CONTENT OF BIOGENIC AMINES IN TASTE BUDS OF FUNGIFORM PAPILLAE IN RATS

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The nature of monoamines in the cells and fibers of taste buds and the ability of these structures to take up precursors of monoamines from the blood stream have now been studied [6, 8]. In the fungiform papillae of the tongue no monoamine-containing cells have been found [6].

This problem has subsequently received little study, but the results obtained by investigation of the taste buds of the vallate and foliate papillae have been extrapolated without justification to the fungiform papillae [2]. At the same time, differences in the structure, origin, times of maturation, and character of innervation of the chemosensory formation of the anterior free surface of the tongue and the chemosensory formation of the body and root of the tongue have been noted [5].

This paper gives the results of investigations of the adrenergic innervation and content of biogenic amines in the taste buds of fungiform papillae of the rat tongue.

EXPERIMENTAL METHOD

Experiments were carried out on tongues of adult male rats weighing 250 g. The content of biogenic amines in the taste buds and nerves was determined by fluorescence microscopy. For this purpose small pieces of mucosa of the tongue, containing different types of chemosensory papillae, were quickly frozen and the frozen sections (6-15 μ) were dried for 10-15 min. Some of the sections were incubated for 1.5-2 h in formaldehyde vapor at 80°C [4], the rest in o-phthalic aldehyde vapor [3] or (in our modification) in phthalic anhydride vapor for 20 min at the same temperature. The preparations thus obtained were examined in the ML-2 luminescence microscope.

The investigations were carried out on three groups of animals. Intact animals of group 1 served as the control. Animals of group 2 received injections of the following precursors, activators of synthesis of biogenic amines, and inhibitors of the enzymes which catalyzed their oxidation: histidine (precursor of histamine) 12.5 ml/kg of a 4% solution intraperitoneally 1 h before taking the material; pyridoxine hydrochloride — vitamin B_6 (activator of histamine synthesis) — 2.5 ml/kg of a 5% solution intramuscularly into the tongue 1 h before taking the material; nialamide (a monoamine oxidase inhibitor) 200 mg/kg intraperitoneally 2.5 h before taking the material; isoniazid (diamine oxidase inhibitor) 100 mg/kg intraperitoneally 2.5 h before taking the material. In the rats of group 3, to reveal the role of the sympathetic innervation in the formation, accumulation, and retention of biogenic amines in cells of the taste buds, all variants of the preceding experiments were carried out on desympathized animals. Chemical desympathization with guanethidine sulfate (25 mg/kg daily for the first 3 weeks of the postnatal period) was used for this purpose.

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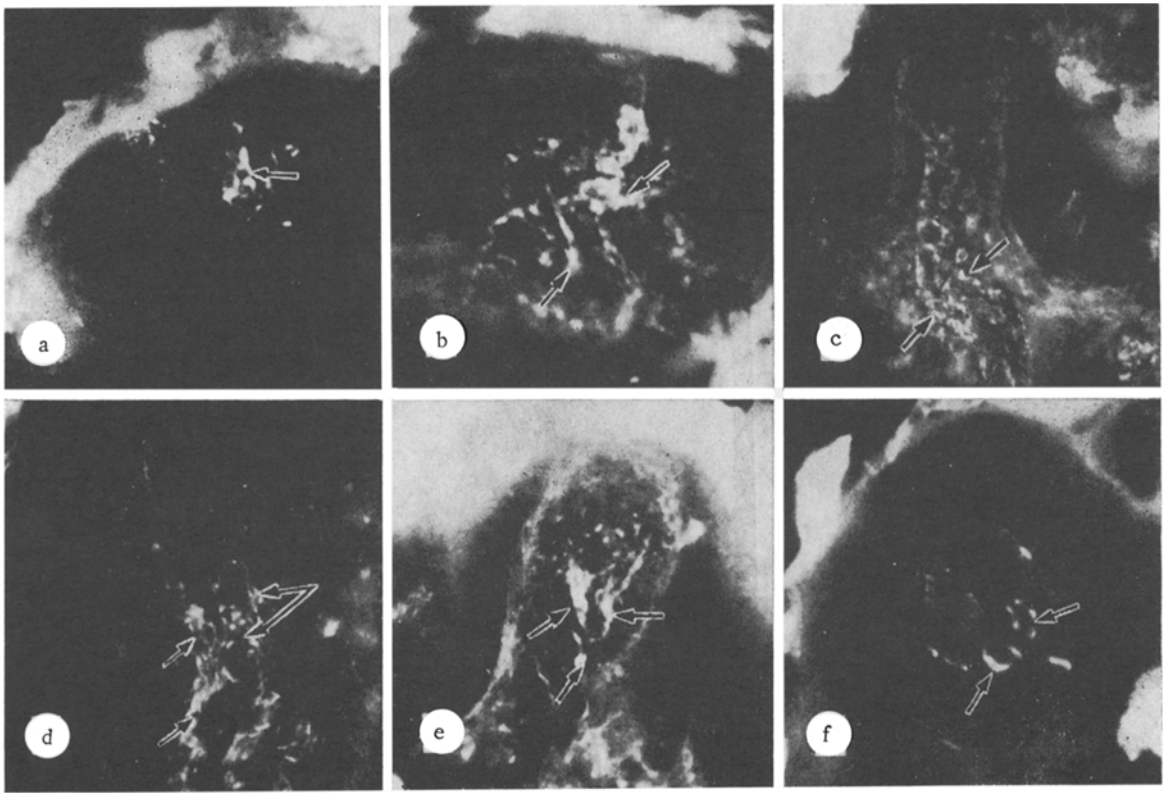

Fig. 1. Adrenergic innervation of fungiform papillae of the tongue of control animals (stained by Falck—Hillarp method). a, b, f) Transverse sections through fungiform papilla; c, d, e) parafrontal sections through fungiform papilla (360 ×). Arrows indicate adrenergic fibers surrounded by autoluminescent collagen fibers of connective-tissue part of papilla.

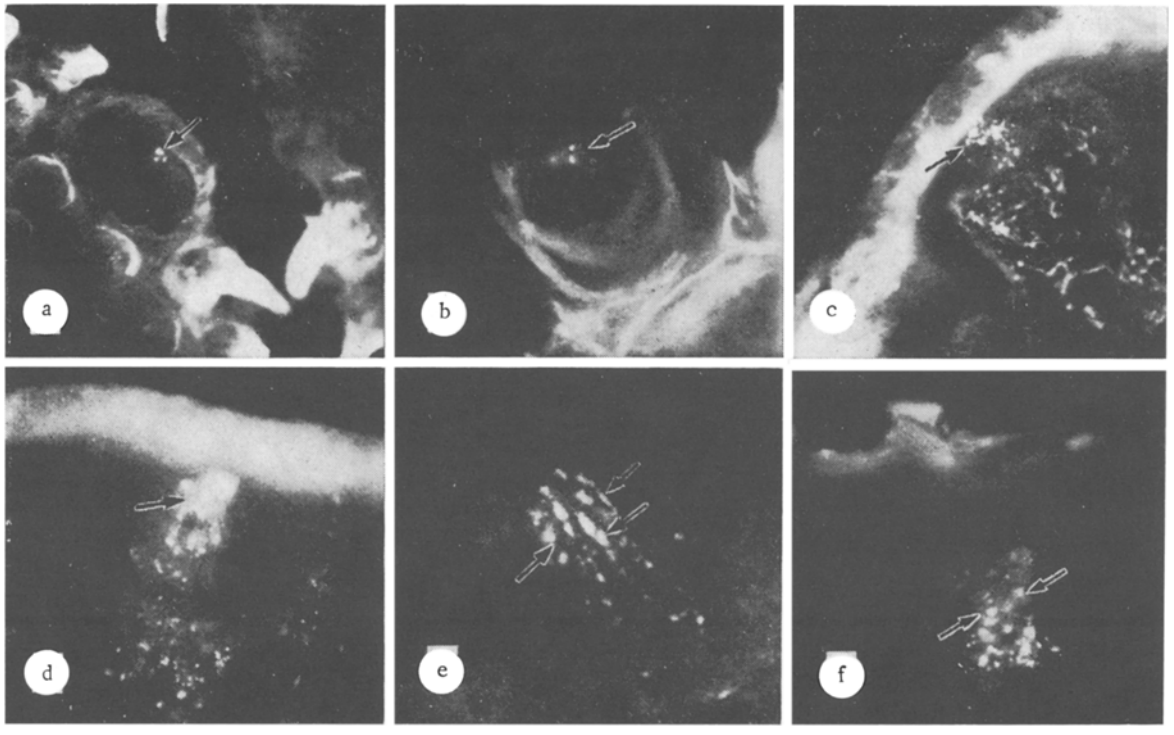

Fig. 2. Content of biogenic amines in apical part of fungiform papillae of normal animals. a, b) Control animals (180 ×); c) after injection of nialamide. Adrenergic fibers visible in stroma of papilla (86 ×); d) after injection of nialamide, isoniazid, and histidine (860 ×); e) after injection of nialamide (860 ×); f) granules in region of basal part of bulb (860 ×); a, b, c, e) stained by Falck—Hillarp method; d, f) treated with phthalic anhydride vapor. Arrows point to histamine-containing granules; autoluminescence of keratin at edge of papilla and collagen in connective-tissue part of papilla.

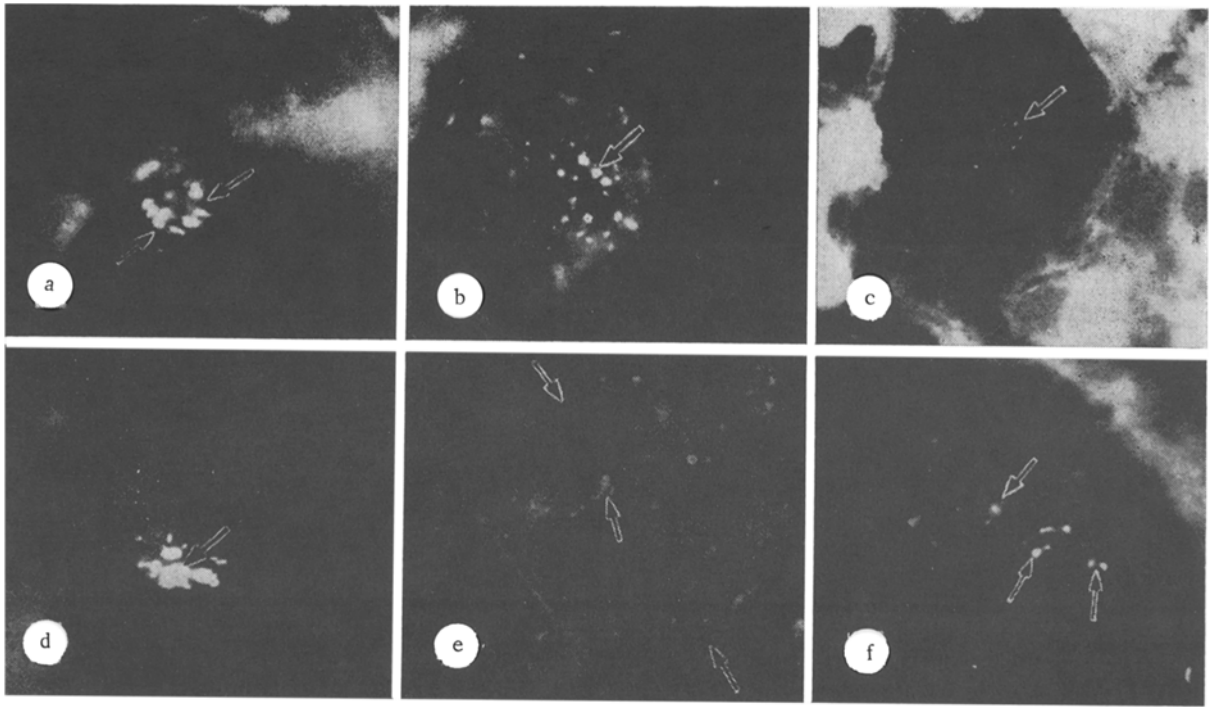

Fig. 3. Content of biogenic amines in different taste papillae of normal and desympathized animals. a) Fungiform papilla of normal animal after injection of nialamide (860 ×); b) the same, after injection of histidine and nialamide (860 ×); c) fungiform papilla of desympathized animal after injection of nialamide (360 ×); d) the same (860 ×); e) taste buds of vallate papilla of normal animal after injection of nialamide, histidine, and isoniazid (850 ×); f) taste buds of foliate papilla of desympathized animal after injection of nialamide (360 ×). Arrows indicate histamine granules in different papillae; a, b, f) Surface of papilla gives white autoluminescence; a, b, c, d, f) stained by Falck—Hillarp method; e) treated with phthalic anhydride vapor.

EXPERIMENTAL RESULTS

Many fibers giving emerald green fluorescence, and evidently adrenergic in nature (Fig. 1a-f) were observed in the connective-tissue part of the fungiform papillae of the control animals. Similar fibers also were found in the tongue of animals receiving nialamide (Fig. 2c). Some of these fibers climbed to the basal region of the taste buds (Fig. 1c, d; Fig. 2c). Analogous fluorescence also was observed in frontal sections through the basal region of the bulbs of the fungiform papilla, possible evidence of a definite connection between cells of the taste buds and these fibers.

Besides adrenergic fibers detectable by the Falck–Hillarp method, varicose fiber-like formations giving yellow-orange fluorescence were found in the basal region of the bulbs in the animals of the first two groups. These formations also were found in material treated by the other method — in phthalic anhydride vapor; this could indicate that the fluorophore of these formations is of a different, noncatecholamine nature.

Besides fluorescent fibers, treatment with formaldehyde revealed irregular granules, giving different fluorescence from serotonin and catecholamines, in the taste buds, specifically in their apical part. These granules had a russet hue, and as a rule they were discovered in the early stage of treatment of the material (incubation for 15-20 min), which suggested that the fluorophore may perhaps be histamine in nature (Fig. 2a, b).

Incubation of sections in phthalic anhydride vapor after preliminary administration of histidine (precursor of histamine), pyridoxine (which stimulates histamine synthesis), and isoniazid (a diamine oxidase inhibitor, preventing histamine breakdown) into the animals confirmed this hypothesis. In fact, this series of experiments revealed russet granules in the apical part of the bulbs, arranged in the form of lobules along the course of the cells (Fig. 2d, e; Fig. 3b). On a transverse section fluorescent granules were seen to be arranged in a circle around the cavity of the taste canal, which often showed fluorescence also (Fig. 2f; Fig. 3a). This fluorescence was evidently connected with the presence of material of the granules inside the taste canal. All these data indicates the presence of histamine-containing granules in taste buds of fungiform papillae, mainly in the apical part but also in the basal region, where their arrangement is less regular. Similar granules also were found in the taste buds of foliate and vallate papillae, but they were smaller and were arranged, not around the taste canal, but along the whole length of the bulb cells (Fig. 3e, f). Histamine granules were still present in the apical part of the bulb of fungiform papillae in desympathized animals (Fig. 3c, d), possible evidence that the processes of accumulation and synthesis of histamine are independent of the sympathetic innervation.

The discovery of histamine-containing granules in the taste bulbs at once raises two questions: in what type of cells are they located, and what is their functional role?

It can be tentatively suggested that cells containing histamine are supporting cells which, according to evidence of light and electron microscopy [5, 7], in all vertebrates contain secretory granules in their apical part. There is evidence that secretion of the granules of these cells may be released into the interior of the taste canal [7]. Fluorescence of material in the taste canal discovered in the present experiment confirms this suggestion.

The question of the functional role of histamine is less clear, especially if its broad spectrum of action is taken into account (stimulation of active transport, influence on various biosyntheses in cells, and so on) [1]. The presence of histamine granules in the taste canal and supporting cells may perhaps play a role in processes regulating the diameter of the pore and changing the permeability of the taste canal for various substances, i.e., regulation may be effected by internal autonomous mechanisms of the taste bulb without any participation by the CNS.

The taste bulb of the fungiform papilla in rats thus contains histamine in the apical part of the cells, in the region of the taste pore, and also in the basal region. The question of the physiological role of histamine requires special experimental study.

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PARENCHYMAL–STROMAL INTERACTION IN HEART MUSCLE DURING AGING

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Although the concept of interrelations between parenchyma and stroma is well established [1-3, 10, 11], there are very few concrete facts on parenchymal–stromal interaction under normal and pathological conditions [5, 6]. In research already undertaken the connective tissue as a rule has been studied in isolation from the parenchyma – the structure with which it constitutes a functional and dynamic, indissoluble unity. Investigations of the parenchyma and stroma of the heart by quantitative morphological methods are rare [7, 12-14]. From the age aspect the problem has been studied only at the ultrastructural level [8, 15].

This paper describes a morphometric and stereologic study of the muscle and connective tissue of the rat myocardium during aging.

EXPERIMENTAL METHOD

A quantitative study of tissue interrelation in the myocardium was undertaken on 10 intact male Wistar rats aged 4 months (three animals) and weighing 213.3 ± 8.8 g, aged 24 months (three animals) and weighing 240.0 ± 25.2 g, and aged 33 months (four animals) and weighing 337.5 ±5.0 g. After decapitation of the animals the heart was removed from the chest and placed in a cold chamber until it completely stopped beating. The relative weight of each heart was then determined. Samples of tissue from the left ventricle were fixed in 4% paraformaldehyde, postfixed in 2% OsO_4 solution, dehydrated in propylene oxide, and embedded in a mixture of Epon and Araldite. Semithin sections through cardiomyocytes were obtained on an LKB (Sweden) Ultrotome, stained with 1% azure II solution, and examined in a universal electron microscope. For each group of animals the mean diameter of the cardiomyocytes was determined in semithin sections by means of the MOV-15^{x} ocular micrometer with a magnification of 639. The same sections were used for stereologic analysis, under a magnification of 1000. A test system of short segments was used (n = 36, P = 72, L = 7.05 μ). By the stereologic methods described previously [6] the following primary parameters were assessed: relative volume (V_V) of the cardiomyocytes, of the cardiomyocyte nuclei, and of the lumen and endothelial cells of the capillaries, cells, and ground substance of the connective tissue, and also the relative surface area (S_V) of the cell structures. These data were used to calculate values of surface–volume ratios of the tissue structures, ratios of volumes of capillaries and stroma of the myocardium to cardiomyocytes and the ratio of the surface density of the capillaries to the bulk density of the cardiomyocytes. Statistical significance of differences between means was determined by Student's t test.

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