HISTOARCHITECTONICS OF THE CHOLINERGIC INNERVATION OF THE FROG TONGUE

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The cholilnergic innervation of the tongue is found in the form of bundles of nerve fibers in the filiform [9, 11], vallate, foliate, and fungiform papillae in rabbits [6, 11], monkeys [2, 9], guinea pigs, mice [3], and other mammals [2]. Specific cholinesterase is found in nerve cells at the base of the vallate papillae in rabbits and monkeys [6, 9, 11]. In amphibians acetylcholinesterase (AChE) has been described only in fungiform papillae [3, 8]. As regards the localization and distribution of postganglionic parasympathetic neurons and nerve fibers innervating the tactile and glandular formations of the amphibian tongue, no attempt has been made to study these problems.

This paper gives the results of an investigation of the cholinergic innervation of chemo- and mechanoreceptor structures and glandular formations of the tongue and also of the AChE distribution in nerve fibers of the hypoglossal and glossopharyngeal nerves, which innervate the amphibian tongue.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana temporaria) kept under standard laboratory conditions at 4°C. The cholinergic component of the innervation was studied by demonstration of AChE, the enzyme of acetylcholine turnover. The tongue was perfused through the lingual artery with Ringer's solution to remove blood components. Next, flaps of epithelium were excised from the dorsal surface of the tongue, separated from the underlying muscles, and placed on a slide and stretched until a film 70-90 μ thick was formed. Total preparations of epithelium thus obtained were fixed in calcium-formol solution (10% formalin in 1% calcium chloride solution) and the histochemical reaction was carried out according to the formula in [10] at 4°C, in incubation medium with pH 6.0. Thio analogs of acetylcholine were used as substiate: acetylthiocholine iodide (ATCh) for demonstration of AChE, and butyrylthiocholine iodide to demonstrate nonspecific cholinesterase (BChE). The incubation time of the preparations in the solution with substrate was determined experimentally relative to the test object on the basis of a distinct pattern of nerve cells in the tissues oi the tongue, and its value was 2 days. To identify enzymes in nerve fibers of the hypoglossal and glossopharyngeal nerves pieces of nerves measuring 7-10 mm were excised from the hypoglossal region, teased apart with a needle on a slide, and treated in the same way as preparations of the lingual epithelium. After incubation the preparations were dehydrated in alcohols, cleared in xylol, mounted in Canada balsam, and studied in the MBI-I and BIOLAR light microscopes. The most complete picture of the character of innervation of the various formations of the tongue could be obtained by the study of a total preparation of the epithelium.

EXPERIMENTAL RESULTS

The presence of copper thiocholine, the histochemical reaction and product, in numerous nerve bundles, in nerve fibers of the fungiform and filiform papillae, along the course of blood vessels, in nerve cells, and in numerous single axons of the interpapillary sub-

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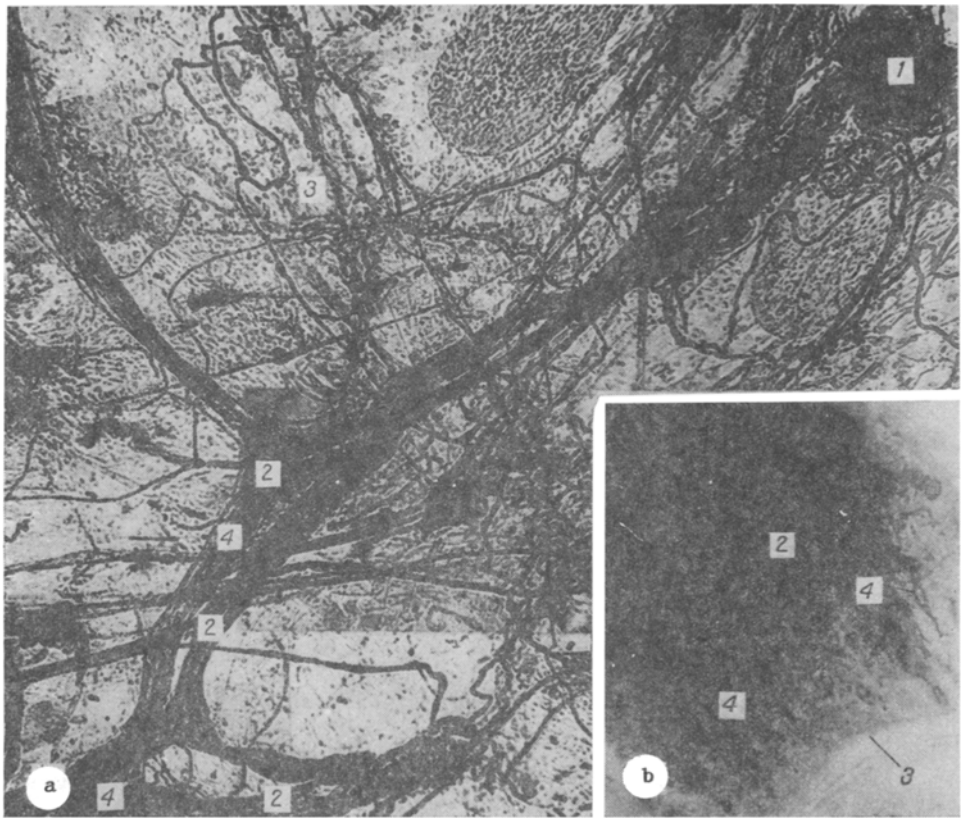

Fig. 1. Histoarchitectonics of cholinergic innervation of frog lingual epithelium (BIOLAR, 100×). 1) Fungiform papilla; 2) bundles of nerve fibers; 3) single nerve fibers; 4) blood vessel. Inset: fungiform papilla (MBI-1, 240×): 1) sensory disk, 2) plexus of nerve fibers in basal part of papilla, 3) apical surface of papilla, 4) fibers running from basal to apical part of papilla.

epithelial connective tissue is evidence that true cholinesterase was present in these nerve structures.

The photomontage, reproducing several fields of vision of the microscope, clearly shows without any description of the details, the abundance and complexity of the histoarchitectonics of the cholinergic innervation of the frog tongue (Fig. 1a).

Bundles of nerve fibers consisting of parallel cholinergic axons ramify and connect neighboring fungiform papillae. The bundles are accompanied by blood vessels, surrounded by a dense cholinergic plexus. The interpapillary epithelial tissue and connective-tissue layer also are abundantly innervated by single axons, with a complex pattern of branching, along the course of which the enzyme is unevenly distributed; many axons are highly convoluted. Fibers entering the fungiform papillae from a plexus at the base of the papilla, from which single fibers rise to the apical part of the sensory disk (Fig. 1b). High AChE activity was found in nerve fibers running into the tactile papillae. At the point where a nerve bundle enters the papilla it is joined by single fibers from neighboring fungiform and tactile papillae (Fig. 2a), an indication of nervous connection between receptor formations of the same or different modalities in the tongue.

Numerous glandular formations in the thickness of the mucosa on the dorsal surface of the frog tongue have a well developed cholinergic innervation. Glands on the dorsal surface of the tongue in amphibians are known to be mainly tubular. Their secretory portion lies at a varied depth in the muscle layer, and the efferent ducts may be either straight or branched [5]. Cholinergic nerve fibers surround the gland ducts, and these circular fibers give off terminals to the duct walls, where a nerve plexus is formed (Fig. 2b). Most glands in the region of the base of the tongue have several external openings, evidence that their efferent ducts are branched. The whole system of ducts of these glands is surrounded by a common cholinergic plexus, from which separate nerve fibers run to each duct, forming

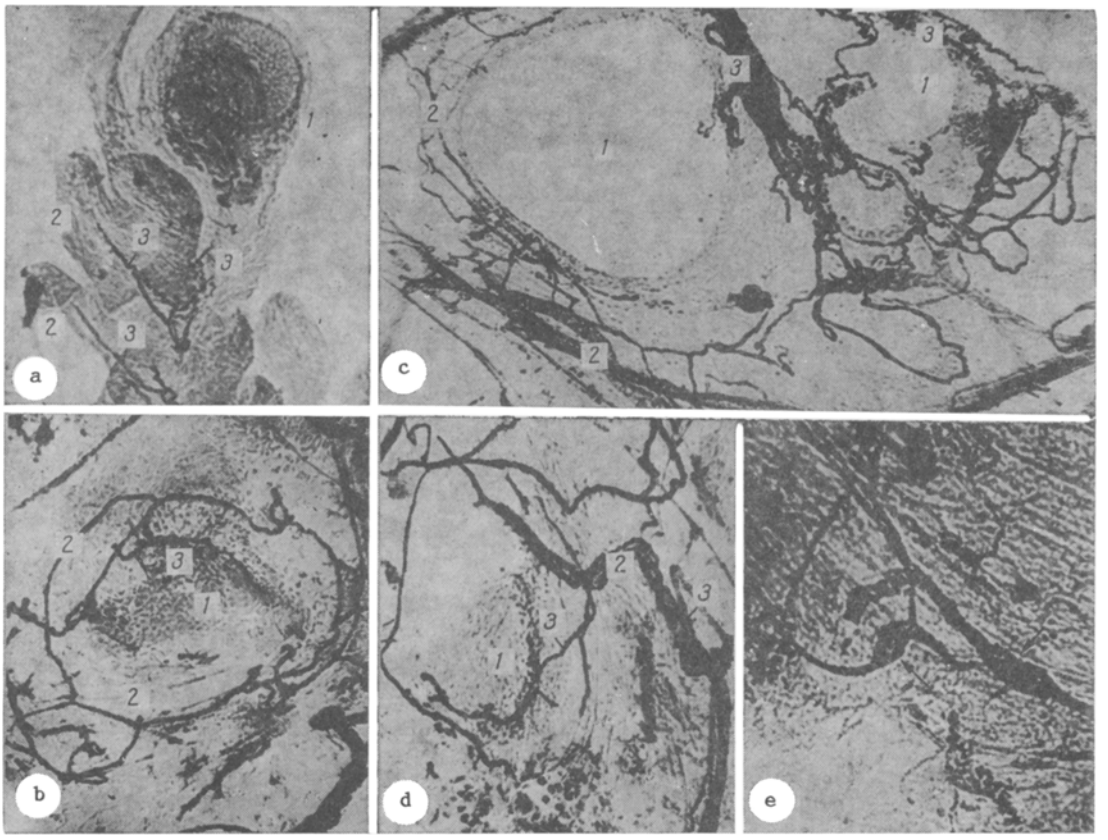

Fig. 2. AChE in structures of mucosa of frog tongue: a) innervation of fungiform and filiform papillae (MBI-1, 60×): 1) fungiform papilla, 2) filiform papilla, 3) nerve fibers; b) innervation of gland with one duct (MBI-1, 60×): 1) outer opening of gland duct, 2) circular nerve fibers, 3) plexus in wall of duct; c) innervation of gland with branched duct and several external openings (BIOLAR, 70×), legend as in Fig. 2b; d) innervation of blood vessel and gland duct by one bundle of nerve fibers (MBI-1, 60×): 1) external opening of gland duct, 2) blood vessel, 3) nerve fibers; e) neurons in interpapillary connective tissue (BIOLAR, 300×); arrows indicate bodies of neurons.

what appear to be secondary plexuses in its wall (Fig. 2c). Sometimes a blood vessel and a gland duct are innervated by the same bundle of cholinergic fibers (Fig. 2d).

High enzyme activity was found also in nerve cells lying in the subepithelial connective tissue (Fig. 2e). The neurons were multipolar of bipolar in shape and were diffusely distributed, without forming any strictly localized concentrations in the form of ganglia. Considering the high specific cholinesterase activity in them, it can be tentatively suggested that they are parasympathetic in nature.

Control experiments with BChE showed the complete absence of nonspecific enzyme activity in neurons with nerve fibers. Meanwhile a small quantity of palely stained residue — the end product of the histochemical reaction with BChE, was found on the walls of blood vessels and on the surface of the sensory disks of fungiform papillae (Fig. 3a). This positive reaction for nonspecific cholinesterase was probably due to the formation of a residue on membranes of one of the cellular types of taste bud. Similar cells, giving a positive reaction for BChE, have been found in mammalian taste buds [9].

A positive reaction for AChE (but not for BChE) was given by the hypoglossal and glossopharyngeal nerves. The quantity of end product of the histochemical reaction, indicating the presence of the specific enzyme in these nerves, differed: in the glossopharyngeal nerve, which is sensory, a denser deposit was found than in the hypoglossal, motor nerve (Fig. 3b, c). Besides cholinergic nerve fibers, bodies of nerve cells also were found in the trunk of the glossopharyngeal nerve. Together with typical multipolar neurons, bipolar

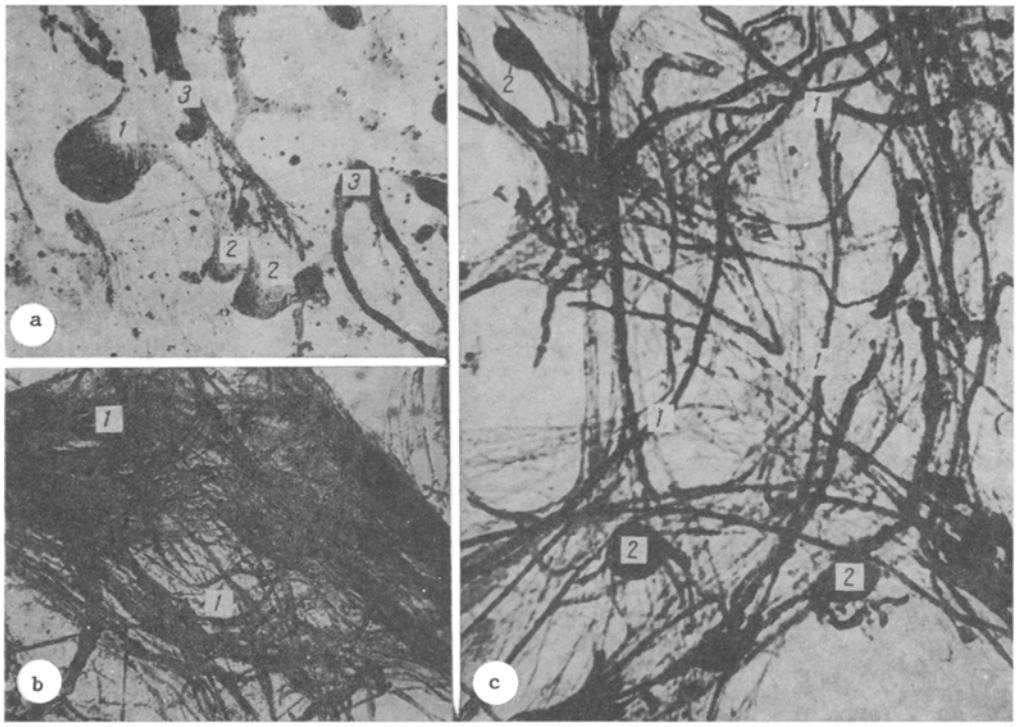

Fig. 3. BChE in lingual epithelium, AChE in nerves of frog tongue: a) nonspecific cholinesterase in lingual ipithelium (MBI-1, 48×): 1) fungiform papilla, 2) filiform papilla, 3) blood vessels; b) hypoglossal nerve (MBI-1, 60×): 1) nerve fibers; c) glossopharynegeal nerve (BIOLAR, 100×): 1) nerve fibers, 2) bodies of nerve cells.

and pseudounipolar neurons also were found (Fig. 3c). The nerve cells mentioned above are probably postganglionic neurons of the vagus nerve, fibers of which run in the trunk of the glossopharyngeal nerve in amphibians.

It can be postulated that neurons lying both along the course of the glossopharyngeal nerve and in the subepithelial connective tissue take part in various peripheral reactions: in changes of vascular and muscle tone, regulation of the diameter of the ducts, and functioning of the glands [2, 11].

These results may serve as the morphological basis for interpretation of the physiological data. The innervation of the different papillae by branches of single axons, and also the presence of nervous connection between the papillae may constitute the structural basis for peripheral interaction of lingual receptors described previously [1]. The discovery of cells giving a positive reaction for AChE, found in the subepithelial tissue of the tongue and along the course of the glossopharyngeal nerve, and data in the literature on the presence of efferent nerve endings in the basal part of the taste buds of the frog, containing vesicles with acetylcholine [3, 4, 7, 8], can provide a rational explanation of the cholinergic nature of centrifugal influences on the chemoreceptor apparatus established previously [1]. The presence of fibers in the apical part of the fungiform papillae and in the tactile papillae, and also of fibers connecting these papillae with one another, suggests that a cholinergic mechanism is involved in reception and transmission of tactile stimuli.

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