including enzymes such as trypsin, chymotrypsin, and pepsin, must be adsorbed onto its surface and incorporated into its gel. In turn, the mucous coating, if detached into the intraluminal medium, constitutes a supplier of the solid part of the intestinal juice.

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LITERATURE CITED

1. Yu. M. Gal'perin, P. I. Lazarev, T. Z. Ivanova, et al., Dokl. Akad. Nauk SSSR, 264, No. 2, 504 (1982).
2. I. E. Mitin, E. V. Petrova, V. K. Mazo, et al., Vopr. Pitan., No. 3, 49 (1983).
3. V. V. Moslov, Proteolytic Enzymes [in Russian], Moscow (1971).
4. A. Allen, Mod. Prob. Paediat., 19, 11 (1977).
5. A. Allen, Br. Med. Bull., 34, 28 (1978).
6. A. J. Barrett and M. F. Heath, "Lysosomal enzymes," in: Lysosomes, Amsterdam (1977).
7. H. Florey, Proc. R. Soc. London B, 143, 147 (1955).
8. J. Forstner, Mod. Probl. Paediat., 19, 1 (1977).
9. M. Kramer and F. Lauterbach, Intestinal Permeation, Amsterdam (1977).
10. T. G. Petters and M. Muller, J. Exp. Med., 136, 1117 (1972).
11. A. Taylor and F. E. Tisdell, Arch Biochem., 210, 90 (1981).
12. D. Waldron-Edwards, Can. J. Surg., 13, 341 (1970).

EFFECT OF SUBSTANCE P ON MONOAMINE-CONTAINING TASTE BUD CELLS

N. A. Solov'eva and A. I. Esakov

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The presence of nerve fibers, immunoreactive toward substance P, has recently been demonstrated in taste buds of the tongue of some species of mammals by indirect immunohistochemical methods [3-5]. These fibers, running through the basement membrane of the papilla, form a dense perigemmal plexus immediately beneath the epithelium. It has been shown that some immunoreactive fibers running from this plexus penetrate into the taste buds and spread there as far as the gustatory pore. The distribution of substance P in the taste buds may indicate a connection and possible interaction of this peptide with the population of serotonin-containing cells located here, as is the case, for example, in certain structures of the CNS [6].

To confirm this hypothesis, a fluorescence-histochemical study was made of the effect of exogenous substance P on serotonin-containing cells of frog taste buds. The frog was chosen as test object because of the possibility of obtaining total stretch preparations from the layer of gustatory epithelium, so that whole populations of monoamine-containing cells can be studied.

EXPERIMENTAL METHOD

Experiments were carried out on frogs *(Rana temporaria)* kept under standard laboratory conditions at 10°C. In the experiments of series I synthetic substance P (from Serva, West Germany) was injected intraperitoneally into normal animals in a dose of 6-7 μg. The peptide was made up in Ringer's solution for cold-blooded animals immediately before injection. The

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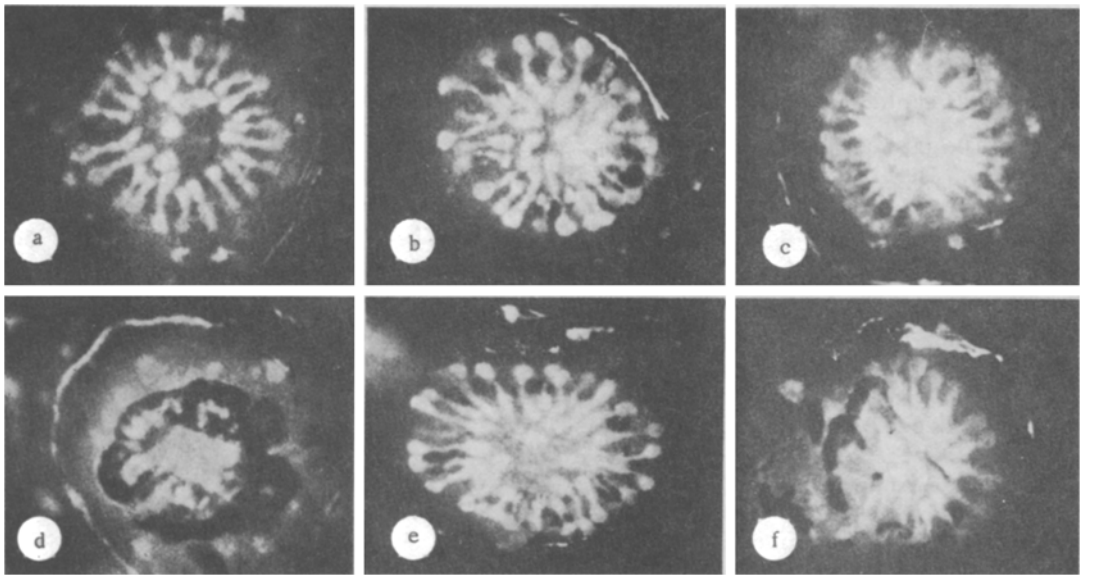

Fig. 1. Effect of substance P on monoamine-containing cells of frog taste buds (120 ×): a) serotonin-containing cells in taste bud of control animals; b) intensification of fluorescence of cells 24 h after injection of substance P; c) intensification of fluorescence of internal thickenings of cells 3-5 days after injection of substance P; d) sharp decrease in fluorescence of cells under the influence of rausedil; e) restoration of fluorescence of cells 24 h after injection of substance P; f) no effect of rausedil on monoamine-containing cells when injected simultaneously with substance P.

gustatory epithelium was studied 24 h and 3, 5, and 10 days after the injection. Taste buds of intact animals served as the control.

In series II substance P was injected by the method described above into animals with an artificial deficiency of biogenic amines induced by preliminary (24 h beforehand) intraperitoneal injection of rausedil (0.5 mg/kg).

In series III the above-mentioned doses of rausedil and substance P were injected simultaneously, 24 h before removal of the material.

All experiments were conducted in the fall and winter. Preparations from the lingual epithelium of the control and experimental animals were obtained by the method described previously [2], treated with formaldehyde vapor at 80°C for 1 h, and studied unembedded in the LM-2b luminescence microscope.

EXPERIMENTAL RESULTS

Serotonin-containing cells in individual taste buds of the tongue 24 h after injection of substance P were indistinguishable in the character of their fluorescence from the control (Fig. 1a). In most taste buds the cells had brighter fluorescence than normally. Under these circumstances the external circular and internal polygonal thickenings of these cells gave fluorescence of equal intensity (Fig. 1b). Some increase in size of the cells also was observed. The intensity of fluorescence 3-5 days after injection of substance P was significantly increased in the region of the internal thickenings of the cells, whereas florescence in the external thickenings became less bright (Fig. 1c). On the 10th day after a single injection of substance P the intensity of fluorescence of ther internal segments of the cells returned to its original level. The number of serotonin-containing cells in the buds, incidentally, was unchanged after injection of substance P compared with the control throughout the period of investigation.

The considerable increase in the intensity of fluorescence of the taste bud cells under the influence of substance P pointed to an increase in their serotonin concentration, which probably took place initially (after 24 h) mainly in the external thickening of the cells, leading to equalization of the intensity of fluorescence in the two parts of the cells. Later (after 3-5 days) a redistribution of serotonin took place and it accumulated mainly in the internal thickenings.

In the next series of experiments the effect of this peptide on monoamine deficiency in serotonin-containing cells of the taste buds caused by injection of rausedil was studied. In this case, as a result of a decrease in the serotonin content there was a sharp fall in the intensity of fluorescence of the cells in the taste buds; fluorescence became weak and was quickly extinguished under the influence of exciting light (Fig. 1d). Injection of substance P in these experiments caused complete restoration of the fluorescence of the cells to the normal level within 24 h, and in some taste buds the normal level was considerably exceeded (Fig. 1e). After simultaneous injection of the two substances, the exhausting action of rausedil on serotonin-containing cells was not observed. As Fig. 1f shows, no changes in the character of fluorescence of the cells were observed in most taste buds and they continued to fluoresce with the same intensity as in control preparations (Fig. 1a). Only in individual buds was weak fluorescence of the cells observed.

The results are evidence that substance P plays an important role in maintenance of a certain serotonin level in the taste bud cells. The increase in the serotonin concentration observed in these experiments under the influence of substance P was in all probability due to its more intensive synthesis, possibly due to activation of the key enzymes, tryptophan hydroxylase, and also to activation of the uptake of the initial amino acid (tryptophan) by the cells.

At the same time substance P is evidently involved also in the control of processes connected with monoamine deposition. As these experiments showed, the exhausting action of rausedil, based on disturbance of the transition of monoamines into the storage granules, is abolished by injection of substance P, and the serotonin level in the cells is restored to normal. Experiments in which the two substances were injected intraperitoneally at the same time showed that substance P, probably by acting as an antagonist of rausedil, activates serotonin uptake into the granules and its deposition, and so maintains its original level in the cells.

Meanwhile the results showing the effect of exogenous substance P on monoamine-containing cells of frog taste buds indicate the presence of endogenous substance P-like material in these structures, located evidently as in other animals in nerve fibers.

Immunohistochemical investigations on cats and rats have shown that substance P-immunoreactive fibers, found in taste buds, run in the composition of the gustatory sensory nerves, and probably perform a trophic function [3, 5]. The peptide, synthesized in the body of neurons located in central sensory ganglia [3], is transported by the axoplasmic flow to the nerve endings in the taste buds where, it can be postualted on the basis of the results of the present experiments, it participates in the realization of trophic influences of sensory neurons on the taste apparatus.

Substance P, secreted from nerve endings, regulates the serotonin level in the monoamine-containng cells of the taste buds by acting on serotonin synthesis and also on its uptake and storage in the cells, whereas the proliferation and differentiation of these cells and the structure of the taste bud are controlled by other neurotrophic factors contained in sensory endings, and also with the aid of catecholamines located in endings of sympathetic nerve fibers [1].

Substance P is a polyfunctional peptide with a wide spectrum of action. The effect of substance P on monoamine metabolism can evidently cause long-lasting changes in the functional state of the taste receptors, but this is evidently not the only point of application of the peptide in the taste apparatus. Substance P may be a modulator in the synpatic transmission of excitation in structures of the taste bud, influencing serotonin release and its reassimilation and modifying the state of the postsynaptic receptors for monoamines. However, these hypotheses require further experimental confirmation.

LITERATURE CITED

1. A. I. Esakov, E. M. Krokhina, and M. A. Savushkina, Tsitologiya, No. 10, 1180 (1976).
2. M. A. Savushkina, E. M. Krokhina, and A. I. Esakov, Byull. Éksp. Biol. Med., No. 11, 118 (1974).
3. J. M. Lundberg, T. Hokfelt, A. Anggard, et al., Acta Physiol. Scand., 107, 389 (1979).
4. I. I. Nagy, M. Goedert, S. P. Hunt, et al., Neuroscience, 7, 3137 (1982).
5. T. Nishimoto, M. Akai, S. Inagaki, et al., J. Comp. Neurol., 207, 85 (1982).
6. M. Schutzbery and T. Hokelt, Br. Med. Bull., 38, 309 (1982).