INVESTIGATION OF THE TASTE BUDS OF THE FROG TONGUE BY LUMINESCENCE MICROSCOPY

M. A. Savushkina, E. M. Krokhina, and A. I. Esakov

UDC 611.87-086.3-019:597.828

Special dumbbell-shaped cells of unknown nature, rich in catecholamines and giving a specific yellowish-green luminescence, were found inside the taste buds. The taste buds responded differently to preliminary (24 h before taking the material) injection of reserpine (0.25 mg). In some taste buds the dumbbell cells lost their property of specific luminescence and became invisible. In other taste buds the intensity of luminescence of the cells, especially of their outer thickenings, was sharply reduced. Adrenergic nerve fibers, giving green luminescence, were found in the lingual epithelium of the tongue as bundles and single varicose axons, winding around the blood vessels entering the taste buds as well as other blood vessels. The epithelial tissue of the tongue was richly innervated with adrenergic fibers.

KEY WORDS: taste receptors; adrenergic structures; luninescence microscopy.

There is no sufficient morphological and physiological evidence of the sympathetic innervation of and sympathetic influences on the activity of receptor structures in the various sensory systems [7, 9, 14, 15, 22, 24]. The sympathetic innervation of the chemoreceptor apparatus of the taste buds of the tongue [2, 4, 10, 11] and chemoreceptors of the carotid body [5, 19] have also been demonstrated. Electron-microscopic investigations have also shown the presence of chemosensitive fibers of vesicles with an electron-dense interior [3, 17, 18], in the synaptic endings, and, in the opinion of the workers concerned, these vesicles contained catecholamines.

Electrophysiological experiments have shown that sympathetic fibers may be one of the channels conveying efferent influences controlling the afferent flow of impulses from chemoreceptor structures [1, 6, 19, 21]. However, despite these investigations the question of the adrenergic structures of the taste bud, their character, and their distribution have received inadequate study. A few observations have been made with the luminescence microscope to show the presence of adrenergic fibers in the taste bud [10] and histochemical investigations have revealed high monoamine oxidase activity in the central part of the taste bud [12, 13].

The object of the present investigation was to study structures containing catecholamines in the fungiform papilla of the frog tongue by means of a luminescence-microscopic method.

EXPERIMENTAL METHOD

An area of the surface epithelium containing taste buds was cut out of the dorsal surface of the frog's tongue, separated from the underlying muscles, placed on a slide, and stretched uniformly to form a film 50-80 μ in thickness. The resulting film was dried in the air and at room temperature and then treated with formaldehyde vapor by the method of Falck and Owman [8]. The relative moisture content of the paraformaldehyde powder was 50-60%.

Laboratory of Experimental and Clinical Physiology of the Sense Organs and B. I. Lavrent'ev Laboratory of Neurohistology, Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 11, pp. 118-121, November, 1974. Original article submitted December 24, 1973.

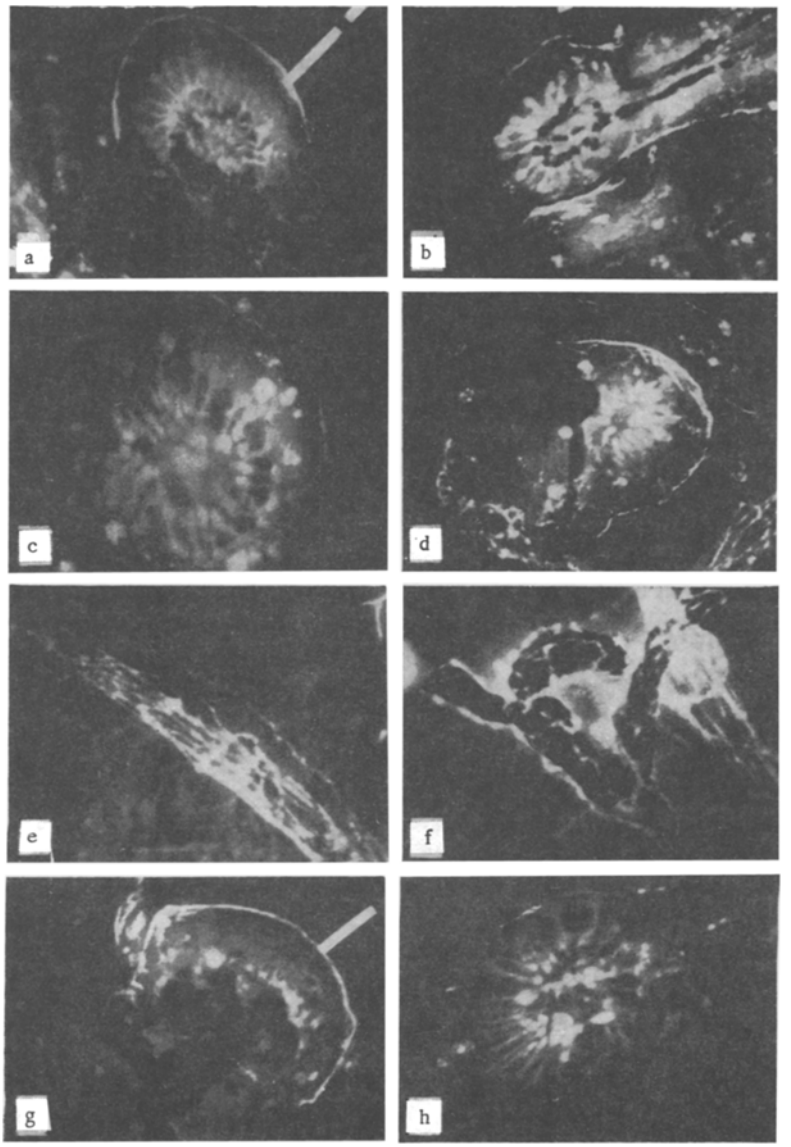

Fig. 1. Catecholamines in taste buds and nerves of the frog tongue: a, b, d, g) 120 ×; c, e, f, h) 360 ×. Explanation in text.

After histochemical treatment the preparations were studied and photographed with the ML-2 microscope. Luminescence was excited with the blue-violet region of the spectrum, wavelength 380-400 nm.

Specific catecholamine luminescence was identified by two methods. The test with reserpine consisted of injecting 0.25 mg of this substance into the frog's lymph sac 24 h before the material was taken for testing. The second test consisted of heating the preparations without paraformaldehyde.

EXPERIMENTAL RESULTS AND DISCUSSION

As a result of the treatment of the epithelial tissue of the frog tongue with paraformaldehyde distinctive dumbbell-shaped cell structures giving a specific yellow-green luminescence for catecholamines were found in the taste buds. On examination of the taste bud from the lateral surface (Fig. 1a) the dumbbell-shaped cells were found to radiate fanwise. The outer thickenings of the "dumbbells" were circular and stood apart from each other, whereas the inner thickenings were smaller and faced the vascular loop that could be seen inside each bud (Fig. 1b). The inner thickenings of the dumbbell cells were more strongly luminescent than the outer. The surface of the taste bud gave off white autoluminescence (marked by arrow).

Examination of the taste buds from above (Fig. 1c) showed that the dumbbell cells were arranged like camomile petals, with their inner expansions facing the center. The dark loop of a blood vessel could be seen beneath these cells with their specific luminescence.

The taste bud was approached by 2 blood vessels, one of which was frequently surrounded by a plexus of thin varicose adrenergic fibers, giving green luminescence typical of noradrenalin (Fig. 1d). The adrenergic fibers in the epithelial layer of the frog tongue were clearly visible as bundles (Fig. 1e), which gave off separate varicose axons to innervate the numerous blood vessels (Fig. 1f) and the epithelial tissue.

After preliminary injection of reserpine into the frogs, fungiform papillae were found in which no dumbbell cells could be seen, but the vascular loop became more clearly visible inside the bud (Fig. 1g). In other taste buds the dumbbell cells appeared shrunken after the administration of reserpine, their outer thickenings ceased to luminesce, and their inner thickenings continued to give the specific luminescence although they appeared reduced in size (Fig. 1h).

If the preparations were heated without paraformaldehyde, incidentally, no dumbbell cells were found but the taste buds were clearly outlined with the vascular loop inside and with autoluminescence of the surface of the bud as a thin, whitish strip (shown by an arrow in Fig. 1a, g).

Cell structures rich in catecholamines were thus found by the specific luminescence-microscopic method in the fungiform papillae of the frog tongue. These cells were found only in the taste buds and not in the epithelium surrounding them. From the color of its luminescence, the fluorogenic substance in these cells may have been one of its precursors; special tests agree with the histochemical data in the literature obtained in experiments on warm-blooded animals and demonstrating high monoamine oxidase activity in the central part of the papilla [12, 13].

Cells containing catecholamines have now been found in various sensory structures such as the retina [16, 23] and the carotid body [18, 20]. Whereas in the retina these cells have been identified as interneurons, specifically as amacrine cells [14], in the tactile corpuscle and carotid body they have been identified as receptor neurons. So far as the fluorescent cells found in the taste bud are concerned, their nature and the role of the high content of catecholamines in them still remain unexplained.

Together with cells, adrenergic fibers with a characteristic green luminescence were found in the tissues of the tongue. The fibers of one group were connected with blood vessels and penetrated inside the taste bud together with them, whereas the others followed a course independent of the blood vessels. It is difficult at present to state how closely the endings of the adrenergic fibers are connected with the cells of the taste bud. Investigation of serial sections of the papilla would probably shed more definite light on the problem. The abundant adrenergic innervation of the taste buds may evidently be concerned not only with the regulation of their blood supply, but they may also have some influence on the activity of the receptor cells. This hypothesis is confirmed by the results of physiological investigations. It has been shown in frogs [1, 6] and rats [21], for instance, that electrical stimulation of the sympathetic system leads to a significant change in the flow of afferent impulses from the taste-receptor apparatus.

In the presence of a catecholamine deficiency produced in animals by administration of reserpine the luminescence of the nerve fibers and dumbbell cells was considerably weakened, and this was matched by a corresponding decrease in the intensity of centrifugal influences on the taste-receptor apparatus observed in electrophysiological experiments. However, the question of the functional significance of the high catecholamine concentration in the dumbbell cells and their actual nature must be matters for further investigation.

LITERATURE CITED

1. A. I. Esakov, in: Problems in the Physiology and Pathology of the Nervous System [in Russian], Vol. 6, Moscow (1962), p. 63.
2. N. B. Lavrent'eva, Arkh. Anat., Gistol. Émbriol., 38, No. 6, 57 (1960).
3. R. A. Pevzner, Tsitologiya, No. 8, 971 (1970).
4. E. T. Yur'eva, Russk. Arkh. Anat., 6, No. 2, 209 (1927).
5. F. Al-Lami and R. G. Murray, Anat. Rec., 160, 697 (1968).
6. K. E. Chernetski, J. Neurophysiol., 27, 493 (1964).
7. E. Eldred and K. Hagbarth, J. Neurophysiol., 17, 59 (1954).
8. B. Falck and C. Owman, Acta Univ. Lund., Section 2, No. 7, 3 (1965).
9. K. Fuxe and B. Y. Nilsson, Experientia, 21, 641 (1965).

10. G. Gabella, J. Neurol. Sci., 9, 237 (1969).
11. F. W. Gairns, J. Physiol. (London), 121, 33P (1953).
12. G. Glenner et al., cited by Goldsmith and Ellis [13].
13. M. Goldsmith and R. A. Ellis, Anat. Rec., 139, 303 (1961).
14. J. Haggendal and T. Malmfors, Acta Physiol. Scand., 64, 58 (1965).
15. C. C. Hunt and A. J. McIntyre, J. Physiol. (London), 153, 88 (1960).
16. A. Iggo and A. R. Muir, J. Physiol. (London), 200, No. 3, 763 (1969).
17. P. Iraldi and E. Robertis, in: Proceedings of the 2nd International Congress of Endocrinology, London (1964), p. 355.
18. K. Ishii and T. Oosaki, J. Anat. (London), 104, 263 (1969).
19. K. Ishii, J. Ishii, et al., J. Exp. Med., 102, 113 (1970).
20. R. D. Yates, J. Li-Chen, and D. Duncan, J. Cell Biol., 46, 544 (1970).
21. K. Kimura, Kumamoto Med. J., 14, 95 (1961).
22. O. Lövenstein, Brit. Med. Bull., 12, 114 (1956).
23. T. Malmfors, Acta Physiol. Scand., 58, 99 (1963).
24. H. Spoendlin and W. Lichtensteiger, Acta Oto-Laring. (Stockholm), 66, 423 (1966).