

In 1894 Emil Fischer published his landmark paper in which he for the first time described the "lock-and-key" concept for enzyme-substrate interactions (Fischer, 1894). A few decades later, this concept was transferred to receptors by Paul Ehrlich and John Newport Langley when they studied receptors and their interactions with ligands (reviewed by (Limbird, 2004; Rang, 2004). When interpreted literally the “lock-and-key” concept suggests that each receptor only has one physiological agonist, which is also in line with the naming of the vast majority of liganded receptors by their main endogenous agonist (e.g. acetylcholine receptor, glutamate receptor etc.). During the following decades receptors were shown to adhere to this scheme although some exceptions were discovered such as the adrenergic receptor subtypes responding to both endogenous epinephrine and norepinephrine albeit with different rank orders (Ahlquist, 1948). However, during the last few years a number of receptors have been cloned and characterized, which are highly promiscuous and thus respond to a range of natural agonists. In addition, several promiscuous taste receptors have been identified in tissues other than taste buds, such as the gut, suggesting that they have other functions than sensing tastants. Interestingly, these receptors respond to organic nutrients or their immediate breakdown products, i.e. fatty acids, sugars, amino acids and proteolytic products, and thus possibly serve as chemosensors for food intake (Conigrave and Brown, 2006; Egan and Margolskee, 2008; Engelstoft et al., 2008).

For decades it has been known that the organic nutrients cause release of hormones from the gut, pancreas and other organs, but the molecular nature of the chemosensors have been enigmatic. The identification of promiscuous seven transmembrane (7TM) receptors responding to the organic nutrients located in the relevant tissues has potentially identified the missing links. In that regard it makes perfect sense that these nutrient-sensing receptors have evolved to be promiscuous in order to respond to the wide range of foods digested by humans,
and it opens for the perspective that mixtures of ligands work in a concerted fashion to activate one receptor as has been shown for the calcium-sensing receptor (CaR) (Conigrave et al., 2004; Conigrave et al., 2000). An analogy may also be drawn to odor perception where just a few hundred promiscuous odorant receptors are able to perceive thousands of odorants. Each odorant may activate several odorant receptors while a complex mixture of odorants will lead to a distinctly perceived smell via combinatorial activation and even blockade of odorant receptors. Several odorants of a complex mixture can thus either act in a synergistic or antagonist fashion on each receptor (Firestein, 2004; Luu et al., 2005). However, much research still has to be performed to elucidate the physiological functions and molecular pharmacology of the 7TM chemosensors and their ligands.

A rising number of receptors thus challenge the “lock-and-key” concept. With the emergence of three-dimensional structures of enzymes and receptors co-crystallized with a variety of ligands it has become possible to get a direct view of the locks and keys and their interactions. By crystallization of the same enzyme/receptor with series of ligand analogs, it has become clear that the protein can make an induced fit to accommodate ligands and that ligands can adopt unexpected conformations in the proteins. Thus, both the locks and keys can be perceived to be "soft" (Sowdhamini et al., 1995). This, however, does not explain why some members of receptor families are exclusively activated by one endogenous agonist while others are highly promiscuous. One such example, which will be discussed in more detail later, is the family C 7TM receptors where the metabotropic glutamate (mGlu) receptors respond exclusively to L-glutamate (L-Glu) among the 20 proteinogenic amino acids (Frauli et al., 2006), and the T1R1/T1R3 taste receptor, CaR and the G protein-coupled receptor family C, group 6, subtype A (GPRC6A) receptor by contrast respond to a broad range of L-amino acids (Bräuner-Osborne et al., 2007; Conigrave and Hampson, 2006). Much more research, like X-ray crystallographic and mutational studies, are needed to elucidate the
molecular mechanisms underlying the differences in monogamous/promiscuous ligand recognition, but some insight has been gained in the last couple of years. These aspects and the molecular pharmacology of the promiscuous 7TM receptors sensing organic nutrients, or their immediate breakdown products (i.e. amino acids from proteolysis), will be the focus of the present review. In addition to the sensors of organic nutrients discussed here, an increasing number of receptors sensing compounds/hormones released upon eating (e.g. N-acylethanolamines activating GPR119 (Overton et al., 2007)) or metabolic intermediates (e.g. succinate and α-ketoglutarate activating GPR91 and GPR99 (He et al., 2004) are known. Even though some of these receptors are also promiscuous (e.g. GPR119), they are indirect/secondary food sensors and will not be discussed further.

**Family C nutrient-sensing receptors**

The family C of 7TM receptors consists of members such as the mGlu receptors, the γ-aminobutyric acid type B (GABA_B) receptor, CaR, T1Rs and GPRC6A (Bräuner-Osborne et al., 2007). These receptors are characterized by a large extracellular Venus flytrap (VFT) domain, revealed by crystallography to contain a dimerization interface and an orthosteric binding site for the endogenous agonist (Kunishima et al., 2000; Muto et al., 2007; Tsuchiya et al., 2002). The VFT domain is connected to the 7TM, G protein-activating domain via a cysteine-rich domain (CRD) (for an overview on topology, refer to Fig. 1). Inferred from the observation that the family C VFT domain is phylogenetically related to a class of bacterial periplasmic binding proteins (Felder et al., 1999; O’Hara et al., 1993), it has been proposed that the agonist binding and signaling domains of family C receptors were once two separate entities that merged during evolution (Conklin and Bourne, 1994). Interestingly, the periplasmic binding proteins are involved in nutrient uptake (Quirocho and Ledvina, 1996).
which would suggest that family C 7TM receptors have preserved binding sites for nutrient-like compounds. Family C receptors serve diverse functions, ranging from neurotransmission by the mGlu and GABA_B receptors and regulation of calcium homeostasis by CaR (Bräuner-Osborne et al., 2007). Still, all identified endogenous agonists for family C receptors are in fact nutrient-like, being amino acids, ions, and sugars. They all bind in the VFT domain and thereby elicit domain closure followed by an activational twist in the 7TM domain (Wellendorph and Bräuner-Osborne, 2009). In addition, several compounds binding at allosteric sites in both the VFT domain, the CRD and the 7TM domain have now been identified, allowing for fine-tuning of the agonist response. A generalized illustration of known family C ligand binding sites is presented in Fig. 1.

Whereas the mGlu receptors are monogamous for L-Glu (Frauli et al., 2006), a phylogenetically distinct group of the family C 7TM receptors comprised by CaR, T1R taste receptors and GPRC6A (Bjarnadóttir et al., 2005; Kuang et al., 2006; Wellendorph and Bräuner-Osborne, 2009), are promiscuous by nature and respond to subsets of L-α-amino acids and divalent cations (CaR, GPRC6A and the heterodimeric receptor T1R1/T1R3) or sugars and D-amino acids (the heterodimeric receptor T1R2/T1R3) (Fig. 2). Such promiscuity for nutrients by family C 7TM receptors in relevant tissues (Fig. 3) allows for a nutrient-sensing capacity of emerging physiological significance (for reviews see (Conigrave and Hampson, 2006; Rozengurt and Sternini, 2007; Sternini et al., 2008)).

**Promiscuous L-amino acid-sensing receptors**

Appreciation of L-amino acid binding in the VFT orthosteric binding pocket of family C receptors has come primarily from X-ray crystal structures of the L-Glu-bound mGlu_1 and mGlu_3 VFT domains (Kunishima et al., 2000; Muto et al., 2007), leading to the identification of five residues in the mGlu receptors that are particularly important for binding of the L-α-
amino acid moiety of L-Glu, and two basic residues that are vital for binding of the distal carboxylic acid of L-Glu. Whereas the five residues responsible for L-α-amino acid recognition are highly conserved, the two basic residues, for binding the distal end of L-Glu are not conserved to CaR, GPRC6A and T1R1 (Acher and Bertrand, 2005; Conigrave and Hampson, 2006; Wellendorph and Bräuner-Osborne, 2009). This observation is consistent with the ability of the latter three receptors to accommodate many different amino acid side chain functionalities in their binding pockets. However, some level of selectivity in amino acid-binding profiles of the individual receptors is introduced by differences in the distal end of the binding pocket.

Although the non-mGluR amino acid sensing-receptors are rather nonselective in their ligand profiles, in vitro studies have shown that they each have a preference for a subset of classes of amino acids (as presented below), potentially providing a means for covering responses to all of the 20 proteinogenic L-amino acids (Conigrave and Hampson, 2006; Wellendorph and Bräuner-Osborne, 2009). However, as well stated in the review by Conigrave and Hampson, it is important to keep in mind that the in vitro-determined apparent selectivity for some L-amino acids over others does not necessarily translate into the in vivo situation where the plasma concentrations of individual amino acids vary up to 10-fold. Also, in relation to the topic of this review, it should be kept in mind that the concentration of free L-amino acids in the stomach and gut may rise as high as to approach millimolar concentrations (Adibi and Mercer, 1973).

Molecular mechanisms for L-amino acid sensing by CaR

As the name implies, the primary physiological agonist for CaR is extracellular Ca\(^{2+}\) which is sensed by tissues relevant for maintaining calcium homeostasis such as the kidney and the parathyroid gland (Brown, 2007; Tfelt-Hansen and Brown, 2005). However, organs not
involved in regulating calcium homeostasis also express CaR, including nutrient-sensing organs such as the stomach, the lower gastrointestinal (GI) tract, liver and pancreas (Fig. 3). Within the last few years, several reports have emerged that demonstrate a role for CaR in protein/amino acid sensing, including CaR-mediated L-amino acid-stimulated gastric acid release from stomach parietal cells (Busque et al., 2005), cholecystokinin (CCK) release from duodenal enteroendocrine cells (Hira et al., 2008), and inhibition of parathyroid hormone release from human parathyroid cells (Conigrave et al., 2004). Also just recently, CaR expression was described in taste tissue of the tongue suggesting that the receptor may sense calcium and even amino acid taste (San Gabriel et al., 2009). The most potent amino acids at CaR in vitro are the aromatic amino acids such as L-phenylalanine (L-Phe) and L-tryptophan (L-Trp), followed by aliphatic and polar amino acids whereas acidic, basic, and branched-chain amino acids are weak or inactive (Fig. 4) (Conigrave et al., 2000). Notably, amino acids work as allosteric enhancers at CaR, requiring a certain level of extracellular Ca$^{2+}$. Experiments have detailed that in vivo L-amino acid sensing per se is enabled at physiological concentrations of Ca$^{2+}$ (around 1 mM) (Conigrave and Hampson, 2006; Conigrave et al., 2004).

As mentioned above, family C receptors, including CaR, contain a highly conserved 5-residue motif binding site in their VFT domain which is predicted to bind the α-amino acid moiety of L-α-amino acids. Whereas molecular pharmacology techniques have confirmed that the amino acid binding site resides in the VFT (Mun et al., 2004; Zhang et al., 2002a), it has been inherently difficult to pinpoint residues specifically involved in amino acid binding and activation, as the amino acids require the presence of Ca$^{2+}$ to work and many of the examined mutations simultaneously reduce Ca$^{2+}$ sensitivity due to partly overlapping binding sites (Silve et al., 2005; Wellendorph and Bräuner-Osborne, 2009) (Fig. 1). Mutation studies have predicted several Ca$^{2+}$ binding sites in the cleft of the VFT (Bräuner-Osborne et al., 1999;
Huang et al., 2007), but Ca\textsuperscript{2+} could also potentially bind between the two bilobed VFT domains stabilizing the closed conformation, as has been shown for Gd\textsuperscript{3+} in mGlu\textsubscript{1} (Fig. 1) (Tsuchiya et al., 2002). Mun et al. identified two mutations, T145A and S170T that specifically impair amino acid sensing while leaving Ca\textsuperscript{2+} sensing intact (Mun et al., 2005). Others have also identified the three serines S169-171 as being important for amino acid binding (Lee et al., 2007; Zhang et al., 2002b). With regards to the distal end of the binding pocket, it remains to be investigated which residues participate in binding of for instance the aromatic moieties of L-Phe/L-Trp. In fact the binding is flexible and large enough to accommodate even the small peptide glutathione (Wang et al., 2006b).

CaR can also be allosterically modulated by small synthetic molecules acting at the 7TM domain (Fig. 1 and Fig. 5). Both positive modulators, termed calcimimetics (e.g. NPS R-568 and calindol), and negative modulators, termed calcilytics (e.g. NPS 2143, Calhex 231 and cinacalcet), have been described (Kessler et al., 2006; Nemeth et al., 2001; Nemeth et al., 1998; Petrel et al., 2003). Binding sites identified thus far for all of these are overlapping, residing within a crevice formed by transmembrane helices TM3, TM5, TM6, and TM7 (Miedlich et al., 2004; Petrel et al., 2004; Petrel et al., 2003). Potentially, compounds of this sort may present novel drugs for affecting nutrient sensing by CaR and subsequent regulating exocrine secretion of various hormones.

*Molecular mechanisms for L-amino acid sensing by GPRC6A*

GPRC6A is a promiscuous L-amino acid receptor cloned just five years ago and for which we are only now beginning to understand the physiological role. The generation of the first GPRC6A knock-out mice will undoubtedly aid in this endeavor (Pi et al., 2008; Wellendorph et al., 2009). Given the strikingly broad sensitivity to L-amino acids and the expression of GPRC6A in organs and tissues such as taste tissue, pancreatic islets, liver, skeletal muscle,
bone and fat (Fig. 3) (Kuang et al., 2005; Pi et al., 2005; Regard et al., 2007; Wellendorph and Bräuner-Osborne, 2004; Wellendorph et al., 2007; Wellendorph et al., 2009), GPRC6A represents a plausible nutrient-sensing receptor (Conigrave and Hampson, 2006). In further support of this, the closely related goldfish odorant receptor 5.24 functions as a nutrient sensor for L-amino acids in sensory tissue of the goldfish (Speca et al., 1999). Pertaining to a possible role for GPRC6A in metabolism, mice lacking GPRC6A, generated by Quarles and co-workers, are reported to have complex metabolic abnormalities including hepatic steatosis, hyperglycemia, glucose intolerance, and insulin resistance, reduced testosterone levels and obvious feminization of male mice, and an osteopenic phenotype (Pi et al., 2008), although no bone phenotype was observed in GPRC6A knock-out mice generated in our laboratory (Wellendorph et al., 2009). So far, the role of GPRC6A in the cells of the GI tract has not been investigated.

GPRC6A responds stereoselectively to 6-8 of the 20 proteinogenic L-amino acids, with some preference for basic amino acids, but may also be activated by small and neutral amino acids (Fig. 4) (Christiansen et al., 2007; Kuang et al., 2005; Wellendorph et al., 2007; Wellendorph et al., 2005). As observed for T1R1/T1R3, amino acid potency rank-orders and efficacies differ among orthologs of mouse, rat and human receptors (Wellendorph et al., 2007). Using heterologous expression systems, we and others have found that the L-α-amino acid response of GPRC6A is augmented by divalent cations Ca^{2+} and Mg^{2+} in physiologically relevant concentrations (Christiansen et al., 2007; Kuang et al., 2005; Wellendorph et al., 2007) and one report has even demonstrated a direct activation of GPRC6A by Ca^{2+} (Pi et al., 2005). GPRC6A can thus be speculated to act in concert with CaR, supposedly in a reciprocal fashion, to sense a wide range of both L-amino acids and Ca^{2+} in plasma or perhaps even in the GI tract. Several synthetic L-α-amino acids carrying positively charged side chains, and coincidentally affecting the nitric oxide synthase and arginase isoenzymes, are also agonists at
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GPRC6A (Christiansen et al., 2006b), whereas to date, no competitive antagonists or allosteric modulators have been identified at GPRC6A.

Based on the assumption that L-lysine (L-Lys) binds in the VFT domain of GPRC6A as does L-Glu in mGlu1, homology modeling has identified conserved residues known to interact with the \( \alpha \)-amino acid moiety of L-Lys GPRC6A, two of which have been validated (S149 and T172) by mutagenesis studies (Wellendorph et al., 2005). The presence of the archetypical family C 5-residue recognition motif has also been substantiated by inactivating mutations in the homologous goldfish 5.24 receptor (Kuang et al., 2003; Luu et al., 2005). By contrast, the precise environment of the distal end of the GPRC6A binding pocket is unaccounted for and the residue(s) responsible for binding the positively charged end of the basic amino acid agonists are not conserved from 5.24 (Wellendorph and Bräuner-Osborne, 2009). Neither has the Ca\(^{2+}\) binding site been mapped. Thus in contrast to the GPRC6A receptor, 5.24 appears to be less spatially restricted in the distal binding pocket corresponding with the fact that it responds to a broader range of L-\( \alpha \)-amino acids (Christiansen et al., 2006a).

*Molecular mechanisms for L-amino acid sensing by T1R1/T1R3*

The T1R class of 7TM receptors consists of three subunits: T1R1, T1R2 and T1R3. In order to form functional receptors, the individual subunits T1R1 and T1R2 depend on co-expression and dimerization with T1R3 (Xu et al., 2004; Zhao et al., 2003). The heterodimer T1R1/T1R3 perceives L-amino acids and functions as an L-amino acid taste receptor in taste buds of the tongue and soft palate where it is highly expressed (Hoon et al., 1999). T1R1/T1R3 is also called the umami taste receptor (umami is the savory taste of L-Glu (and L-aspartate (L-Asp))) (Li et al., 2002; Nelson et al., 2002). For a complete picture, it should be mentioned that there is also biochemical and physiological evidence for at least one other umami
receptor (Damak et al., 2003), and one purported candidate is a truncated version of the mGlu4 receptor, although this “umami” receptor is not potentiating by purinic ribonucleotides such as 5′-inosine monophosphate (IMP) (Chaudhari et al., 2000), a hallmark of umami taste (Yamaguchi, 1991). Taste signal transduction is largely mediated by the G protein gustducin (Ruiz-Avila et al., 2001), and the finding that both α-gustducin (Höfer et al., 1996) and the different T1R subunits are expressed in the GI tract (Bezençon et al., 2007; Dyer et al., 2005) (Fig. 3), suggests the presence of taste-sensing mechanisms in the gut, more appropriately referred to as chemosensing (reviewed by (Rozengurt and Sternini, 2007)). In fact, very recent studies have provided the first functional demonstration of T1R1/T1R3 in chemosensing (nutrient absorption) based on the ability of the receptor to down-regulate expression of the oligopeptide transporter, PepT1, and upregulate expression of the glucose transporter GLUT2 and the L-Glu/L-Asp transporter EAAC1 (Mace et al., 2009). Interestingly, the sweet sensing T1R2/T1R3 is also able to decrease PepT1 expression and increase GLUT2 expression in the jejunum through activation of the same intracellular signaling molecule (PKC βII) (Mace et al., 2009). It is thus suggested that a taste receptor-coordinated transport network exists within the GI tract which cross-regulates expression of nutrient transporters (i.e. sugars regulate expression of amino acid/peptide transporters via T1R2/T1R3 and amino acids regulate glucose transport via T1R1/T1R3), which could well incorporate other nutrients as well (Mace et al., 2009).

T1R1/T1R3 responds broadly to aliphatic including branched-chain, amidic, charged, sulfur- and hydroxyl-containing L-amino acids but not to aromatic amino acids. The signaling of T1R1/T1R3 is augmented dramatically by IMP, and all L-amino acids but L-Trp can activate the receptor in the presence of IMP (Fig. 4) (Nelson et al., 2002). There are, however, notable species differences between human and rodent T1R1/T1R3. Whereas the human receptor is more than an order of magnitude more sensitive to L-Glu than to other amino
acids, and is also sensitive to the synthetic L-Glu analogue L-AP4 in the presence of IMP, the
rodent orthologs have approximately equal sensitivity to L-Glu and the other L-amino acids
and no sensitivity to L-AP4 (Li et al., 2002; Nelson et al., 2002).

Since L-Glu and IMP have no effect on the T1R2/T1R3 sweet taste receptor, it is
inferred that these compounds bind to the VFT domain of T1R1 (Li et al., 2002; Xu et al.,
2004), recently confirmed by mutagenesis studies (Zhang et al., 2008). Hence, the differences
in ligand sensitivity may likely be assigned to the rather low percentage sequence identity
(70% amino acid identity between rodent and human T1R1s (Nelson et al., 2001)). The work
by Zhang et al. presents a homology model for T1R1 and confirms by mutagenesis the
involvement of four of the five key residues predicted to bind the α-amino acid moiety (Acher
and Bertrand, 2005; Wellendorph and Bräuner-Osborne, 2009). Furthermore, they identify
residues responsible for the binding of IMP and map the purinic ribonucleotide binding site at
an allosteric site adjacent to the L-Glu site (Fig. 1). Binding of IMP is proposed to stabilize
VFT domain closure by electrostatic interactions between the charged phosphate group of
IMP and a cluster of positive charges on the other lobe (Zhang et al., 2008). Other allosteric
small molecules acting at the T1R1/T1R3 receptor include the allosteric enhancer cyclamate
and the negative modulator lactisole. Both these ligands bind in the 7TM domain at
overlapping sites, similar in location to the CaR 7TM modulator site (Cui et al., 2006) (Fig.
1).

Carbohydrate-sensing promiscuous 7TM receptors

As already outlined for L-amino acid-sensing taste receptors, the molecular machinery for
mediating taste sensation is also present in the GI tract and in enteroendocrine cells. Thus far,
the only described sweet sensing receptor is the heterodimeric receptor T1R2/T1R3 which
displays an intriguingly broad sensitivity for naturally occurring sweet substances such as
glucose, fructose, sucrose, and sweet-tasting D-amino acids but also for synthetic sweeteners like aspartame (NutraSweet®), cyclamate, saccharin, and acesulfam K (Li et al., 2002; Nelson et al., 2001). T1R2/T1R3 is expressed both in taste buds of the oral cavity (Nelson et al., 2001), but also throughout the GI tract (Bezençon et al., 2007; Dyer et al., 2005; Mace et al., 2009). Thus, the gut senses sweet stimuli, and within the last couple of years exciting links between dietary sugars and chemosensing have surfaced, notably the regulation of glucose absorption (Le Gall et al., 2007; Mace et al., 2007; Mace et al., 2009; Margolskee et al., 2007) and enteroendocrine GLP-1 hormone secretion (Jang et al., 2007).

The fact that T1R2/T1R3 is so promiscuous for sweet-tasting substances suggests that the binding site is not very restricted. In order to localize the orthosteric binding site in the T1R2/T1R3 heterodimer for the synthetic sweeteners aspartame and neotame, chimeric receptor studies have been particularly useful and clarified that the VFT domain of T1R2 is the subunit responsible for binding (Fig. 1). Alignments of T1R2 and mGlu1 show that 3 of the 5 residues predicted to bind the α-amino acid moiety of the ligands are conserved (Li et al., 2002). Correspondingly it has been verified by mutational analyses that neotame, aspartame and sweet-tasting D-amino acids (for instance D-Trp) bind via residues S144 and E302 of the human T1R2 (Xu et al., 2004), corresponding to residues binding respectively the carboxylate group and amino group of the α-amino acid function of L-Glu in mGlu1 (Wellendorph and Bräuner-Osborne, 2009). Interestingly, the stereoselectivity for amino acids is reversed at T1R2/T1R3 compared to T1R1/T1R3, i.e. only the D-forms but not the L-forms of i.e. Phe and Asn can activate T1R2/T1R3 (Nelson et al., 2002).

In addition to compounds acting in the orthosteric site of the VFT domain, structurally diverse compounds acting at allosteric sites can stimulate or inhibit receptor signaling (Fig. 1 and Fig. 5). Compounds such as cyclamate and lactisole act at the common subunit T1R3 and are thus able to affect receptor signalling of both umami and sweet taste receptors but do this
by different modes of interaction. Cyclamate acts as an allosteric agonist at the T1R3 subunit of T1R2/T1R3, and notably, exclusively at the human T1R3 (Jiang et al., 2005b). By contrast, cyclamate acts as an allosteric enhancer at the T1R3 subunit of T1R1/T1R3, thus requiring the presence of an orthosteric agonist such as Glu (Xu et al., 2004). Also, the allosteric compound lactisole acts differently depending on the heterodimeric partner of T1R3, and appears to be a competitive antagonist at sweet-T1R3 but a negative allosteric modulator at umami-T1R3 (Xu et al., 2004). Both cyclamate and lactisole act at defined allosteric sites in the 7TM region (Jiang et al., 2005a; Jiang et al., 2005b). However, as these small molecules are not selective there is a need for allosteric compounds acting selectively in the 7TM regions of T1R1 and T1R2. Recently Zhang et al. have identified such small molecules, S807 and S819, respectively (Fig. 5), from a high-throughput screening effort (Zhang et al., 2008). These compounds are interesting as pharmacological tool compounds and might have future relevance as artificial sweeteners or therapeutics.

Finally, adding to the list of allosteric modulators and unique sites for family C 7TM receptors, the sweet-tasting protein brazzein is an allosteric agonist of T1R1/T1R3 with a novel binding site residing in the CRD (Jiang et al., 2004).

**Family A nutrient-sensing receptors**

In addition to the family C 7TM receptors, several family A 7TM receptors are promiscuous in their ligand preferences, and several respond to organic nutrients from food, notably protein degradation products and free fatty acids (FFAs).

**Promiscuous protein/proteolysis-sensing receptors**

*GPR93 (IUPHAR name GPR92)*
The family A receptor GPR93 was fairly recently described as a nutrient-sensing receptor responsive to peptone, a peptide mixture resembling proteolytic degradation products (Fig. 2). GPR93 is highly expressed in the small intestine (Choi et al., 2007a) and studies have suggested it as the missing link for a previously described protein hydrolysate-mediated CCK expression and release in intestinal cells (Nemoz-Gaillard et al., 1998; Nishi et al., 2001). Thus it has been shown that peptone stimulation of GPR93 in enterocytes and enteroendocrine cells lead to G protein-signaling cascades ultimately promoting CCK gene transcription and CCK release (Choi et al., 2007a; Choi et al., 2007b).

Despite the fact GPR93 is most closely related to purinoreceptors and FFA1 (see below) by 25-32% amino acid identities, it is not activated by nucleotides or FFAs (Choi et al., 2007b) but displays nanomolar potency for lysophosphatidic acid (Kotarsky et al., 2006; Lee et al., 2006). A pronounced synergistic effect between peptone and lysophosphatidic acid implies that these substances act at separate sites in the receptor (Choi et al., 2007b), although the mechanism for the activation of GPR93 remains to be determined. It would be highly relevant to further elucidate the physiological role of GPR93 in nutrient sensing by knock-down in cell lines or knock-out of the receptor \textit{in vivo}.

**Free fatty acid-sensing receptors**

FFAs are known to possess a wide range of physiological effects, typically recognized as functions mediated by actions on cellular metabolism. However, not all biological effects can be ascribed to intracellular metabolism. Some of the effects instead bear the characteristics of cell surface receptor involvement (Louet et al., 2001; Sauer et al., 2000). Thus, the recent deorphanization of several receptors activated by FFAs, belonging to the family A of 7TM receptors, has offered an alternative mechanism of action for FFAs. Moreover, the action of
FFAs on cell surface receptors is known to play significant roles in the regulation of food intake.

A variety of medium- and long-chain FFA has been identified as ligands for the FFA1 (previously termed GPR40), GPR84 and GPR120 receptors (Briscoe et al., 2003; Hirasawa et al., 2005; Itoh et al., 2003; Kotarsky et al., 2003; Wang et al., 2006a), whereas short-chain FFAs activate FFA2 and FFA3 (previously termed GPR43 and GPR41, respectively) (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). The short-chain FFAs are produced by anaerobic fermentation of dietary carbohydrate fibers, and FFA2 and FFA3 could thus be classified as carbohydrate intake sensors rather than lipid intake sensors (Fig. 2). FFA1, FFA2 and FFA3 share a relatively limited degree of sequence similarity (33-43% amino acid identity), while the GPR84 and GPR120 receptors are distantly related to each other and other family A receptors (Gloriam et al., 2007).

The FFA1 receptor

A wide range of medium- and long-chain FFAs have been identified as agonists on the FFA1 receptor, with eicosatriynoic acid being the most potent example (Table 1) (Briscoe et al., 2003; Itoh et al., 2003; Kotarsky et al., 2003). Intriguingly, the carbon chain lengths of the saturated fatty acids correlate with the potency, with pentadecanoic acid (C15) and palmitic acid (C16) being the most potent. In contrast, the chain length or degree of saturation does not correlate with the potency of the unsaturated fatty acids (Briscoe et al., 2003). The EC50 values in the micromolar range might seem high, however the blood plasma concentration of the medium- and long-chain FFAs covers this range (Swaminath, 2008). Thus, the FFA1 receptor could be a nutrient sensor for FFAs generated by hydrolysis of ingested fat and oil, which is consistent with the expression of the receptor in the islets of Langerhans from the pancreas and the gut (Fig. 3) (Briscoe et al., 2003; Edfalk et al., 2008; Kotarsky et al., 2003;
Ma et al., 2008). This hypothesis has been substantiated by several studies using FFA1 knockout mice and selective FFA1 small molecule agonists, showing that FFA1 regulates the FFA-mediated release of gastric inhibitory peptide (GIP) and GLP-1 from the gut and the FFA-mediated enhancement of insulin release from β-cells of the pancreas (Briscoe et al., 2006; Christiansen et al., 2008; Edfalk et al., 2008; Itoh et al., 2003; Steneberg et al., 2005; Tan et al., 2008).

Little is known about the molecular mechanism of the ligand interaction with the FFA1 receptor, but it seems that the carboxylic group of the FFA is required for receptor activation, since the methyl ester of linoleic acid is unable to activate FFA1, while linoleic acid is an activating ligand (Itoh et al., 2003). Likewise, many potent small molecule FFA1 ligands with a carboxylic acid residue and only few other functional groups have been developed (Briscoe et al., 2006; Christiansen et al., 2008; Garrido et al., 2006; Tikhonova et al., 2008), which suggest that the FFAs and these small molecules share an overlapping binding site anchored by the carboxylic acid (Sum et al., 2007; Tikhonova et al., 2008; Tikhonova et al., 2007). Molecular modeling and mutagenesis studies have pointed to three polar residues in the 7TM binding cavity as the potential anchor point of the carboxylic head group of both linoleic acid and the small molecule agonist GW9508 (Fig. 5) (Sum et al., 2007; Tikhonova et al., 2007). These amino acids (R183, N244 and R258) are situated at the top of transmembrane segments 5, 6 and 7, respectively. Especially the residues R258 and N244 are known to be important, as they are required for achieving full potency and efficacy for linoleic acid and essential for any activation by GW9508 (Sum et al., 2007). Furthermore, a number of other residues have been identified to be involved in FFA1 receptor activation by long-chain FFAs, such as H86 and H137 that could form aromatic contacts with GW9508 (Sum et al., 2007; Tikhonova et al., 2007). Additionally, the residue T91 has been implicated to be responsible for the greater potency of the synthetic agonist GW9508 compared to that of
the endogenous linoleic acid, as this residue can make a hydrophilic interaction with GW9508 but not with linoleic acid (Sum et al., 2007; Tikhonova et al., 2007). The residues H137, R183, R258 of the human FFA1 are conserved in FFA2 and FFA3, and the polarity of the residue corresponding to N244 in the human FFA1 is also retained in FFA2 and FFA3 (as H242 and H245, respectively). The corresponding arginine residues in FFA2 and FFA3 have recently been identified by a mutational study to be very important for the activation of the receptors by FFAs (Stoddart et al., 2008a). Thus it is conceivable that the carboxylate group of the FFA interacts in a similar fashion with FFA2 and FFA3 as it does with FFA1.

**FFA2 and FFA3 receptors**

In 2003, short-chain FFAs were identified as ligands for both FFA2 and FFA3 by three independent groups (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). The potencies of FFAs with different chain lengths are somewhat similar, as formate, acetate, propionate, butyrate and pentanolate activate both FFA2 and FFA3 (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). However, pentanolate is more potent at FFA3 than acetate, in contrast to FFA2, where acetate is more potent than pentanolate (Table 1) (Brown et al., 2003; Le Poul et al., 2003). Recently, the first potent and selective FFA2 agonist, phenylacetamide 1 [(S)-2-(4-chlorophenyl)-3-methyl-N-(thiazol-2-yl)butanamide] (Fig. 5), was identified by high-throughput screening (Lee et al., 2008). Interestingly, phenylacetamide 1 is an allosteric agonist as the compound is both able to activate FFA2 alone and to enhance the response of acetate and propionate. Molecular modeling and docking studies have suggested that the carboxylate group of the FFAs bind to residues at the top of TM5 (Arg180), TM6 (His242) and TM7 (Arg255) as described for FFA1 above, whereas phenylacetamide 1, containing no carboxylate group, binds to an adjacent non-overlapping pocket (Lee et al., 2008).
Analysis of mRNA levels in tissues has revealed that FFA3 mRNA is primarily found in immune cells such as neutrophils, monocytes and B-lymphocytes (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). Significant levels of FFA3 mRNA is also found in immune tissues such as bone marrow and spleen, which could be due to the high concentration of immune cells in these tissues (Le Poul et al., 2003). Additionally, FFA3 has also been reported to be expressed in adipose tissue (Xiong et al., 2004). The FFA2 displays a more widespread expression pattern when compared to FFA3, with the highest expression in adipose tissue, pancreas, spleen, lymph nodes and bone marrow (Fig 3.) (Brown et al., 2003; Le Poul et al., 2003).

When the FFA1-3 receptors were initially cloned, a fourth gene, GPR42, was cloned from the same cluster (Sawzdargo et al., 1997). The GPR42 receptor shares 98% sequence identity with FFA3, but is not activated by FFA and thus hypothesized to be a pseudogene (Brown et al., 2003). A mutagenesis analysis of the six amino acids that differ between the human FFA3 and GPR42, identified R174 in the second extracellular loop as an essential residue for activation (Brown et al., 2003). When R174 was mutated in FFA3 to its respective GPR42 residue, tryptophan, the receptor could not be activated by propionate anymore. Conversely, introduction of R174 into GPR42 enabled the receptor to be activated by propionate (Brown et al., 2003). Presumably this R174 residue forms a salt bridge with the carboxylate ligand (Brown et al., 2003) albeit the arginine residue is not conserved to FFA1 and FFA2 and thus not part of the previously discussed common anchor site.

GPR84 and GPR120

Two additional 7TM receptors, GPR84 and GPR120, have recently been shown to be activated by FFAs but have so far not been studied in as great detail as FFA1-3. The GPR84 receptor responds to medium-chain FFA (C9-C14) and is expressed in leukocytes and
monocytes/macrophages (Wang et al., 2006a). The receptor has only been the subject of one pharmacological study and very little is thus known about the receptor at the present stage. However, its expression pattern suggests it may be involved in linking fatty acid metabolism to immunological regulation (Wang et al., 2006a).

Like FFA1, GPR120 is a receptor for both saturated (C14 to C18) and unsaturated (C16 to C22) fatty acids (Table 1), and also require the carboxylate group in the ligand for activation (Briscoe et al., 2006; Hirasawa et al., 2005; Tanaka et al., 2008). GPR120 mRNA and protein has been detected in the intestine, adipose tissue and lung (Fig. 3) (Hirasawa et al., 2005; Miyauchi et al., 2009; Tanaka et al., 2008). Interestingly, the FFA α-linolenic acid has been shown to stimulate release of glucagon-like peptide-1 (GLP-1) in STC-1 intestinal endocrine cells and in vivo in mice and rats (Hirasawa et al., 2005; Miyauchi et al., 2009). However, α-linolenic acid is an agonist of both GPR120 and FFA1, which are both expressed in the intestine (Fig. 3, Table 1), and the latter has also been shown to mediate GLP-1 release as previously mentioned. Given the present lack of GPR120 specific ligands and/or a GPR120 knockout mouse, the precise role of GPR120 in mediating GLP-1 release and other physiological effects remain to be shown in vivo.

Interestingly, the N/H, R, R residues at the top of TM5, TM6 and TM7, anchoring the carboxylate group in FFA1-3 are absent in GPR84 and GPR120 (D. Gloriam, personal communication) suggesting that the binding mode of FFAs in the GPR84 and GPR120 receptors is different from FFA1-3. This is in line with a recent study of small molecule GPR40/GPR120 agonists where modeling suggests that the carboxylate group of these agonists are anchored by R99 at the top of TM2 (Suzuki et al., 2008). It is thus plausible that the carboxylate group of FFAs are anchored in the same position, but the role of R99 remains to be validated by mutagenesis for both FFAs and the small molecule agonists.
Therapeutic perspectives

It is tempting to hypothesize that the metabolic syndrome and diabetes could be treated by activating the organic nutrient sensors thereby "tricking" the body to believe it has eaten, which would initiate physiological effects such as release of hormones from the gut (e.g. GLP-1 and GIP), pancreas (e.g. insulin) and fatty tissue (e.g. leptin). It might not be that simple, but many of the promiscuous receptors discussed in the review are expressed in relevant organs (Fig. 3) and now receive increased focus as potential drug targets. As noted throughout the review, selective ligands and/or genetically modified mice are now becoming available for some of the receptors, which is making it possible to address their physiological roles as organic nutrient sensors. These tools are most advanced for FFA1 and have shown very interesting results in terms of potential treatment of diabetes/obesity via release of GLP-1 and insulin (Christiansen et al., 2008; Stoddart et al., 2008b). However, the FFA1 receptor might also mediate the long-term toxicity of FFAs as shown in some but not all studies using FFA1 knockout mice (Brownlie et al., 2008). More studies are thus needed to validate the FFA1 receptor as a drug target for long-term treatment of obesity/diabetes.

A second case showing that the road to novel drugs might not be that straightforward is the T1R2/T1R3 receptor which was recently found to mediate GLP-1 release from the gut by sugars and artificial sweeteners (Jang et al., 2007). One would thus think that the artificial sweeteners could be used as drugs to treat type 2 diabetes via the GLP-1 mediated increase in insulin secretion and decrease in glucagon secretion, gastric emptying and appetite (Holst et al., 2008). However, epidemiological studies have pointed to increased incidence of obesity and/or metabolic syndrome in diet soft-drink consumers (Dhingra et al., 2007; Lutsey et al., 2008). Consequently, additional studies are needed to sort out the therapeutic potential of the T1R2/T1R3 receptor.
The field of nutrient-sensing 7TM receptors is evolving quickly and it is thus an exciting time as we have just begun to understand the molecular pharmacology, physiological function and therapeutic potential of the receptors described in the present review - and other related 7TM receptors which yet remain to be deorphanized.
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Footnotes:

1 These authors contributed equally.

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Legends for figures.

**Fig. 1.** Model of a dimeric family C 7TM receptor in its open-closed/active conformation. The localizations of the Venus flytrap (VFT) domain, cysteine-rich domain (CRD) and 7 transmembrane domain (7TM) and ortho- and allosteric ligand binding sites are indicated (see text for details). The model was constructed with the program MacPyMol using coordinates from PDB files 1EWK (mGlu$_1$ open-closed/Active VFT), 2E4U (mGlu$_3$ CRR) and 2R4S (ß2-adrenergic receptor 7TM).

**Fig 2.** Classification of mammalian promiscuous nutrient-sensing 7TM receptors into different classes based on sensitivity to the breakdown products from major organic nutrients protein, lipid and carbohydrate. Receptors are drawn so as to illustrate their overall topology depending on their classification into either family A or family C. Family C 7TM receptors function either as homodimers (CaR and GPRC6A) or heterodimers (T1R1/T1R3 and T1R2/T1R3), the latter group indicated by different gray-scaling of the subunits. The absence of full lines between lipids and carbohydrates serves to illustrate that FFA2 and FFA3 receptors sense short chain free fatty acids produced by anaerobic fermentation of dietary carbohydrate fibers in the gut and thus indirectly sense carbohydrate intake.

**Fig. 3.** Illustration of human tissues displaying predominant expression of the promiscuous organic nutrient-sensing 7TM receptors (see text for details).

**Fig. 4** L-Amino acid selectivity profiles at CaR, GPRC6A and the T1R1/T1R3 heterodimer. Amino acids are grouped according to side chain charge and polarity. Branched-chain amino acids are rendered as a distinct subgroup of aliphatic amino acids. Data have been normalized to allow for comparison of relative amino acid preferences. The profile for CaR was
generated by calculating the potentiating effect of each of the 20 amino acids (at 10 mM concentrations) on the Ca\(^{2+}\) response. Numbers represent percentage response relative to that of L-His (100 \%) (Conigrave et al., 2000). Amino acids marked with asterices were not included in the original study. The profile for GPRC6A is based on reported EC\(_{50}\) values of all 20 L-amino acids from mouse GPRC6A measured in the presence of 1 mM Ca\(^{2+}\) and 1 mM Mg\(^{2+}\) (Christiansen et al., 2007), here normalized to the L-Lys response (set to 100\%). Mouse T1R1/T1R3 data originally in the form of “number of responsive cells” measured in the presence of 2.5 mM IMP (Nelson et al., 2002) and converted to percentage normalized response by calculating the response relative to that of L-Cys (set to 100\%). The # denotes that L-Tyr was not tested at T1R1/T1R3 (due to insolubility).

**Fig. 5.** Chemical structures and pharmacological activity of selected allosteric and orthosteric receptor ligands discussed in the text.
Table 1. Overview of potencies of fatty acids activating human 7TM receptors along with their expression patterns and suggested physiological functions.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Major expression</th>
<th>Physiological function</th>
<th>Agonists</th>
<th>pEC_{50}</th>
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<td>Glucose-dependent insulin release</td>
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* Briscoe et al., 2003.  † Brown et al., 2003; Le Poul et al., 2003.  ‡ Wang et al., 2006a.  § Hirasawa et al., 2005.
Figure 1

Orthosteric agonists (L-aa, divalent cations, sugars)

Allosteric enhancers (L-aa, divalent cations)

Allosteric enhancers (purinic ribonucleotides)

Allosteric enhancers (Gd³+, Ca²⁺?)

Allosteric agonists (brazzein)

Allosteric modulators (drug-like small molecules)
Figure 4
### Figure 5

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