Taste perception: from the tongue to the testis

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ABSTRACT: In mammals, the sense of taste helps in the evaluation and consumption of nutrients, and in avoiding toxic substances and indigestible materials. Distinct cell types expressing unique receptors detect each of the five basic tastes: salty, sour, bitter, sweet and umami. The latter three tastes are detected by two distinct families of G protein-coupled receptors: T2Rs and T1Rs. Interestingly, these taste receptors have been found in tissues other than the tongue, such as the digestive system, respiratory system, brain, testis and spermatozoa. The functional implications of taste receptors distributed throughout the body are unknown. We therefore reviewed the remarkable advances in our understanding of the molecular basis of taste perception in ‘taste’ and ‘non-taste’ tissues. We also present our speculations on the direction of further research in the field of male reproduction.

Key words: G protein-coupled receptor / male reproduction / spermatozoa / taste perception / testis

Introduction

The mammalian testis can produce millions of spermatozoa every day. Although the regulation of proliferation and differentiation of spermatogonial stem cells and the control of spermatogenesis have been extensively studied, our knowledge of these processes is largely rudimentary. From the perspective of molecular biology and endocrinology, gene expression in germ cells is regulated on three levels: the intrinsic, interactive and extrinsic levels (Eddy, 2002). A possible model for the self-renewal and proliferation of spermatogonial stem cells has been proposed (de Rooij, 2001). Recently, several potential molecular markers of spermatogonial stem cells have been identified (Nakagawa et al., 2010). A detailed 3D clone morphology and live-imaging analysis of spermatogonial migration has revealed the surprisingly rapid rate of spermatogonial stem cell replacement in vitro (Klein et al., 2010).

Although several generations of researchers have investigated fertilization, this fundamental biological process remains poorly understood. Millions of genetically unique sperm cells must undertake the long journey to their destination deep inside the female genital tract. To this end, spermatozoa are endowed with a distinct chemoosensory ability to scan their environment and alter their spatial orientation. Many candidate sperm attractants have been identified in aquatic and terrestrial organisms (Riffell et al., 2002). However, there remains some controversy about receptor proteins that detect chemical compounds in the different fluids of the female reproductive tract and the chemoattractive cues that successfully guide the sperm towards the oocyte (Jaiswal et al., 1999; Sun et al., 2005). The chemical senses, i.e. smell and taste, are sensory modalities that generate an internal representation of chemical information from the outside world. Olfactory receptors, conventionally found on the ciliary membranes of nasal olfactory sensory neurons, have recently attracted much attention, due to their expression in the sperm flagella of different mammalian species (Spehr et al., 2006). In addition, several studies have reported the expression of taste receptors and a signalling transduction cascade in non-taste tissues, including the digestive system (Fujita, 1991; Rozengurt, 2006; Margolskee et al., 2007; Treesukosol et al., 2011b), respiratory system (Shah et al., 2009; Deshpande et al., 2010; Tizzano et al., 2011), urinary bladder (Elliott et al., 2011), pancreas (Taniguchi, 2004; Nakagawa et al., 2009; Nakagawa, 2011; Treesukosol et al., 2011b), liver (Taniguchi, 2004), brain (Ren et al., 2009) and testis (Max et al., 2001; Kiuchi et al., 2006; Fehr et al., 2007; Li and Zhou, 2012; Meyer et al., 2012; Voigt et al., 2012). In this review, we examine the recent advances in our understanding of the possible biological function of taste receptors in the testis.

Taste receptors and signal transduction

In mammals, taste provides valuable sensory information required for evaluating the nutritional components of food and preventing the ingestion of toxic substances. Although we can taste a vast array of chemical entities, these are generally believed to evoke a few distinct taste sensations: bitter, sweet, umami, sour and salty. The anatomical substrates and units of taste detection are the taste receptor cells (TRCs), which are assembled into the taste buds distributed on the...
epithelium of the tongue and palate. Theoretically, the basic taste sensations are each recognized by different cells that express specialized receptors and distinct transduction pathways (Chandrashekar et al., 2000; Margolskee, 2002; Zhang et al., 2003; Huang et al., 2006; Kinnamon, 2009). Sour and salty tastes modulate the function of TRCs by the direct activation of specialized membrane channels (Horio et al., 2011; Huang et al., 2006). In contrast, sweet, bitter and umami taste transduction is mediated by a common G protein-coupled receptor (GPCR) signalling pathway (Perez et al., 2002; Zhang et al., 2003). Tastants interact with cell surface receptors and initiate downstream signalling cascades that transduce the chemical energy of ligand binding into ion fluxes and resulting in changes in membrane potential. Several reports on the expression of sweet/bitter/umami receptors in rather unexpected tissues have led to the assumption that these receptors represent excellent candidates for high-affinity chemodetectors in cells outside the tongue (Finger and Kinnamon, 2011; Trivedi, 2012).

**Taste receptors: bitter, sweet and umami**

The finding of the G protein α-gustducin marked the beginning of the molecular era of taste sensory research (Margolskee, 1993; Wong et al., 1996). The landmark discovery of several taste receptors jump-started the field of taste research, leading to the creation of a new framework for multi-level studies in taste physiology (Wong et al., 1996; Chandrashekar et al., 2000; Max et al., 2001; Zhang et al., 2003; Mueller et al., 2005).

**Bitter taste receptors**

In general, sweet and umami tastes have evolved to recognize a limited subset of nutrients. In contrast, bitter taste, as a defence mechanism, prevents the ingestion of numerous structurally distinct toxic compounds in food. In rodents, bitter taste is mediated by a family of ~30 highly divergent GPCRs (taste receptors, type 2; T2Rs). A subset of TRCs, distinct from sweet and umami receptor cells, selectively express T2R genes, which are clustered in genomic regions in humans and mice (Adler et al., 2000). Many studies using heterologous expression assays have found a large number of T2Rs that may act as bitter taste receptors (Chandrashekar et al., 2000; Buhe et al., 2002). Knockout and mis-expression studies in mice have provided direct proof that T2Rs are necessary and sufficient for bitter taste. Animals lacking mT2R5 (candidate Cx3 receptor) exhibit a dramatic and selective loss of response to Cx3, and retain basically normal responses to all other bitter tastants tested (Mueller et al., 2005). Another remarkable feature of bitter-receptor biology is that most T2R genes are co-expressed in the same subset of TRCs on the tongue and palate epithelium; this is consistent with the observation that mammals need to detect a wide range of bitter substances, but have no need to distinguish between them (Adler et al., 2000; Mueller et al., 2005).

**Sweet and umami taste receptors**

Sweet and umami, the attractive taste modalities, are mediated by a small family of three GPCRs: taste receptor, type 1 (T1R1), T1R2 and T1R3. T1Rs are expressed in subsets of TRCs, and T1R expression patterns define three TRC subtypes: TRCs co-expressing T1R1 and T1R3 (T1R1 + T1R3 cells), TRCs co-expressing T1R2 and T1R3 (T1R2 + T1R3 cells) and TRCs expressing T1R3 alone (Nelson et al., 2001). Functional expression studies in heterologous cells have revealed that T1R3 combines with T1R2 to form a sweet taste receptor that responds to all classes of sweet tastants, including natural sugar, artificial sweeteners, D-amino acids and intensely sweet proteins (Nelson et al., 2001; Li et al., 2002; Nelson et al., 2002). Furthermore, T1R2- and T1R3-knockout mice provide definitive proof that T1R2 + T1R3 is the principal mammalian sweet taste receptor (Damak et al., 2003; Zhao et al., 2003). Homozygous mutants for either receptor subunit show a devastating loss of sweet taste. Moreover, cats carry a naturally occurring T1R2 gene deletion and do not respond to sweets, indicating that sweet taste requires T1R2 (Li et al., 2005, 2006). The T1R2 + T1R3 receptor complex responds to a wide range of sweet-tasting compounds, from simple six-carbon sugars to guanidinoacetic acids, large peptides and polypeptides (Nelson et al., 2001; Li et al., 2002).

Most mammals are strongly attracted to the taste of a broad range of L-amino acids (Pritchard and Scott, 1982; Zhao et al., 2003). Cell-based assays have shown that T1R1 and T1R3 GPCRs combine to form a broadly tuned L-amino acid receptor (Nelson et al., 2002). Studies on T1R1- and T1R3-knockout mice have proved that T1R1 + T1R3 functions in vivo as an umami taste receptor (Damak et al., 2003; Zhao et al., 2003). Homozygous mutants lacking either the T1R1 or T1R3 subunit showed an overwhelming loss of umami taste, including all responses to inosine monophosphate and behavioural attraction to monosodium glutamate and L-amino acids. In rodents and humans, T1R1 + T1R3 heterodimers resulted in strong potentiation in response to purine nucleotides (Li et al., 2002; Nelson et al., 2002).

**Taste signal transduction**

In general, the following molecular model has been suggested for signal transduction by taste receptors. When a tastant binds to T1Rs or T2Rs, taste GPCRs activate the heterotrimeric G protein α-gustducin. Ligand binding results in the release of the Gβγ13 subunits and the subsequent stimulation of phospholipase C-β2 (PLCβ2). Activation of PLCβ2 hydrolyses phosphatidylinositol-4,5-bisphosphate to produce two intracellular messengers, inositol-1,4,5-trisphosphate and diacylglycerol, and ultimately leads to calcium release from internal stores and activation of the taste transduction channel (the transient receptor potential protein, TRPM5; Perez et al., 2002; Ruiz et al., 2003; Zhang et al., 2007). As expected from this model, mice lacking gustducin, PLCβ2 or TRPM5 show major deficits in sweet, umami and bitter tastes. However, it should be noted that multiple signalling pathways are involved in taste perception. For example, taste receptors including T1R3 may couple with Gsα14 and other Gs subunits (Tizzano et al., 2008). Several studies have also shown that a PLCβ2/inositol-1,4,5-trisphosphate receptor type 3-independent signalling pathway may be involved in the detection of taste stimuli (Hacker et al., 2008). Another study has suggested that Trpm5-null mice showed markedly diminished, but not abolished, responses to bitter, sweet and umami compounds (Damak et al., 2006).

**Ectopic expression of taste receptors in non-taste tissue**

Taste receptors were originally discovered in taste buds. However, an increasing number of studies, most of which focused on the gut and airways, have detected taste receptor expression and downstream
signalling molecules in non-taste tissues. Zancanaro et al. (1999) reported the presence of gustducin-expressing cells in the vomeronasal organ. T2Rs and T1Rs and the taste transduction cascade have also been found throughout the airways (Tizzano et al., 2010, 2011). Curiously, T2Rs are present on the cilia of ciliated epithelial cells (Shah et al., 2009). Recently, several researchers have reported that the smooth muscle cells of the human airways express T2Rs along with α-gustducin and some components of the taste-associated PLC signalling cascade (Deshpande et al., 2010; Doggrell, 2011). Considering the distribution of taste receptors in the airway, some authors have reported that bacterial metabolites and bitter molecules activate nasal solitary chemosensory cells, which act as a defence mechanism against the inhalation of irritants (Tizzano et al., 2010). In cilia, increases in intracellular Ca2+ mediated by T2Rs and signal transduction might increase ciliary beat frequency (Shah et al., 2009). However, in airway smooth muscle cells the binding of various bitter substances to T2Rs increases intracellular Ca2+, leading to relaxation rather than contraction of the muscles by opening calcium-activated big potassium (BKCa) channels (Deshpande et al., 2010).

Taste receptors in the gut might be related to diet-related diseases and other public health issues (Egan and Margolskee, 2008). Early in 1996, G-protein gustducin-specific taste cells were found in the brush cells of the stomach and the intestine (Hofer et al., 1996). Subsequently, T1R1/T1R2/T1R3 and T2R were also found in the guts of rodents and humans (Raybould, 1998). Several studies have revealed that taste signal transduction occurs in a variety of cell types throughout the gut, from the stomach to the large intestine (Fujita, 1991; Rozengurt, 2006). Indeed, the sweet receptor (T1R2 + T1R3) and umami receptor (T1R1 + T1R3) are found in gastric secretory cells, which induce the release of ghrelin, an appetite-inducing peptide, after the activation by an appropriate substance (Hass et al., 2007, 2010). Further down the gastrointestinal tract, gustducin expression is found in three subtypes of enteroendocrine cells (K, L, K/L cells), which secrete the gut hormones glucagon-like peptide-1 (GLP-1) and/or glucose-dependent insulino tropic peptide (also called gastric inhibitory peptide). The sweet receptor is selectively expressed in enteroendocrine L cells, which can release GLP-1 after sugar ingestion, and GLP-1 in turn augments insulin release from the pancreas (Jang et al., 2007; Mace et al., 2007; Margolskee et al., 2007; Kokrashvili et al., 2009; Nakagawa et al., 2009). Unlike T1R expression, T2R expression in the gut is more difficult to explain. T2Rs are found in an enteroendocrine cell line (STC-1 cells; Wu et al., 2002). After the activation of T2Rs by binding to bitter compounds, STC-1 cells release the peptide hormone cholecystokinin (CCK), which might reduce gut motility (Masuho et al., 2005).

Immunostaining analysis has revealed the presence of sweet taste receptors (T1R2 + T1R3) in the plasma membrane of three urothelial cell types, particularly, umbrella cells from human and rat bladders. Functional experiments have further shown that stimulation by artificial sweeteners (saccharin) can strengthen rat bladder smooth muscle contraction when the sweeteners are added to organ baths containing urothelium and bladder strips, indicating that the sweet taste receptor T1R2/T1R3 may have physiological effects on the urinary bladder (Elliott et al., 2011). In situ hybridization and immunostaining have revealed that T1R1, T1R2 and T1R3 and their associated G-proteins (α-gustducin, Gnb3 and Gγ13) are expressed in the mammalian brain, particularly in the hypothalamus, hippocampus and cortex.

Curiously, the expression of taste receptors and taste-related G-proteins has been detected in both neurons and non-neuronal cells. Thus, the sweet receptors, T1R2 and T1R3, may be associated with brain glucosensors (Ren et al., 2009). Another study has revealed that neurons in the pyramidal cell and granule cell layers constitutively express the sweet receptor T1R2+T1R3 and α-gustducin, and activated astrocytes in ischaemic hippocampi show up-regulated expression of genes related to these taste receptors (Shin et al., 2010).

Since T1Rs are expressed in the gut and brain, sweet taste is assumed to be related to glucose sensing, feeding and obesity (Egan and Margolskee, 2008). Behavioural studies are yet to provide conclusive evidence for the hypothesis that T1Rs are important in glucose sensing in vivo. Like same-sex littermate wild-type controls, T1R2- and T1R3-knockout mice showed normal chow and fluid intake and body weight. Furthermore, the knockout mice exhibited polyose intake similar to that of their wild-type counterparts in 30 min intake tests over a broad range of concentrations (Treesukosol et al., 2011a, b). These results collectively indicate that taste receptors might have unusual physiological roles in addition to taste sensation.

**Spermatogenesis**

Spermatogenesis, one of the most well-characterized stem cell systems, is a paradigm of development that continues throughout adult life in most mammals. In mouse testes, spermatogenesis occurs within the seminiferous tubules with the support of somatic Sertoli cells, and results in the formation of mature spermatozoa from spermatogonial stem cells (Eddy, 1998, 2002). A small spermatogonial subpopulation, consisting of Aa (Apaired; cysts of interconnected cell pairs) and Aal (Aaligned; interconnected cysts in saccular cysts of 4, 8, 16 and occasionally 32 cells), is now considered to constitute spermatogonial stem cells (de Rooij and Grootegoed, 1998; de Rooij, 2001). Three subtypes of spermatogonia can be distinguished by variable levels of gene expression (GFrα1, nanos2 and Ngn3). GFrα1 and nanos2 are expressed in spermatogonia showing steady-state self-renewal, while Ngn3-expressing spermatogonia tend to differentiate, but can switch to GFrα1 expression and self-renewal (Nakagawa et al., 2007; Klein et al., 2010; Nakagawa et al., 2010). Thus, the stem cell population undergoes continuous turnover, but the total number of stem cells remains constant (de Rooij, 2001; de Rooij and Mizrak, 2008); therefore, a regulatory mechanism must exist to control the ratio between self-renewing and differentiating spermatogonial stem cells. However, such a mechanism is yet to be discovered.

As germ cells move from the periphery to the lumen of seminiferous tubules, three successive phases are observed: mitotic (spermatogonia), meiotic (spermatocytes) and post-meiotic (spermatids) phases. The mitotic phase occurs in the basal compartment, while the meiotic and post-meiotic phases occur in the luminal compartment. A precise, well-coordinated programme must regulate the constantly changing patterns of gene expression during this process. Such regulation is generally believed to occur at three concentric levels: intrinsic, interactive and extrinsic. Gene expression is regulated by the genetic code of the germ cells themselves (intrinsic), but signals from glands (extrinsic) and from the Sertoli and Leydig cells (interactive) provide an important microenvironment and are essential for germ cell development (Eddy, 2002). In addition, cytoplasmic bridges, a specific structure in
the testis, maintain germ cell communication among isogenous groups (Nagano, 1961; Connell, 1984).

**Taste receptors and spermatogenesis**

The chemical senses, smell and taste, guide an animal’s struggle for survival throughout evolution. Olfactory receptors and components of their downstream signalling cascade (especially Gαolf and ACIII) are expressed in the testis and mature sperm (Parmentier et al., 1992; Vanderhaeghen et al., 1997; Defer et al., 1998; Gautier-Courteille et al., 1998; Goto et al., 2001). Interestingly, we found that G gamma subunit 13 (Gγ13) is expressed in the testis (Li and Zhou, 2012); this subunit is an important co-unit of Gαolf (Kulaga et al., 2004; Kerr et al., 2008). Fehr et al. (2007) reported that gustducin, another partner of Gγ13, is expressed in the testis and mature sperm. In agreement with this report, our study showed the wide distribution of G protein α-gustducin in the testis, from the basal compartment to the luminal compartment (Li and Zhou, 2012). We generated T2R5-GFP/Cre transgenic mice and crossed them with Rosa26-LacZ or Rosa26-DTA (diphtheria toxin fragment A). In this double transgenic model, immunostaining revealed mT2R5 expression in the testis (Li and Zhou, 2012). In the original report, the mT2R5 receptor was suggested to be expressed in round sperm (Fig. 1A and B). Subsequent analysis indicated that bitter receptors may be expressed during three phases of spermatogenesis. First, LacZ staining was distributed in both the luminal and basal compartments (T2R5-Cre/GFP-Rosa26-LacZ), and surprisingly, was also present in the spermogonemia attached to the basal membrane (Fig. 1C–E). Secondly, in the T2R5-Cre/GFP-Rosa26-DTA model, Leydig cells and Sertoli cells were observed, and spermatids were observed in a partial section (Fig. 1F–H). In addition, we observed very few epididymal spermatozoa. These findings suggest that normal spermatogenesis is maintained after T2R5+ cell ablation. Third, in situ hybridization revealed mT2R expression from the basal compartment to the luminal compartment. The expression levels of the 35 identified mouse T2R genes have been determined by quantitative real-time PCR in samples of testis tissue, which showed that most T2R genes are highly expressed in the testis (Xu et al., 2012). Finally, after detailed analysis of sections from a model of T2R5+ cell ablation, we found that most seminiferous tubules contained spermatogonia and spermatocytes. Given that most bitter receptors are expressed in the same receptor cell in taste buds, we speculated that the presence of T2R5+ cells in the seminiferous tubules may be a driving force in human evolution (Leonard, 2002). Diversification of T2R functions during phylogenetic evolution may reflect an adaptation to changing nutritional environments. The positive selection of the human bitter receptor T2R16 (N172 allele) is related to an increased sensitivity to bitter compounds (salicin, arbutin and five different cyanogenic glycosides) and may represent selection pressure at an early stage of human evolution (Soranzo et al., 2005). Another study also revealed natural selection acting on functional human T2R38 alleles (Wooding et al., 2004).

Another model involving T1R3-Cre/GFP transgenic mice has revealed unexpected results (Fig. 2A). Several researchers have reported the expression of T1Rs (T1R1, T1R2 and T1R3) in the testis (Max et al., 2001; Iwatsuki et al., 2010; Li and Zhou, 2012; Meyer et al., 2012). T1R3 is the most important receptor, as it forms functional heterodimers with T1R1 and T1R2 that detect sweet and umami taste, respectively; T1R3 is present in the testis (Li and Zhou, 2012; Meyer et al., 2012). Male double transgenic mice (T1R3-Cre/GFP-Rosa26-DTA) are infertile. Immunohistochemical analysis with anti-gustducin showed similar spermatogenesis in these transgenic and wild-type control mice (Fig. 2B and C). Immunostaining with anti-T1R3 revealed that T1R3 is partially expressed in spermatocytes, round sperm and Leydig cells (Li and Zhou, 2012; Meyer et al., 2012). In taste buds, T1Rs are expressed in different subsets of TRCs from those expressing T2Rs. Furthermore, many T2Rs are co-expressed in the same receptor cell in taste buds, which may explain the difference between the two models. mT2R5 may be expressed in the spermatogonemia phase in these transgenic mice. DTA expression has a toxic effect on the development of germ cells belonging to the same isogenous group. In contrast, mT1R3 expression is concentrated in spermatids. Meanwhile, mCherry signals were detected during earlier stages of spermatogenesis and Sertoli cells in the transgenic T1R1BLR/BLR+/mouse. In addition, hrGFP signals were also found during spermatogenesis in the transgenic T2R13BLR/BLR+ mouse (Meyer et al., 2012; Voigt et al., 2012). In situ hybridization revealed T2R8 expression in the seminiferous tubule, and qPCR analysis further showed the expression of all 35 bitter genes in the testis (Xu et al., 2012). T1R2 expression has been observed in primary spermatocytes and spermatogonial cells in the T112-LacZ knock-in mouse (Iwatsuki et al., 2010). In summary, the current data collectively suggest that mT2Rs and mT1Rs may be involved in the regulation of spermatogenesis, and are likely to participate in the formation of acrosomes and flagella, in addition to chromatin remodelling and condensation.

To elucidate the function of taste receptors and their downstream signalling cascade during spermatogenesis, we assessed green fluorescent protein (GFP) expression in transgenic mice, including T2R5-GFP/Cre, T1R3-GFP/Cre, gustducin-GFP (Wong et al., 1999) and Trmp5-GFP mice (Clapp et al., 2006). We observed GFP expression in the interstitium and basal compartment of seminiferous tubules in these mice (Supplementary data, Figs S1A–D, S2A–D, S3A–D and S4A–D). Thus, taste receptors and downstream signal transduction are expressed during the three phases of germ cells and in Leydig cells (Fig. 3A–D), which indicates that testes can ‘taste’ the taste-like compounds. Meanwhile, the germ cells isolated from the gustducin-null mice were observed to be unresponsive to bitter compounds when assayed with calcium imaging (Xu et al., 2012). These findings are not surprising, because animals that can avoid toxic foods by taste perception, especially bitter taste, may generate higher quality spermatozoa and produce more progeny. Thus, dietary changes may be a driving force in human evolution (Leonard, 2002). Diversification of T2R functions during phylogenetic evolution may reflect an adaptation to changing nutritional environments. The positive selection of the human bitter receptor T2R16 (N172 allele) is related to an increased sensitivity to bitter compounds (salicin, arbutin and five different cyanogenic glycosides) and may represent selection pressure at an early stage of human evolution (Soranzo et al., 2005). Another study also revealed natural selection acting on functional human T2R38 alleles (Wooding et al., 2004).
after the binding of specific chemical compounds to taste receptors (Chaudhari and Roper, 2010). The resulting changes in cAMP concentrations induce many physiological responses, including proliferation, differentiation and apoptosis of various types of cells (Stork and Schmitt, 2002; Javelaud and Mauviel, 2005; Wettschureck and Offermanns, 2005; Bierie and Moses, 2006). The Notch pathway and

Figure 1  T2R5 expression in the testis. (A) A cross section of seminiferous tubules in the mouse testis. The seminiferous tubules contained a mixture of germ cells and Sertoli cells and were surrounded by a thin wall of peritubular cells. Leydig cells were located in the areas between the tubules. The Sertoli cells divided the seminiferous epithelium into two compartments: basal compartment and luminal compartment. (B) In T2R5-Cre/GFP transgenic mice, immunostaining with anti-GFP revealed that T2R5 is expressed throughout the seminiferous epithelium, from the basal compartment to the luminal compartment. (C–E) T2R5-Cre/GFP transgenic mice were crossed with Rosa26 \(^{\text{LoxP\_ LacZ\_ LoxP}}\) mice, and X-Gal staining revealed a wide distribution of Cre activity. Except for some areas in the luminal compartment, blue staining was mostly observed in the basal compartment. Furthermore, most of the blue staining was present in the basal layer, which contained spermatogonial stem cells. In addition, blue staining was detected in the Leydig cells. (F–H) T2R5-Cre/GFP transgenic mice were further crossed with Rosa26 \(^{\text{LoxP\_ bp\_ LoxP\_ DTA}}\) mice. Spermatids were found to have moved out of most seminiferous tubules and the ratio of testis to weight had decreased significantly. A cross section of seminiferous tubules showed that Sertoli cells were present in the almost empty tubules, which contained spermatogonia and spermatocytes. In addition, spermatocytes, which were originally located in the basal compartment, had moved into the luminal compartment. Leydig cells were still present in the area between the tubules. Scale bar: (A) 30 \(\mu\)m, (B) 90 \(\mu\)m, (C–E) 60 \(\mu\)m, (F–H) 50 \(\mu\)m.
Hes1 are also widely distributed in the testis (Tang et al., 2008; Hasegawa et al., 2012). Hes1 may regulate the expression of the taste signal transduction cascade in developing taste buds (Ota et al., 2009). Thus, it is reasonable to speculate that taste receptors and their signal transduction may participate in the regulation of the proliferation/differentiation of germ cells by generating crosstalk with the Notch-Hes1 signalling pathway.

**Taste receptors and sperm physiology**

In the mature spermatozoa of rodents, gustducin is first detected on the acrosomal cap, and then in the mid-piece region and finally the principal piece. Gustducin has also been observed in bovine and human mature spermatozoa (Fehr et al., 2007). Moreover, the umami receptor T1R3 + T1R1 is expressed in mature spermatozoa. T1R3 is expressed in the acrosomal cap and principal piece, and T1R1 in the acrosomal cap and principal and end pieces of the sperm tail. Owing to the lack of anti-T1R2, the authors of the above study could not assess T1R2 expression in mature spermatozoa (Meyer et al., 2012). In one study, T1R1 knockout mice and wild-type controls showed similar reproductive characteristics, such as sperm concentration, testis histology, sperm morphology, sperm mobility and testosterone level (Meyer et al., 2012). In the tasl-null animals, treatment with zona pellucida induced significant acrosome reaction, indicating that tasl deletion did not influence the ability of sperm to bind to the zona pellucida and activate coupled intracellular signalling cascades. Surprisingly, tasl-null sperm showed similar physiological responses to wild-type sperm after treatment with glutamate (tastant for T1R1 + T1R3 umami receptor in taste buds), indicating that spermatozoa may have other active ligands for...
glutamate besides the Tas1r1 receptor. In addition, the authors further examined the acrosome reaction and co-localization of T1R1-T1R3-gustducin in the acrosomal cap in tas1r1-null mice. Curiously, they found a significantly higher rate of spontaneous acrosome reaction in both freshly isolated (uncapacitated) and capacitated sperm, and this was linked to increased levels of Ca$^{2+}$ and cAMP. Both Ca$^{2+}$ and cAMP are key regulators of the acrosome reaction (Breitbart, 2003; Abou-haila and Tulsiani, 2009). Finally, the authors of the study on T1R1 knockout mice suggested that taste receptors may regulate the spontaneous acrosome reaction by balancing cAMP levels in spermatozoa (Meyer et al., 2012). To understand the biological function of taste receptors in the testis, it may be reasonable to speculate that spermatozoa in the cauda epididymidis of T1R1-null mice express a mixture of taste receptors. The ablation of T2R5+$^+$ cells or T1R3+$^+$ cells failed to completely eliminate gustducin+$^+$ and Trmp5+$^+$ spermatids in the testis. In addition, T1R3+$^+$ spermatids were detected in the testis, even after the ablation of T2R5+$^+$ cells (Li and Zhou, 2012). The staining pattern of anti-gustducin in epididymal spermatozoa varies among individual mice (Fehr et al., 2007). Taste bud cells can be divided according to their taste receptors into T1R cells, T2R cells and PKD2L1 cells, besides salty cells and supporting cells (Finger, 2005; Chaudhari and Roper, 2010). In addition, T2R131 expression has also been detected in the middle section (mid-piece) of sperm flagella (Voigt et al., 2012). Calcium imaging has revealed that the amplitudes of the response to bitter compounds are variable, depending on the location of the sperm. The acrosome and mid-piece responses were equally strong, whereas the responses from the principal pieces were mostly undetectable (Xu et al., 2012). If spermatozoa can be divided into similar categories based on taste receptors, then T1R1 gene deletion may only partially impair the biological function of the spermatozoan population. T1R1-null sperm were expected to show similar capacitation and acrosome reaction as wild-type sperm, because several critical molecular events that occur during sperm capacitation and the acrosome reaction may be regulated by unknown mechanisms. However, T1R1-null sperm showed a significantly higher rate of

![Figure 3](image-url) Several important components involved in taste signal transduction are observed in the testis. (A) The gustducin expression was observed throughout spermatogenesis, from spermatocytes to spermatids, and in the basal layer and Leydig cells. (B) Gyl3 expression was found in the basal layer and Leydig cells, but not during spermatogenesis (from spermatocytes to spermatids). (C) PLCβ2 expression, another important component of taste signal transduction, was found during spermatogenesis (from spermatocytes to spermatids), and in the basal layer and Leydig cells. (D) Trmp5 expression was detected in elongated spermatids, the basal layer and Leydig cells. Scale bar, 50 μm.
spontaneous acrosome reaction and a higher cAMP level, indicating that T1Rs may be involved in the regulation of acrosomal vesicle release by controlling cAMP levels (Meyer et al., 2012).

**Perspectives**

Although taste receptors and their downstream signalling transduction cascades are widely dispersed in diverse organ systems, the function of these receptors in many tissues remains unclear despite recent advances. The wide distribution of these receptors and their downstream signalling components in spermatogenesis and spermatozoa is even more difficult to explain. Histological examination has shown the distribution of taste receptors in the testis (Fig. 4A and B). The bitter receptor mT2R5 is expressed in spermatogonia, spermatocytes and spermatids. T1R3 is expressed in spermatocytes and spermatids. Correspondingly, several important signalling components (gustducin–Gy13–PLCβ2) related to bitter and sweet tastes have been detected in these three types of cells. In contrast, Trmp5 expression is largely linked to spermatids. Moreover, Trmp5-independent mechanisms exist for the detection of bitter and sweet compounds (Damak et al., 2006). Research on taste perception has now been extended to the reproductive field, and may lead to potential applications in reproductive medicine and clarify the reproductive toxicology of Chinese herbal medicines. However, practical application of the current theoretical knowledge requires conclusive proof and long-term exploration. Thus, the recent findings are merely a first step towards the general understanding of taste receptor-mediated spermatogenesis.

The umami receptor T1R3+T1R1 and gustducin are found in spermatozoa, while the bitter receptor T2R131 is found in the mid-piece of sperm flagella. Germ cells from gustducin-null mice are unresponsive to bitter tastants; however, no other taste components are detected in spermatozoa. The molecular mechanism of taste signal transduction in spermatozoa remains unclear. Originally, we speculated that taste receptors may be involved in chemosensation when determining the route to the egg. However, recent studies have revealed that the sperm-specific CatSper channel plays an important role in regulating intracellular Ca^{2+} concentrations and the swimming behaviours of sperm. Odorants directly activate CatSper without involving GPCRs or cAMP (Strunker et al., 2011; Brenker et al., 2012). Another possibility is that spermatozoa use taste receptors to detect toxins in the female reproductive tract. In previous reports, T2Rs and the taste transduction cascade have also been found in the respiratory system, including the respiratory epithelium of the nasal cavity (Tizzano et al., 2010, 2011), airway smooth muscle (Deshpande et al., 2010) and motile cilia of airway epithelia (Shah et al., 2009). Thus, bitter receptors appear to act as defence mechanisms by sensing toxic substances that are invading airways. Correspondingly, it appears likely that bitter receptors play an important role in sensing and avoiding toxins secreted by bacteria present in the female reproductive tract.
Regarding the expression of T1R3/T1R1 in spermatozoa, it is difficult to predict the biological function of those receptors in spermatozoa, due to increasing numbers of biological functions being reported both in vivo and in vitro (Damak et al., 2003; Jiang et al., 2005; Bachmann and Beauchamp, 2007; Tordoff et al., 2008, 2012). These recent discoveries are merely the first step towards a greater understanding of the involvement of taste perception in sperm physiology.

Sertoli cells and Leydig cells provide important extrinsic cues that influence germ cell development. While taste receptors have not been found in Sertoli cells, these receptors and their signal transduction cascades have been detected in Leydig cells (Figs 1C–E, 3A–D, 4A and B). The widespread expression of the components of the taste transduction cascade in the testis may indicate that some of these components are involved in spermatogenesis. Activation of taste receptors in the testis, as in the gut or airway, produces the same sensation as that produced by the activation of identical receptors in the taste buds. The ‘taste’ receptors of the testis should not be regarded as an internal sense of taste, but rather as a completely different chemoreceptor system. Not all extrinsic regulators of germ cell development act via Sertoli cells and Leydig cells. Follicle-stimulating hormone (FSH) and testosterone play an important supportive, non-development act via Sertoli cells and Leydig cells. Follicle-stimulating hormone (LH) and FSH, which indicates an effect on the hypothalamic–pituitary–gonadal feedback loop (D’Cruz et al., 2010). Many plants have beneficial effects against various ailments, but their clinical applications are limited because they also have detrimental effects on reproduction (Patwardhan et al., 2005; Choi, 2008; D’Cruz et al., 2010).

Thus far, the molecular mechanisms that underlie plant-induced anti-fertility effects remain unclear. Plant extracts contain various chemical compounds, including sugars, amino acids, proteins, fats, waxes, enzymes, pigments, vitamins, organic acids, tannins, inorganic salts, volatile oils, alkaloids and glycosides (Kahkonen et al., 1999; Fiehn et al., 2000). Taste receptors may play a critical role in the molecular mechanisms that regulate cell physiology in response to Chinese herbal medicines. The wide distribution of taste receptors in the human body offers a new line of research to elucidate the pharmacological and toxicological effects of Chinese herbal medicines. T1Rs induce complex physiological responses in their target cells in vivo after binding to a wide range of chemical compounds, including simple six-carbon sugars, large peptides, polypeptides, L-amino acids and purine nucleotides (Damak et al., 2003; Chandrashekar et al., 2006). The diversity and complexity of bitter molecules and their receptors in humans (40–80) and rodents (25–35) further complicate the study of chemical senses in vivo (Chandrashekar et al., 2000; Mueller et al., 2005; Behrens and Meyerhof, 2009). Most known bitter molecules have not been associated with a specific bitter receptor, and little is known about the key structural groups and structure–activity relationships of bitter molecules (Wiener et al., 2012). The analysis of a database of 833 bitter molecules suggests that, in general, the presence of a lactone group in a molecule is associated with a bitter taste (Rodgers et al., 2005). The intriguing question remains as to how hundreds of structurally diverse compounds are detected by a limited number of receptors.

Supplementary data
Supplementary data are available at http://molehr.oxfordjournals.org/.

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Author’s role
Dr F.L. conceived, designed the experiments, analyzed the data and wrote the paper.

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Conflict of interest

None declared.

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