TASTE BUDS OF THE TONGUE AFTER APPLICATION OF COLCHICINE TO THE GLOSSOPHARYNGEAL NERVE IN RATS

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Nerve fibers control the state of the taste buds of the tongue [2, 8]. This conclusion was drawn from an investigation of taste buds after division of the afferent nerves. It has been suggested that chemical factors which, after secretion from nerve endings, can exert a trophic influence on the taste receptor [4, 9, 12], are transported along nerve fibers which form connections with the taste receptor cells. The idea of the trophic influence of chemical factors transported along the nerve and the role of axoplasmic transport in maintenance of the differentiated state of the taste buds of the tongue have been confirmed experimentally [5, 7]. The authors cited showed that blocking of axoplasmic transport by colchicine in the glossopharyngeal nerve induces destruction of the lingual taste buds.

The aim of the present investigation was to study the structural and biochemical characteristics of the taste buds of the rat tongue after blocking of axoplasmic transport (division of the nerve, application of colchicine) in the glossopharyngeal nerve.

EXPERIMENTAL METHOD

Experiments were carried out on 75 noninbred albino rats weighing 150-200 g. After intraperitoneal injection of urethane (500 μg/kg) a segment of the glossopharyngeal nerve 5 mm long was excised in the submandibular region of the animals of one group. Colchicine (from Merck, West Germany), in a concentration of 5 mM, was applied for 10 min to the same region of the glossopharyngeal nerve in animals of another group. In the control the nerve was treated with physiological saline under conditions similar to those for application of colchicine. Material containing the foliate papillae both on the ipsilateral (experiment) side and on the contralateral side (control) of the tongue was taken for analysis after 2, 4, 5, 7, and 10 days. Pieces of tissue were fixed by Carnoy's method and in 10% neutral formalin, and then stained with hematoxylin and picroindigocarmine. Some material was processed in a mixture of ink, iodine, and osmic acid [11].

To study the protein spectrum, 10 days after application of colchicine to the nerve pieces of epithelium of the foliate papillae and the epithelium surrounding them on both sides were removed. After equalization by weight the material was homogenized in glass homogenizers, using an extracting solution containing β-mercaptoethanol [10], and centrifuged at 8000g for 20 min. The supernatant was subjected to electrophoretic fractionation in 10% polyacrylamide gel with sodium dodecylsulfate [10] in the modification for gel blocks 0.45 mm thick and 12 cm long. The gels were fixed in 50% TCA and stained in 0.25% Coomassie in a mixture of methanol, acetic acid, and water. After rinsing of the excess of dye the gels were subjected to densitometry on the IFO-451 microphotometer. The results of planimetry of the densitograms were analyzed by statistical methods [3].

EXPERIMENTAL RESULTS

After division of the glossopharyngeal nerve signs of destruction of the taste buds of the foliate papillae were observed on the rats' tongue. The changes were most marked on the 7th-8th days after division. Destruction of the taste buds of the foliate papillae also took place after application of colchicine to the nerve. However, the destructive processes under these circumstances developed more slowly. A change in shape of some of the taste buds and a decrease in their size as a result of the development of destruction of cells of the taste buds were observed 4-5 days after application of colchicine to the nerve. Under these conditions,

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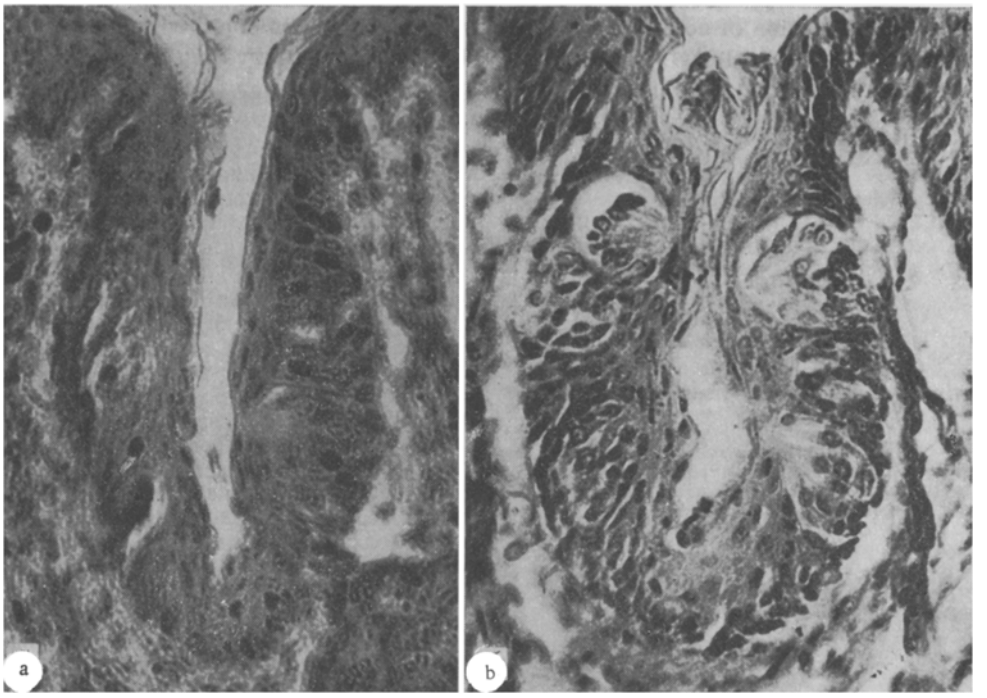

Fig. 1. Epithelium of foliate papilla of rat tongue 10 days after application of colchicine to glossopharyngeal nerve: a) experiment, b) control. Hematoxylin and picroindigocarmine, 200×.

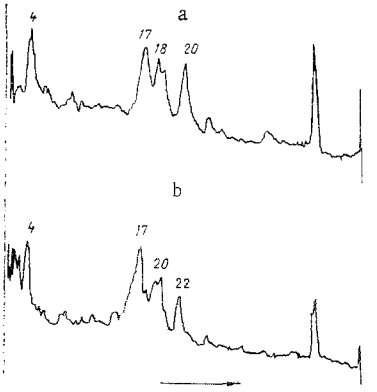

Fig. 2. Protein spectrum of homogenates of epithelium of foliate papillae (a) and epithelium surrounding them (b) from the rat tongue.

intact taste buds were still preserved in the foliate papillae on the ipsilateral side. Only single taste buds could be found in the foliate papillae 9-10 days after application of colchicine to the nerve. The intensity of the destructive changes was greatest in this period: The size and shape of the buds differed sharply from normal, their orientation in the layer of epithelium was disturbed, and the gustatory pore was absent (Fig. 1a). On the contralateral side of the tongue taste buds remained intact both after division and after application of colchicine to the nerve (Fig. 1b). This observation shows that changes in the state of the taste buds after application of colchicine to the nerve were not connected with any systemic cytotoxic effect of the alkaloid on the epithelium of the tongue but that it was mediated through the nerve.

Processing the material in a mixture of zinc, iodine, and osmic acid revealed destruction of nerve fibers in the subepithelial plexus and in the epithelium of the tongue starting with the 2nd day after division of the

nerve. At all times after application of colchicine to the nerve, nerve fibers could be traced in the epithelium of the tongue and in the subepithelial plexus. Consequently, the changes observed in the state of the target were not the result of denervation but were probably due to the specific action of colchicine on the nerve fibers, giving rise to blocking of axoplasmic transport.

The protein spectrum of the epithelium of the foliate papillae differed from the spectrum of the surrounding epithelium not containing taste buds (Fig. 2). Values were compared for the most clearly defined fractions Nos. 4, 17, 18, and 20 in the epithelium of the foliate papillae after 10 days, when the most marked morphological changes were observed in the taste buds after application of colchicine to the nerve. A decrease ($P < 0.005$) in the intensity of staining was observed in fraction No. 18 compared with that on the contralateral side. This change can be regarded as the result of a change in the biochemical state of the taste buds as the result of blocking of the axon current in the fibers approaching them. The most clearly defined fractions Nos. 4, 17, 20, and 22 in the protein spectrum of the surrounding epithelium were unchanged on the ispilateral side. Consequently, these data confirm the results of the histological investigation and indicate that colchicine has no direct effect on the epithelium or taste buds of the tongue. Investigations have shown that injections of colchicine into the tongue inhibit the activity of chemoreceptors [1, 6] and cause their destruction [6]. As a result of this exclusion of the possibility of any direct effect of colchicine on the epithelium following application of the alkaloid to the nerve, causing destruction of the taste buds, it can thus be postulated that axoplasmic transport in the fibers of the glossopharyngeal nerve is directly concerned with maintenance of the structural integrity of the chemoreceptors of the lingual foliate papillae.

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