PHARMACO-HISTOCHEMICAL STUDY OF MONOAMINE-CONTAINING TASTE BUD CELLS IN CAPSAICIN-INDUCED SUBSTANCE P DEFICIENCY

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Research workers have recently been drawn to the study of functional interaction between substance P (SP) and various monoaminergic systems, both in the CNS and at the periphery. SP located in sensory nerve fibers innervating taste buds [7, 8] has been shown to affect the serotonin level in the serotonin-containing cells located in them. Administration of SP increased the serotonin concentration in the cells, and, in artificial monoamine deficiency induced by rausedil, normalized its level. If SP was administered along with rausedil, it had a protective effect on serotonin-containing cells [4]. The mechanisms of this phenomenon have not been explained. In a study of the physiological role of SP in sensory systems a toxin which has been successfully used is capsaicin, the active ingredient of various species of capsicum, which exhausts the reserves of this peptide in central and peripheral processes of primary sensory neurons and disturbs their function. These effects of capsaicin persist in adult animals for several months [5, 6]. However, capsaicin evidently has no direct action on monoamines [9].

The aim of this investigation was to make a pharmaco-histochemical study of serotonin-containing cells in taste buds in capsaicin-induced SP deficiency. The results could help to shed light on the precise mechanisms of action of this peptide in the taste organ.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (*Rana temporaria*) kept under standard conditions at 10°C. Capsaicin (Merck, West Germany) for injection was prepared by the method in [5] and injected in a volume of 0.3 ml twice in a dose of 30 mg/kg subepithelially into the tongue. Control animals received an injection of the solvent of capsaicin: 5% ethanol + 5% Tween-80 + 90% physiological saline. The gustatory epithelium of the control and experimental animals was investigated every 7 days during the next 42 days. The substances used were obtained from Serva (West Germany): SP (6-7 μg), serotonin creatinine-sulfate (50 μg), DL-tryptophan and DL-5-hydroxytryptophan (1-1.5 mg/kg). The substances, dissolved in 0.5 ml of Ringer's solution (pH 7.3) immediately before injection, were injected intraperitoneally 24 h before the beginning of investigations of the control and experimental animals, treated with capsaicin 4 weeks beforehand. All the experiments were carried out in the fall and winter. Preparations from the lingual epithelium of the control and experimental animals were made by the method described previously [3], exposed to formaldehyde vapor at 80°C for 1 h, and examined unmounted under the LM-2b luminescence microscope.

EXPERIMENTAL RESULTS

The intensity of luminescence of the cells in the taste buds decreased as a result of the chronic action of capsaicin, evidence of a fall in their serotonin concentration. This effect was seen most clearly on the 21st-28th day after injection of capsaicin. At these times the cells in the overwhelming majority of taste buds were reduced in thickness and showed hardly visible pale green fluorescence (Fig. 1b, c). Meanwhile some taste buds contained cells whose inner thickenings still showed quite intense fluorescence, whereas the outer thickenings had almost ceased to fluoresce (Fig. 1d). The cells themselves were greatly reduced in size. In the later stages of the action of capsaicin (35-42 days after

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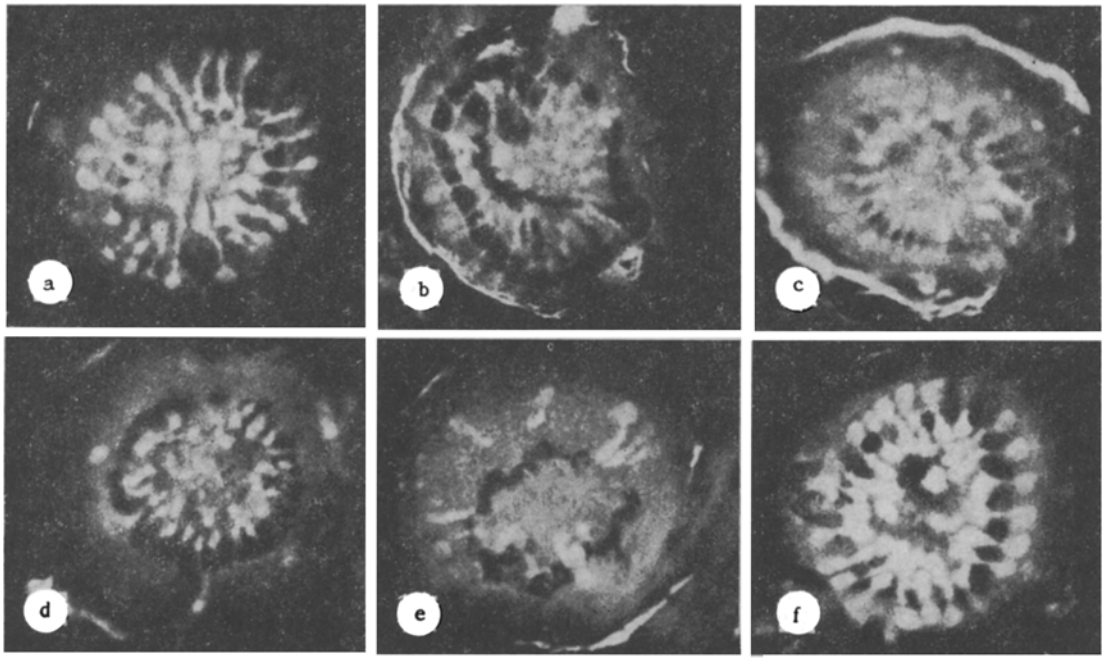

Fig. 1. Effect of capsaicin-induced SP deficiency of serotonin-containing cells of frog taste buds. a) Serotonin-containing cells in taste bud of normal animal; b, c) decrease in intensity of fluorescence of cells 28 days after injection of capsaicin; d) decrease in size of cells; e) decrease in number of serotonin-containing cells in taste bud 43 days after injection of capsaicin; f) restoration of fluorescence of cells 24 h after injection of SP. Here and in Fig. 2: magnification 120×.

injection), besides the taste buds described above, sometimes others were found which resembled those usually observed in sensory denervation of the tongue (Fig. 1e).

Changes in the population of serotonin-containing cells were reversible in character: Injection of exogenous SP into animals treated with capsaicin led to complete recovery of the serotonin concentration in the cells after 24 h, as shown by the bright fluorescence acquired by the cells (Fig. 1f). The widened inner thickenings fluoresced most intensely under these circumstances. These data are a powerful argument in support of the view that the fall in the serotonin level during the chronic action of capsaicin was not the result of its harmful action on the cells themselves, but was due to endogenous SP deficiency in the fibers of the gustatory sensory nerves. The result of this was evidently a disturbance of the synthesis and uptake of serotonin, which, as was shown previously, maintain the optimal level of this monoamine in taste bud cells [1]. Proof of this hypothesis was obtained by studying the ability of these cells to synthesize serotonin from its precursors, and to assimilate extrinsic serotonin, after treatment with capsaicin.

These experiments showed that 24 h after injection of serotonin the intensity of fluorescence of the cells and, in particular, of their inner thickenings, was sharply increased in the control animals, evidence of active uptake of exogenous serotonin from the blood stream and of evaluation of its intracellular concentration (Fig. 2a). Meanwhile, in animals previously treated with capsaicin, no changes in fluorescence of the cells were observed. As before, the cells appeared thinner and continued to show pale green fluorescence (Fig. 2d). A similar picture was observed after injection of DL-tryptophan, which induced activation of serotonin synthesis in the taste bud cells of the control animals (Fig. 2b) but had no effect on taste bud cells of animals treated with capsaicin (Fig. 2e). Meanwhile injection of another serotonin precursor, 5-hydroxytryptophan, was more effective and intensified fluorescence of the cells (Fig. 2f), but the increase was not as great as in the control animals (Fig. 2c).

The results show that injection of capsaicin — an agent leading to exhaustion of SP in fibers of gustatory sensory nerves — is accompanied by a decrease in the intracellular serotonin concentration in the taste buds, and sometimes by its almost total disappearance. Exogenous SP completely restores the serotonin level in the cells. This precise relationship is

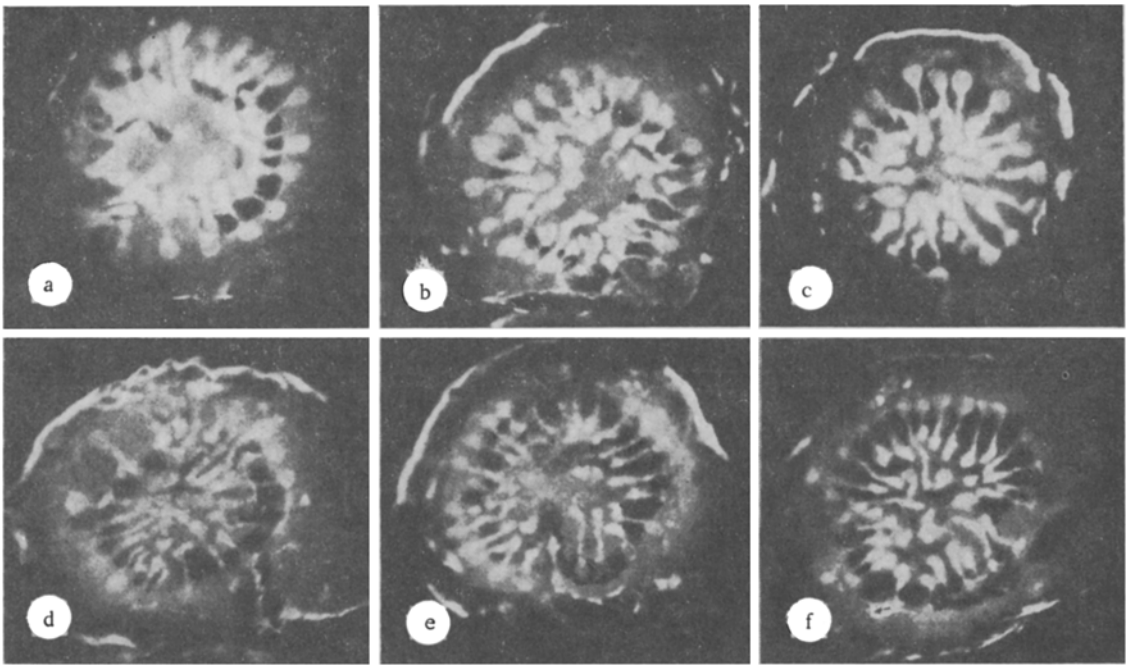

Fig. 2. Effect of SP deficiency of uptake and synthesis of serotonin in taste bud cells. a, b, c) Intense fluorescence of cells in normal animals 24 h after injection of serotonin, tryptophan, and 5-hydroxytryptophan respectively; d, e) no effect of serotonin and tryptophan respectively in animals treated under capsaicin; f) slight increase in intensity of cell fluorescence under the influence of 5-hydroxytryptophan.

probably due to the activating effect of SP on serotonin uptake and synthesis. This is shown by experiments with SP deficiency, when both processes were disturbed: Serotonin formation from the original amino acid (tryptophan) was completely blocked, but it still took place from 5-hydroxytryptophan, although not as intensively as in the control. These data indicate that SP affects serotonin synthesis at a stage which limits the velocity of the process, i.e., at the stage of the reaction of hydroxylation of tryptophan, through activation of the enzyme tryptophan hydroxylase and transport of the original amino acid into the cell. SP may perhaps be part of the regulatory system by which the body monitors and maintains a constant physiological level of intracellular serotonin, which plays an important role in the mechanisms generating afferent impulse activity in sensory nerve endings in response to activation of the gustatory receptor apparatus [2]. SP also evidently participates in modulation of the efficiency of synaptic transmission in afferent synapses, affecting the time of stay of the neurotransmitters in the synaptic space by changing the rate of serotonin uptake.

It has been shown by immuno-electron microscopy that SP-containing nerve fibers do not form synaptic junctions of any kind in taste buds with cells of any type, and that in their nature they are not afferent fibers, transmitting primary taste information to the nucleus solitarius, but they are effector fibers, maintaining structural and functional connections between the CNS and the taste organ [10]. SP is evidently one of the endogenous factors by means of which the CNS exerts its influence on the gustatory receptor apparatus. Because of its extrasynaptic release the peptide can diffuse in the taste bud for considerable distances and can effect wider forms of communicative connections. It may be expected that SP not only participates in the regulation of monoamine synthesis and uptake, but is also a component of other regulatory mechanisms of activity of the gustatory receptor apparatus.

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MORPHOMETRIC CHARACTERISTICS OF NEUROMUSCULAR SPINDLES OF HYPERTROPHIED SKELETAL MUSCLE

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The principal function of neuromuscular spindles (NMS) is to regulate muscle stretching and contraction through spinal reflex arcs [6, 8]. The use of horseradish peroxidase [7] has revealed a specialized microcirculatory bed in the region where NMS and axo-muscular synapses are located [4], which maintains their nutrition.

Investigations [1, 5] have shown that NMS possess considerable reactivity in response to measured physical exercise. However, to determine morphological equivalents of strengthening of the functional potential of NMS during hypertrophy of skeletal muscle arising under the influence of repeated physical exercises, objective quantitative information is needed on the degree of adaptive reorganization of all components of proprioceptors.

The aim of this investigation was a quantitative study of changes in NMS and the components of their microcirculatory bed arising during hypertrophy of skeletal muscles.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male rats aged 1 month and weighing 62.4 ± 2.7 g, five of which served as the control. Skeletal muscular hypertrophy was produced by regular measured physical exercises of submaximal intensity (daily running on a treadmill at a speed of 35-65 m/min for 4 months). Blood vessels were injected and material collected under ether anesthesia. The rectus femoris muscle was the test object. Injection of the vessels with 0.25% silver nitrate solution, or a suspension of Paris green, combined with staining of sections by the Bielschowsky–Gros and Kupriyanov [2, 3] methods were used to reveal the microvessels and individual components of NMS. The MVO-1-15* screw-adjusted ocular micrometer was used for the morphometric investigation. The results were subjected to statistical analysis by Student's test.

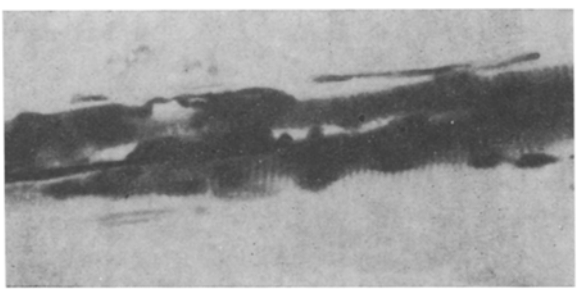

Fig. 1. IFMF of hypertrophied rectus femoris muscle. Impregnation by Bielschowsky-Gros method, stained with Ehrlich's hematoxylin and eosin. 600×.

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