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The Gustatory System of Lampreys

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Key Words

Agnathans · Biciliated cells · Glossopharyngeal nerve · Gustatory system · Lampreys · Nucleus of the solitary tract · Serotonin · Taste buds · Vagal nerve

Abstract

The present is a review of the gustatory system of lampreys, which are representative of the earliest vertebrates. They are the oldest extant vertebrates that possess taste buds. Because of the phylogenetic position of lampreys, the study of their gustatory system will provide important information to help understand the early evolution of this system in vertebrates. The taste buds of larval lampreys, which are papillae located on the first six pairs of gill arches facing the water current, respond to classical taste substances. They consist of two types of tall differentiated cells, serotonergic biciliated taste receptors ('light' cells) and microvillous sustentacular cells ('dark cells'). The taste buds also contain basal proliferative cells. Afferent gustatory fibers of the glossopharyngeal and vagal nerves innervate the taste buds of lampreys and contact the basal surface of the biciliated cells without entering the bud. Central processes of the glossopharyngeal and vagal cranial nerves terminate in a caudal rhombencephalic region that may correspond to the nucleus of the solitary tract of gnathostomes. To date, most studies in lampreys have focused on characterizing taste buds; future research should focus on the central processing of the gustatory information. Here we will review the current knowledge about the gustatory system of lampreys to provide a basis for establishing the direction of further studies of this chemosensory system.

Introduction

Aquatic organisms such as lampreys evolved in a world of dissolved chemicals, and detection of such surrounding chemicals was important for their survival. One of the major chemosensory systems is the gustatory system, which is dedicated to the evaluation of food contents. A sense of taste is important for the detection of both nutritive molecules and harmful substances, which is vital to enable animals to adapt to specific habitats.

The gustatory system in gnathostome vertebrates comprises peripheral receptors (taste buds) that are innervated by fibers of three branchiomeric nerves (facial, glossopharyngeal and vagus), and a series of central nuclei and pathways. Lampreys, which are representatives of the jawless vertebrates (agnathans), are the oldest vertebrates that posses taste buds [Northcutt, 2004]. Lampreys occupy a key phylogenetic position, as the sister group to gnathostomes, between protochordates (amphi-

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oxus and tunicates) and gnathostome vertebrates. It is therefore generally accepted that lampreys represent the closest living forms to early vertebrates and that they may be good models for studying the evolution of the chemosensory systems. Since the first studies by Baatrup in the 1980s on the ultrastructure and physiology of the taste buds of lampreys [Baatrup, 1983a, b, 1985a, b], the gustatory system of lampreys has not received much attention. However, recent neurochemical data on the taste buds of sea lampreys have revealed some interesting new aspects of the early evolution of taste buds in vertebrates [Barreiro-Iglesias et al., 2008c]. In the present review, we aim to summarize the current knowledge of the organization and physiology of the gustatory system of lampreys. This will provide a basis for further studies of the gustatory system of lampreys, which in turn will provide valuable information to help understand how these animals have adapted to their habitat as well as to help understand the early evolution of the gustatory system in vertebrates.

Lampreys: The Earliest Vertebrates with Taste Buds

Hagfishes are chordates, and perhaps also vertebrates [Ota et al., 2007], which have a system of sensory organs resembling taste buds, the Schreiner organs [Braun, 1998]. Schreiner organs are located extensively – on the skin, the prenasal sinus, the nasopharyngeal duct, the pharynx and, at lower densities, in the oral and velar cavities of hagfishes [Braun, 1998]. These structures were initially described on the tentacles and the skin of the head and body of hagfishes [Retzius, 1892; Schreiner, 1919; Georgieva et al., 1979]. In these early studies, Schreiner organs were initially identified as end buds. End buds are found in large numbers on the skin of many fishes and they are, in most cases, external taste buds similar in structure and innervation to taste buds within the oropharyngeal cavities [Lane and Whitear, 1982]. Out-group analysis has indicated that taste buds were primitively restricted to the oropharynx, and that external taste buds (end buds), distributed over the head and, in some cases, even the trunk, evolved independently a number of times [Northcutt, 2004].

Recent studies have revealed important systematic differences between the organization of the Schreiner organs system of hagfishes and the end bud/taste bud system of vertebrates: (1) Schreiner organs are innervated by sensory trigeminal branches, the glossopharyngeal/vagal nerve and by the cutaneous branches of spinal nerves [Braun, 1998], while gnathostome taste buds are innervated by the facial/glossopharyngeal/vagal nerves [Northcutt, 2004]. (2) The central projections of the nerves innervating Schreiner organs of hagfishes form a continuous tract in the trigeminal sensory zone and the dorsolateral funiculus of the spinal cord, but only some Schreiner organs may be represented in the nucleus of the solitary tract [Braun, 1998], whose rostral part (the gustatory nucleus) is the primary recipient of the gustatory afferents in gnathostomes [see Smith and Davis, 2000]. (3) Supporting cells of Schreiner organs are not associated with high levels of ecto-ATPase [Finger, 2006], which is a key feature of the structurally similar type I cells of vertebrate taste buds in which ATP serves as a neurotransmitter [Finger et al., 2005]. For these reasons, it appears that Schreiner organs are not homologous to end buds/taste buds [Braun, 1998; Finger, 2006]. This sensory modality of hagfishes has no direct homologue in vertebrates, and appears to be a specialization of hagfishes [Braun, 1998]. It is therefore generally accepted that lampreys are the oldest extant vertebrates that possess true taste buds [Finger and Simon, 2000; Northcutt, 2004] and, therefore, that taste buds evolved in the common ancestor of lampreys and gnathostome vertebrates.

General Organization

Lampreys have clearly differentiated larval and adult stages. Larval lampreys spend most of their time buried within the sand of freshwater streams. They are filter feeders and feed exclusively on microorganisms in suspension, which become embedded in mucus in the pharynx [Moore and Mallat, 1980]. The mouth of larval lampreys is divided into buccal and oral cavities (fig. 2a). The anterior opening of the mouth leads into a large buccal cavity through a narrow oral aperture (fig. 2a). Posterior oral cirri form a ring of branched projections around and across the oral aperture, and prevent the passage of large objects into the oral cavity [Mallat, 1979, 1981]. The muscles used for feeding are innervated by the trigeminal nerve [Homma, 1975]. In larvae, a muscular velum that is also innervated by the trigeminal nerve pumps water through the pharynx and gills [Johnston, 1905] (fig. 2a). The water current used for respiration and feeding flows into the pharynx by the coordinated action of the velum movements and by the sequential contraction and expansion of the branchial region. The water comes out through the seven pairs of branchiopores. The pharyngeal region is also the site where food particles are trapped before passing into the esophagus. Tracts of ciliated cells present

in the pharynx help to move the food cord, which is formed by mucus secretion and trapped particles, toward the esophagus. These ciliated tracts are prominent in the bilateral pseudobranchial grooves, in the hypobranchial groove, and in the anterolateral surfaces of the gill seams [Mallat, 1979]. The first six pairs of gill arches bear volcano-like papillae that face the water current (fig. 1, 2a). These gill structures were identified in larval lampreys in early light [Schreiner, 1879; Retzius, 1893; Schaffer, 1895; Alcock, 1899] and electron microscopic studies [Mallat, 1979] as taste buds. According to Alcock [1899] and Pietschmann [1929], these organs of larval lampreys are innervated by branches of the glossopharyngeal and vagal nerves.

The anatomy of adult lampreys differs considerably from that of the larvae because of the changes that take place during metamorphosis. In the adult, a secondarily formed esophagus begins as a dorsal orifice of the oral cavity at the level of the velum and communicates directly with the oral cavity (fig. 2b), also called 'pharynx' by early authors. During transformation, the larval pharynx becomes a respiratory region, formed by the water tube with the associated branchial chambers. The secondarily formed esophagus is separated from the more ventrally located water tube by cartilaginous processes [Weissenberg, 1926] (fig. 2b). After metamorphosis, lampreys are carnivorous and feed on a variety of bottom fauna, including worms and crustacea, in addition to fish tissues. Half of all living species of lampreys [38] are parasitic and feed on fishes by sucking their blood and tissues [Hardisty, 2006]. In adult lampreys, taste buds have also been described in the inner branchiopores joining the water tube and branchial chambers of the pharynx (fig. 2b) [Retzius, 1893; Fahrenholz, 1936]. Like larval taste buds, the buds of adult lampreys are innervated by branches of the glossopharyngeal and vagal nerves [Alcock, 1899; Johnston, 1905; Pietschmann, 1929].

Experimental studies in gnathostomes have shown that taste buds are innervated by branches of the facial, glossopharyngeal and vagal nerves [see Northcutt, 2004]. If the taste buds of lampreys lack facial nerve innervation, this would indicate that this is an evolutionary novelty of gnathostome vertebrates. However, it is now known that in lampreys the rostral part of the first gill, which bears taste buds [Barreiro-Iglesias et al., 2008c], is innervated by the facial nerve [Guimond et al., 2003]. Therefore, the possible facial nerve innervation of the taste buds located on the first gill of lampreys should be determined by use of modern tract-tracing techniques.

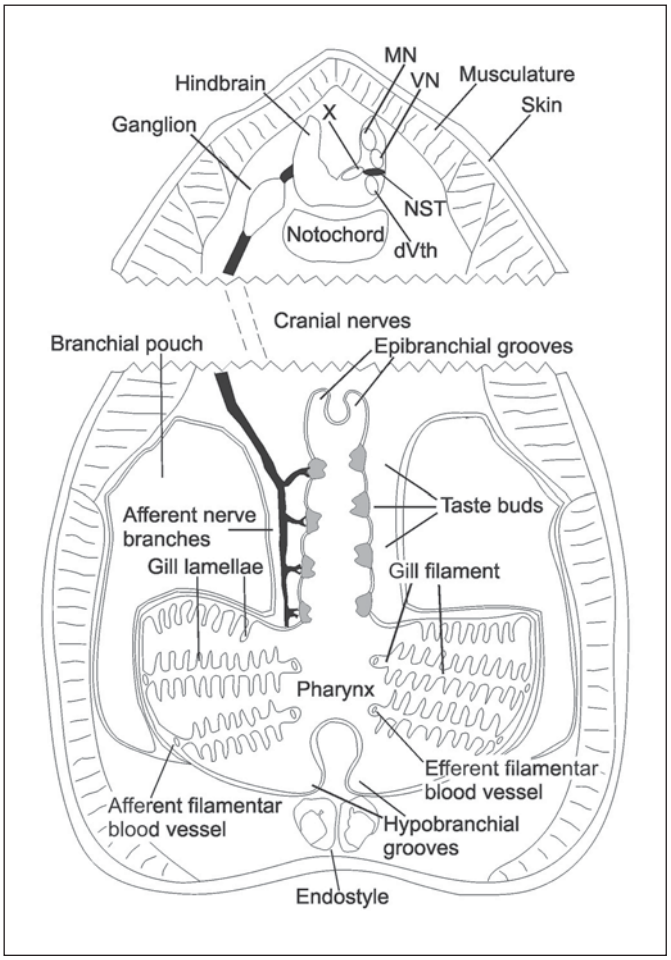

Fig. 1. Schematic drawing showing the location of taste buds in relation to the gill support elements, the afferent innervation (at the left) and the location of the nucleus of the solitary tract in relation to other rhombencephalic nuclei (at the right) in larval lampreys. Top schematic drawing: transverse section at the level of the caudal rhombencephalon where the nucleus of the solitary tract is located (at the right). Bottom schematic drawing: oblique, more caudal section of the branchial region in which rostral is at the top. Modified from Barreiro-Iglesias et al. [2008c].

Taste Bud Structure

Transmission electron microscopic studies have revealed that the structure of the taste buds of larval and adult lampreys is very similar [Baatrup, 1983a, b]. Taste buds are located close to the origin of the gill filaments on the surface, which turns towards the inside of the pharyngeal cavity. Each papilla appears as a volcano-like elevation (100–150 μm in diameter) extending between 50 and 100 μm beyond the surrounding general surface [Baatrup, 1983a].

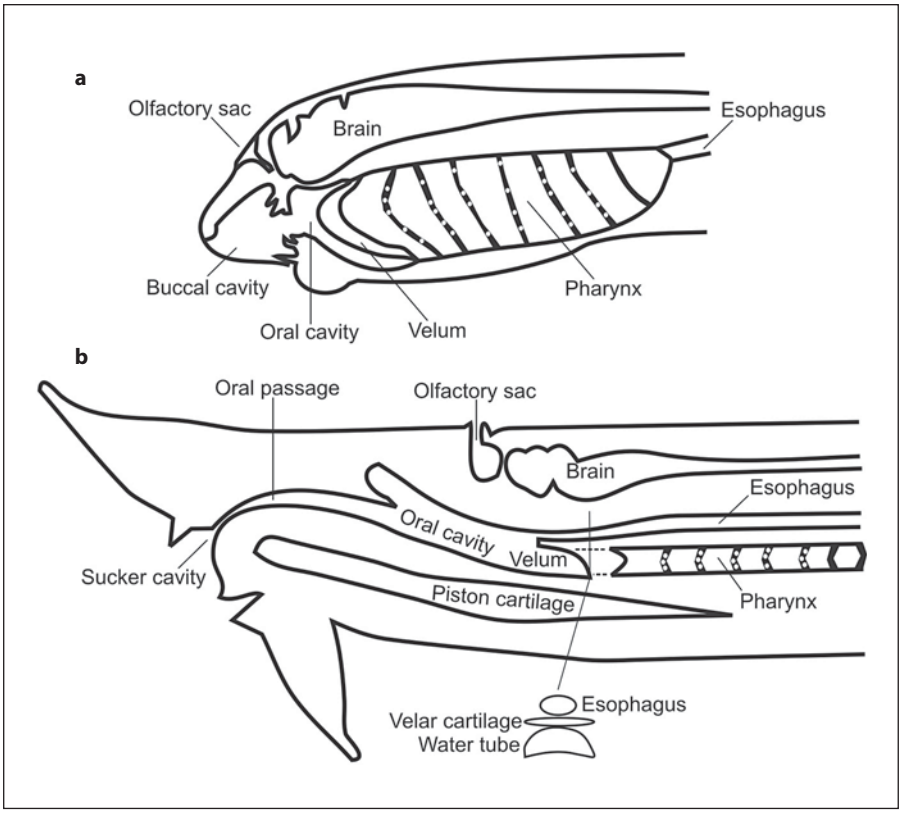

Fig. 2. Schematic drawings of sagittal head sections comparing the organization of the oral/pharyngeal regions between larval (**a**) and adult (**b**) lampreys. Note that in larvae the taste buds (white circles) are located in a common respiratory and digestive canal, while in adults the taste buds are located in the respiratory canal because of development of the secondary esophagus. Modified from Kawasaki and Rovainen [1988].

The taste buds consist of tall elongated cells that extend through the entire length of the epithelium. With light microscopy, it is possible to distinguish between 'light' and 'dark' elongated cells in the taste buds of lampreys. These cell types can also be distinguished on the basis of their apical processes by electron microscopy: those with electron-dense cytoplasm end apically in a tuft of microvilli, while the more electron-lucent cells are ciliated. The ciliated cells are separated by the microvillar cells that surround them [Baatrup, 1983a].

Each ciliated cell bears two 2- to 5-μm-long cilia (biciliated cells) with a 9 + 2 microtubular arrangement; they originate from basal bodies with 2–5 basal feet in larvae [Baatrup, 1983a] and 2–4 basal feet in adults [Baatrup, 1983b]. Neither accessory centrioles nor rootlets are associated with the basal bodies [Baatrup, 1983a]. The basal region of these cells is filled with vesicles with electron-lucent contents and also with a small number of dense-cored vesicles (100–120 nm). The microvillar cells extend 1.5 μm beyond the surface of the neighboring ciliated cells. The microvilli are circular in cross section and have a central core of microfilaments, which continues proximally into the apical cytoplasm [Baatrup, 1983a].

Afferent nerve fibers do not enter the taste buds of lampreys [Baatrup, 1983a, b], unlike mammals and other jawed vertebrates [Finger, 2006]. However, nerve fibers are numerous in the connective tissue below the taste buds. They are 1.3–2 μm in diameter. Contacts, which may be interpreted as chemical synapses, are only found between biciliated cells and fiber varicosities. These connections are established through holes in the basal lamina between the ciliated cell and the subjacent afferent fiber. There is some degree of membrane thickening at these sites.

A third cell type has occasionally been observed by electron microscopy in taste buds of lampreys. These are spherical 'basal cells' with a thin layer of cytoplasm enveloping a cell nucleus showing deep invaginations [Baatrup, 1983a]. They have been considered as Merkel cells, because they are structurally similar to those on the skin [Whitear and Lane, 1981], since they have microvilli and spur synapses [Baatrup, 1985a]. These basal 'Merkel cells' are only observed in some buds and are absent from others. No mitotic activity has been observed in this type of cell [Baatrup, 1983a].

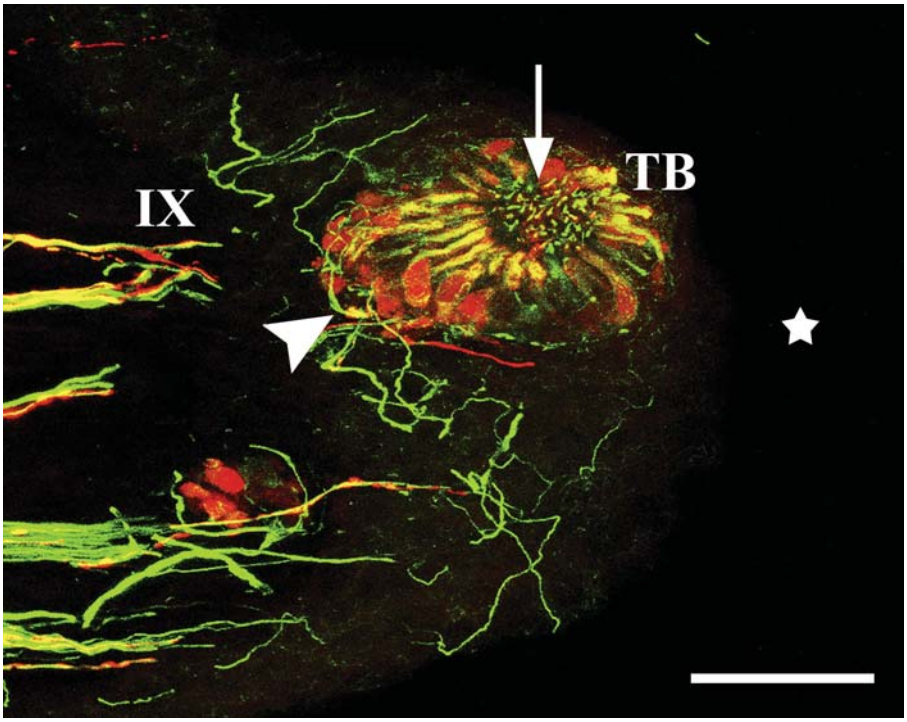

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Fig. 3. Photomicrograph of a horizontal section of the pharynx of a larval sea lamprey showing colocalization of serotonin (red channel) and tubulin (green channel) immunoreactivities in the biciliated taste bud cells [for experimental procedures see Barreiro-Iglesias et al., 2008c]. Note the presence of the apical cilia of the putative taste receptors (arrow). Note also the presence of the afferent gustatory fibers of the glossopharyngeal nerve surrounding and contacting the taste bud cells (arrowhead). The star indicates the pharyngeal cavity. Scale bar = 60 μm.

Cell Proliferation

Baatrup [1983b] reported the absence of mitotic activity in cells surrounding the taste buds of adult lampreys, but proliferation has only been evaluated by use of proliferation markers in the taste buds of larval lampreys [Barreiro-Iglesias et al., 2008c]. The taste buds of larval lampreys mainly consist of non-proliferating cells. As indicated by proliferating cell nuclear antigen immunohistochemistry and bromodeoxyuridine labeling experiments, the tall cells (ciliated and microvillar cells) of taste buds of larval lampreys are non-proliferating. In contrast, a few spherical cells surrounding the taste buds are proliferating cells. These cells are located below the basal cell layer of the epithelium. Occasionally, proliferating cells can be observed above or below the basal lamina or intermingled with the elongated cells of the taste bud. The cells above and below the basal lamina may be considered as progenitor cells of the tall taste bud cells, as described in gnathostome vertebrates [Finger, 2006]. Further studies with proliferation markers should confirm the early observations of Baatrup [1983b], i.e. the absence of mitotic activity in cells surrounding the taste buds of adult lampreys.

Neurochemical Characterization

Early morphological data on the structure of the taste buds of brook and river lampreys, obtained by light and electron microscopy [Baatrup, 1983a, b, 1985b], have recently been supported by findings obtained by use of anti-acetylated α-tubulin immunofluorescence techniques [Barreiro-Iglesias et al., 2008c]. Antibodies against acetylated α-tubulin can be used as ciliary markers [Piperno and Fuller, 1985] and as general markers of neuronal-like elements in both peripheral and central nervous system [Barreiro-Iglesias et al., 2008a, b]. In taste buds of the sea lampreys, only the biciliated cells are α-tubulin immunoreactive [Barreiro-Iglesias et al., 2008c] (fig. 3). These immunofluorescence experiments have confirmed the presence of two cilia in the sensory cells of the taste buds of the sea lamprey and the neuronal-like microtubular cytoskeleton of the cells. In addition, α-tubulin-immunoreactive afferent fibers contact these biciliated tubulin-ir cells without entering the taste bud [Barreiro-Iglesias et al., 2008c] (fig. 3), as previously reported in electron microscopic studies (see above). Biciliated cells and most afferent gustatory fibers are also immunoreactive to the neuronal marker calretinin (an EF hand calcium-binding protein) [Barreiro-Iglesias et al., 2008c]. Calretinin was initially discovered as a marker for amphibian taste buds

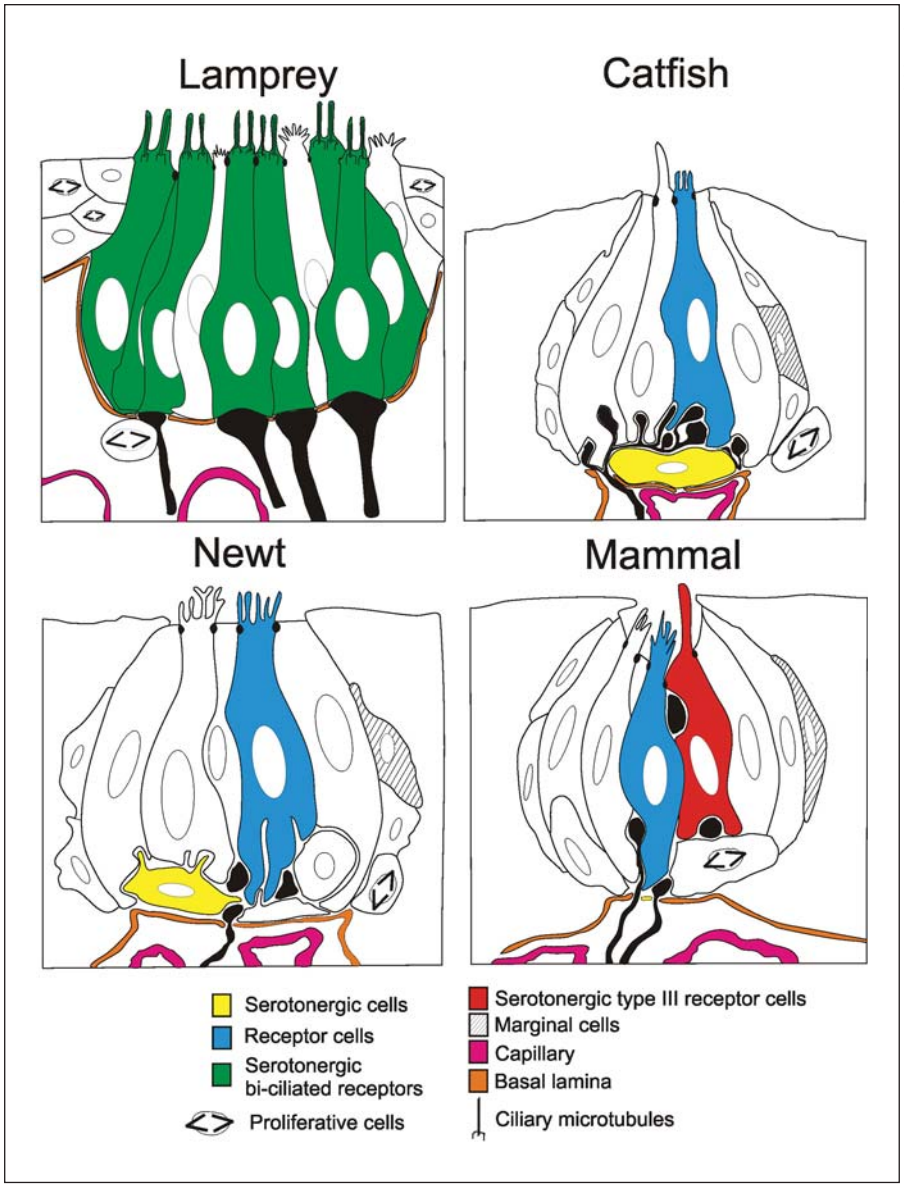

Fig. 4. Schematic drawing showing organization of the TBs of lampreys and of representative species of fishes, amphibians and mammals. White cells in the larval sea lamprey bud represent sustentacular microvillar cells. Afferent gustatory fibers are in black. Slightly modified from Barreiro-Iglesias et al. [2008c].

[Barlow and Northcutt, 1997]. Calretinin expression has also been observed in taste cells of teleost fishes [Díaz-Regueira et al., 2005; Northcutt, 2005].

A schematic drawing comparing the structure of the taste buds in representative vertebrates is shown in figure 4. Immunofluorescence experiments have also shown that the biciliated cells of the taste buds of lampreys are serotonin (fig. 3) and calcitonin gene-related peptide (CGRP) immunoreactive [Barreiro-Iglesias et al., 2008c]. As far as we are aware, the existence of CGRP-immunoreactive cells has not been reported in the taste buds of any other vertebrate species, which indicates that this is probably a unique characteristic of lampreys. Serotonin has only been observed in scarce Merkel-like basal cells in the taste buds of other anamniotes [Toyoshima and Shimamura, 1987; Delay and Roper, 1988; Delay et al., 1993; Reutter and Witt, 1993]. Merkel-like basal cells have been observed in the taste buds of lampreys by electron microscopy [Baatrup, 1983a], but have not been observed in immunofluorescence studies with an anti-serotonin antibody [Barreiro-Iglesias et al., 2008c]. In mammals, some type III taste receptors are tall serotonergic cells [Yee et al., 2001; Huang et al., 2009] and/or noradrenergic [Huang et al., 2009], which may resemble lamprey sero-

tonergic taste bud cells morphologically. However, homology between these cell types appears unlikely because of the absence of a similar cell type in jawed fishes or amphibians and the ciliary nature of serotonergic cells of lampreys. In addition, the negative results with TH (the common and rate-limiting enzyme of the catecholamine synthetic pathways) in the taste buds of the sea lampreys strongly suggests that taste cells of lampreys do not use noradrenaline [Barreiro-Iglesias et al., 2008c]. The morphological/neurochemical resemblance between serotonergic biciliated cells of lampreys and mammalian type III serotonergic taste receptors may be the result of convergent evolution.

In mammals, the main taste receptors are the type II taste cells (the light cells of mammalian taste buds). Different subtypes of type II cells have been reported, and are distinguishable by the expression of different proteins such as gustducin, phospholipase Cβ2 [Matsumura et al., 2009] and TRPM5 [Oike et al., 2006]. As regards the transmitters used by mammalian type II cells, recent studies reveal that they are purinergic (release ATP) [see Huang et al., 2009]. Further neurochemical studies should investigate whether serotonergic biciliated cells of lampreys express these protein markers specific to different subsets of mammalian type II taste receptor cells, and also whether some cells in the taste buds of lampreys use purinergic mechanisms of neurotransmission. In addition, cloning of serotonergic and purinergic receptors of lampreys and the study of their possible expression in taste bud cells and/or afferent gustatory fibers may also provide important information about the neurochemical transmission of the gustatory inputs in lampreys and about the evolution of the gustatory system in vertebrates.

Physiology

The physiology of the taste buds of lampreys has only been studied in larvae of *Lampetra planeri* [Baatrup, 1985a]. In this study, evaluation of the functional properties of the taste buds of *L. planeri* was performed by means of recording from nerve fibers obtained from the gill arch during mechanical and chemical stimulation. No mechanical responses were detected, but the buds were sensitive to chemical stimulants. Of the classical taste substances in gnathostomes, sodium chloride and quinine proved to be the most effective (threshold concentrations of 10^{-6} M). The threshold concentration of sucrose and acetic acid was 10^{-3} M. The most potent stimulants were the amino acids L-arginine and L-alanine (threshold concentrations of 10^{-7} M). For L-serine and L-glutamic acid, the threshold concentrations were 10^{-5} and 10^{-4} M, respectively. The physiological study by Baatrup [1985a] confirmed the role of the larval taste buds of lampreys in the reception and transmission of gustatory inputs and the homology between lampreys and gnathostome taste buds. However, the location of the taste buds of adult lampreys in the water (respiratory) tube and not in a common respiratory and digestive tract as in larvae suggests that pharyngeal buds of lampreys may serve other purposes, e.g. possible evaluation of oxygen levels in respiratory water [Baatrup, 1983b], as recently suggested for other serotonergic peripheral cell types in gills of lampreys [Barreiro-Iglesias et al., 2009]. In any case, the location of the taste buds in the respiratory water tube does not exclude the possibility that they play a role in the detection of taste substances that are dissolved in the surrounding water. Further studies analyzing the physiology of adult taste buds and the cloning of the possible taste receptors of lampreys should clarify this point.

Central Gustatory Nuclei

As far as we are aware, the organization of the central gustatory system has not been specifically studied in lampreys. In mammals, the gustatory afferents of the facial nerve enter into the gustatory nucleus directly (rostral part of the nucleus of the solitary tract) and the afferents of the glossopharyngeal and vagal nerves enter more caudally and ascend with solitary tract until they reach their targets in the gustatory nucleus [see Smith and Davis, 2000]. Koyama [2005] observed central targets of the glossopharyngeal and vagal nerves in the brain of *Lampetra japonica* by means of tract-tracing experiments. It should be taken into account that this study reported the overall central projections of the glossopharyngeal and vagal nerves and was not specific to the gustatory central afferents.

Thick ascending afferent fibers labeled from both the glossopharyngeal and vagal nerves of lampreys mainly terminate in the ipsilateral cerebellar area, medial octavolateral nucleus, and between the ventral octavolateral nucleus and descending tract and nucleus of the trigeminal nerve [Koyama, 2005]. In the area between the descending tract and the ventral octavolateral nucleus (fig. 1), central fibers of both nerves course near the dorsal margin of the rostral part of the descending tract, and shift dorsally in the caudal part. Koyama [2005] did not determine whether the ascending fibers pertain to the

vagal and glossopharyngeal nerves or are actually lateral line fibers coursing in peripheral branches of these nerves. Thinner varicose fibers are observed in the marginal region at the level of the vagal nerve entry [Koyama, 2005].

In lampreys, a nucleus equivalent to the nucleus of the solitary tract has been neurochemically identified by use of antibodies against different neuropeptides [Auclair et al., 2004 (tachykinins); Pombal et al., 2006 (neuropeptide FF); Pombal et al., 2008 (adrenomedullin)] in a region dorsal to the descending trigeminal tract in the caudal rhombencephalon. This cell population forms a well-delineated nucleus ventrally to the ventral octavolateral nucleus at the level of the vagal motor nucleus (fig. 1). These neuropeptides are also expressed by neurons of the nucleus of the solitary tract of different gnathostome species [Zhang and Ashwell, 2001 (tachykinins); Kivipelto et al., 1989, 1992; Crespo et al., 2003 (neuropeptide FF); Muñoz et al., 2001 (adrenomedullin)], which suggests that the neurochemical features of this nucleus were mainly established before separation of agnathans and gnathostomes. However, differences can be observed between lampreys and gnathostomes with regard to the expression of neuronal markers within this nucleus. In gnathostomes, some neurons of the nucleus of the solitary tract are catecholaminergic [Kivipelto et al., 1992; Cheng et al., 2006], whereas tyrosine hydroxylase immunoreactivity [Pierre-Simons et al., 2002; Pombal et al., 2006; Barreiro-Iglesias et al., 2010a] and tyrosine hydroxylase mRNA expression [Barreiro-Iglesias et al., 2010b] have not been observed in the nucleus of the solitary tract of lampreys.

In gnathostomes, the gustatory nucleus (rostral part of the nucleus of the solitary tract) projects to the parabrachial complex, thalamus and the reticular formation [see Smith and Davis, 2000]. Tract-tracing experiments in lampreys have only demonstrated the existence of afferent projections from the nucleus of the solitary tract to the middle and posterior reticular formation of the rhombencephalon [González et al., 1997], and the presence of a parabrachial complex homologue (or a secondary gustatory nucleus, as reported in teleosts) is uncertain. Projections to the lateral hypothalamus from the gustatory neurons of the parabrachial complex [Smith and Davis, 2000; Lei et al., 2008] appear to be related to the role of hypothalamic nuclei in feeding control [Lei et al., 2008]. Recent neurochemical data in lampreys also suggest that the lateral hypothalamic nucleus plays a role in the control of feeding in lampreys [Barreiro-Iglesias et al., 2010a]. Further studies should be performed to decipher the ascending gustatory pathways of lampreys and the central circuits of lampreys that participate in processing the gustatory information.

Hypothesis on Gustatory Information Transmission and Processing

Physiological studies have shown that the taste buds of lampreys, at least in larval lampreys, respond to common taste stimulants. Studies performed to date suggest that the biciliated cells of the taste buds in lampreys are probably the taste receptors and that microvillar cells are sustentacular cells. Serotonin is probably the main neurotransmitter for the transmission of the gustatory input from the biciliated taste receptors to the afferent gustatory fibers that contact the base of these cells, while CGRP may act as a neuromodulator during the transmission of the gustatory input to the afferent fibers. The presence of calretinin (a cytoplasmic calcium buffer) in the biciliated cells may be associated with modulation of serotonin and CGRP release by calcium influx following activation of cells [Evans et al., 2000]. The results of anatomical and neurochemical studies suggest that the central target of the gustatory fibers of the cranial nerves may be homologous to the nucleus of the solitary tract of gnathostomes. The ascending secondary pathways of the gustatory system of lampreys must be investigated to provide new insights into the early evolution of this system in vertebrates.

Acknowledgments

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