POSSIBLE NATURE OF THE BIOGENIC AMINE IN THE DUMBBELL CELL OF THE FROG TASTE BUD

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An attempt was made by histochemical analysis to identify a specific monoamine present in the dumbell cells of the taste buds of the frog tongue. By luminescence microscopy it was shown that injection of exogenous serotonin into the blood stream in the tongue led to a sharp increase in specific luminescence of the dumbbell cells, whereas blocking of serotonin synthesis by DL-parachlorophenylalanine caused weakening and disappearance of the specific luminescence. The authors conclude from their results that the biogenic amines of the dumbbell cells are possibly of serotonin nature.

KEY WORDS: *taste bud; serotonin; luminescence microscopy.*

Previous investigations by the method of luminescence microscopy showed that the taste papillae of the frog tongue contain a population of distinctive, radially arranged cellular structures of dumbbell shape giving a positive reaction for biogenic amines [2, 7]. The number of cells in the population in the fall and winter (September-February) was 18-24, rising in the spring and summer (March-June) to 50-70, and 2 or 3 such cell populations were seen to be formed in one papilla (the intensity of specific luminescence and the size of the cells were considerably reduced under these circumstances [3, 8]. It was also shown that the number of dumbbell cells with biogenic amines in the taste bud and the intensity of their specific luminescence are under neuronal control, with the participation of sensory and sympathetic fibers innervating the taste apparatus [1, 8]. However, the chemical nature of the biogenic amines in these cells was not studied, although the yellowish green color of their luminescence, distinct from the luminescence of adrenergic fibers, giving a specific emerald green luminescence, and also the relatively rapid fall in the intensity of luminescence in response to continuous blue-violet irradiation pointed to the possible serotonin nature of the fluorogenic substance in these cells.

The object of this investigation was to study the nature of the biogenic amine contained in the dumbbell cells by a pharmaco-histochemical method involving perfusion with serotonin and the blocking of its synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (*Rana temporaria*) kept under standard laboratory conditions at 10°C.

In the experiments of series I a single injection of 10 μg serotonin (creatinine-sulfate), dissolved in 1 ml Ringer's solution, was given into the blood stream of the frogs' tongue, and the state of the taste papillae was investigated 30 and 60 min later. The experiments of this series were carried out in summer (June and July).

In series II, 24 h before fixation of the material the frogs were given an intraperitoneal injection of DL-parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, in doses of between 150 and 300 mg/kg. The experiments of this series were carried out in January and February. The taste buds of intact animals taken at the corresponding seasons served as the control.

Specimens from the lingual epithelium of the control and experimental animals were prepared by the method described previously [2], treated histochemically in formaldehyde vapor at 80°C for 1 h [5], and studied unmounted in the ML-2 luminescence microscope.

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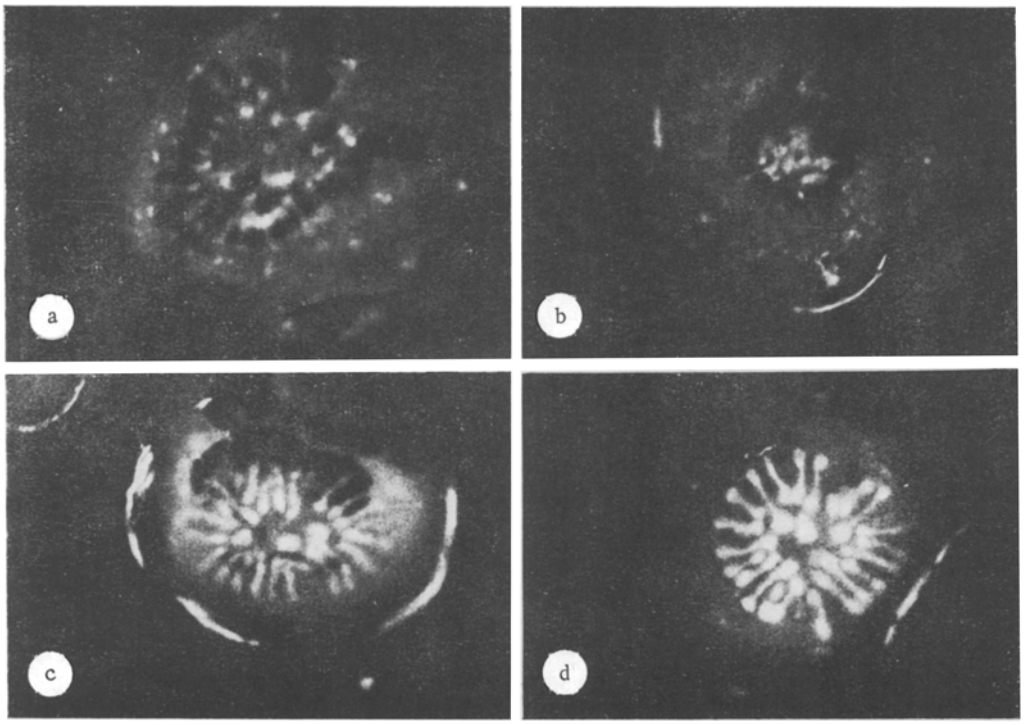

Fig. 1. Effect of exogenous serotonin on dumbbell cells of taste buds of frog tongue (120×). A, B) Control taste buds with very weakly luminescent dumbbell cells (June-July); C) recovery of luminescence of cells 30 min after serotonin injection; D) intensive luminescence of cells 60 min after injection of serotonin.

EXPERIMENTAL RESULTS

An attempt was made by cytospectrofluorometry* to evaluate the emission spectrum of the fluorogen in the dumbbell cells. On excitation of luminescence in the blue-violet region of the spectrum (wavelength 380 nm) the maximum emission of the cells was found to be in the 520 nm region, corresponding to the serotonin nature of the cellular fluorophore.

The specific luminescence of the dumbbell cells of most papillae is known to fall sharply in summer (Fig. 1A), and in some papillae none of these cells were found whatsoever (Fig. 1B). After intravascular injection of serotonin under these conditions, specific luminescence recovered significantly after 30 min (Fig. 1C). After 60 min the dumbbell cells had acquired bright characteristic yellowish green luminescence and their usual shape and distribution (Fig. 1D). The outer and inner segments of the cells emitted luminescence of equal intensity, evidence of the uniform distribution of accumulated serotonin in the cell. The dimensions of the cells observed were smaller than those of the cells obtained in the fall and winter, thus corresponding to seasonal changes in this index of cellular dynamics [3, 8].

Injection of serotonin into the blood stream of the tongue did not change the specific weak pale green luminescence of the adrenergic nerve fibers characteristic of this season. The increase in the intensity of luminescence of the dumbbell cells under the influence of serotonin and the absence of any such effect in the adrenergic fibers point to selective accumulation of serotonin by the cells and the existence of different monoamines in the dumbbell cells and adrenergic fibers.

Besides the effect of exogenous serotonin, the state of the dumbbell cells also was studied after blocking of serotonin synthesis by PCPA, an inhibitor of tryptophan hydroxylase [6]. It was decided to carry out these experiments in winter (January-February), when the intensity of luminescence of the cells is characteristically high, probably on account of the high content of their biogenic amine. The typical structure of a taste bud in the control animal in winter is illustrated in Fig. 2A: Intensively luminescent dumbbell cells, radially arranged, can be seen.

*The authors are grateful to Dr. Med. Sci. P. N. Aleksandrov for help with this test.

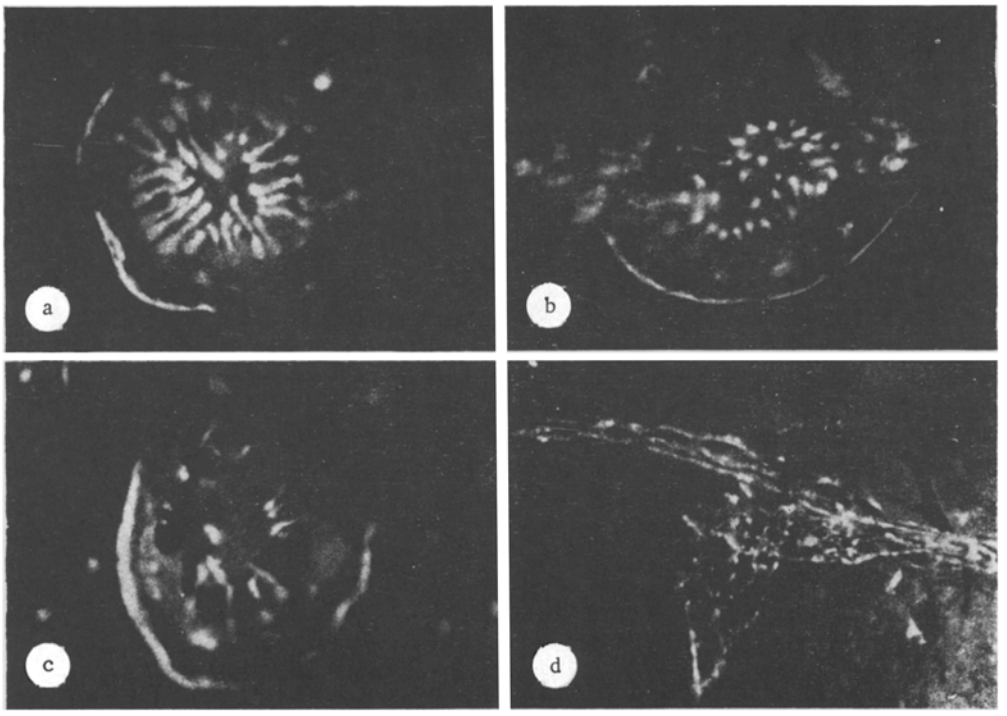

Fig. 2. Effect of parachlorophenylalanine (PCPA) on dumbbell cells of taste bud of frog tongue (120×). A) Normal appearance of cells with biogenic mines in winter; B) small, weakly luminescent dumbbell cells 20 h after injection of PCPA; C) taste bud with reduced number of small cells after action of PCPA; D) adrenergic nerve fibers of lingual epithelium still visible after action of PCPA.

Marked weakening of luminescence of the cells was observed 24 h after injection of PCPA, their thickness was reduced, their outer segments could not be seen, and the inner segments appeared as shining dots (Fig. 2B). The number of cells in these papillae showed no significant change. Meanwhile papillae were found in which there were only 3 to 5 luminescent cells or none whatever (Fig. 2C). It is intersting to note that luminescence of the adrenergic fibers in these sections did not differ significantly from that in the control (Fig. 2D).

The presence of a maximum of the emission spectrum in the 520 nm region is thus evidence of the possible serotonin nature of the fluorogen. This is confirmed in experiments in which exogenous serotonin was injected, for the cells were shown to take up the mediator from the blood stream. The sharp weakening of the intensity of luminescence and the reduction in the number of luminescent cells or their complete disappearance under the influence of PCPA, a specific blocker of serotonin synthesis, points to the presence of a specific enzyme of indoleamine synthesis in the cells. This conclusion is in agreement with the results of the experiments of Nada and Hirata [10] on sections through the lingual taste papillae of the frog *Rana catesbiana* which showed that the serotonin analog 5,6-dihydroxytryptamine, which specifically exhausts indoleamine-containing structures, causes a decrease in luminescence in the taste bud. Probably the mechanism of accumulation of monoamine is not rigidly fixed, so that the cells can take up other biogenic amines also, such as adrenalin [3, 8]. However, precursors of catecholamines, notably L-dopa, as preliminary investigations in the writers' laboratory have shown, have no action analogous to that of the indoleamine precursors DL-tryptophan and 5-hydroxytryptophan.

Cells containing a serotonin-like amine have recently been found in the taste buds of several different vertebrates, notably fishes [12] and the foliate papillae of the rabbit [9]. The circumvallate papillae of the rabbit contain cells which are potentially capable of synthesizing serotonin from its precursors [11]. The significance of these amines is not yet very clear. Our earlier investigations [4] show that global exhaustion of biogenic amines by reserpine weakens or abolishes regulatory influences on the chemoreceptor system.

It may be that it is along these lines that efforts must be made to discover the role of indoleamine. Perhaps they participate in an afferent function, but it is evidently not by accident that the whole population of these cells is located far out at the periphery of the chemoreceptor system.

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SUBMICROSCOPIC CHANGES IN THE THYROID GLAND IN BURNS

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The submicroscopic changes in the thyrocytes and perifollicular blood capillaries were studied on the 1st, 2nd, 7th, 14th, 21st, and 28th days after experimental burns on sexually mature male guinea pigs. During the period of burn shock the picture in the thyroid gland was dominated by macrofollicles formed by thickened thyrocytes. The lumen of the capillaries was dilated and the ultrastructure of their wall disturbed. On the 7th-14th day an increase in the height of the cells, with hypertrophy and hyperplasia of the intracellular organelles, were observed. Together with hyperplastic changes in the thyrocytes and endothelial cells, destruction of the membranous components of the cells increased; in the later stages of burns (21st and 28th days) this led to the development of severe necrobiotic lesions of the cells and to a sharp disturbance of vaso-parenchymatous relationships in the gland.

KEY WORDS: *thyroid gland; ultrastructure; burns.*

Considerable material has been collected in recent years on disturbance of the functions of the anterior pituitary and adrenal cortex in burns [3, 4]. The morphology of the endocrine glands and, in particular, of the thyroid gland, has received much less study [2, 6]. There are only a few brief and contradictory reports in the literature on the character of the histological changes in the thyroid gland in burn shock [7-11] and one paper on a study of the thyroid gland in the later stages of burns [2]. There is absolutely no information in the accessible literature on changes in the thyroid gland in burns detected by investigation at the ultrastructural level.

With these facts in mind, and also considering the important role of thyroid hormones in the pathogenesis of burns, the investigation described below was undertaken.

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