CELL COMPOSITION OF TASTE BUDS OF THE RAT TONGUE AFTER DENERVATION AND APPLICATION OF COLCHICINE TO THE GLOSSOPHARYNGEAL NERVE

Yu. A. Chelyshev, T. L. Zefirov, and Z. Kh. Timergaleeva

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KEY WORDS: denervation; application; colchicine; taste buds of the tongue.

The taste buds of the tongue contain cells of several types [3, 10]. In mammals it is usual to distinguish dark, pale, and intermediate cells. The functional role of the different types of cells is not yet clear. They are known to make contact with nerve fibers which enter the taste bud [10]. Taste buds disappear in denervated lingual epithelium and they reappear simultaneously with regenerating nerves [1, 2, 7]. In rat embryos the appearance of taste buds coincides in time with growth of nerve fibers into the epithelium [5, 6]. Transplantation of lingual epithelium and sensory neurons into the anterior chamber of the eye induces the formation of taste buds in the epithelium [14]. These data are evidence of a trophic influence of sensory neurons on the epithelium of the taste bud [2, 8]. Axon transport has been shown to have a direct bearing on the realization of this effect [1, 3, 4], but it is not clear whether neurotrophic control is differentiated with respect to different cell types in taste buds.

To study this problem the distribution of cell types in taste buds of foliate papillae of the rat tongue was investigated after division of the glossopharyngeal nerve and application of colchicine to it.

EXPERIMENTAL METHOD

Under urethane anesthesia (500 μg/kg) a segment of the glossopharyngeal nerve was resected unilaterally in the submandibular region in 43 noninbred albino rats weighing 150-200 g. In animals of another group, a 5 mM solution of colchicine was applied for 10 min to the same region of the glossopharyngeal nerve. The posterior region of the foliate papillae, which is innervated only by the glossopharyngeal nerve [13], was studied 3 and 7 days after division of the nerve and 5, 7, and 10 days after application of colchicine to it. Foliate papillae on the opposite side served as the control. After isolation and dissection the material was fixed in 2.5% glutaraldehyde solution and postfixed in 1% OsO_4 solution at 4°C and pH 7.4, then dehydrated and embedded in Epon-Araldite. Semithin sections up to 1 μ thick, stained with methylene blue, were used to count the number of cells in the taste buds.

EXPERIMENTAL RESULTS

Dark (61.8%), pale (25.5%), and intermediate (12.5%) cells were found in the taste buds of the foliate papillae (Fig. 1). A marked decrease in the relative number of dark cells and an increase in the number of intermediate cells were observed 3 days after division of the nerve, on both ipsilateral and contralateral sides. Seven days after division of the nerve a decrease in the number of dark and pale cells and an increase in the number of intermediate cells compared with the corresponding parameters in intact animals were found on the contralateral side (Fig. 2). At the same period after division of the nerve, taste buds on the ipsilateral side showed considerable destruction.

Application of colchicine to the nerve caused a decrease in the number of dark and pale cells on the contralateral side (7 and 10 days after the operation) and an increase in the number of intermediate cells, which could be observed at all times after the operation. Comparison of the taste buds on the ipsilateral and contralateral sides after application of col-

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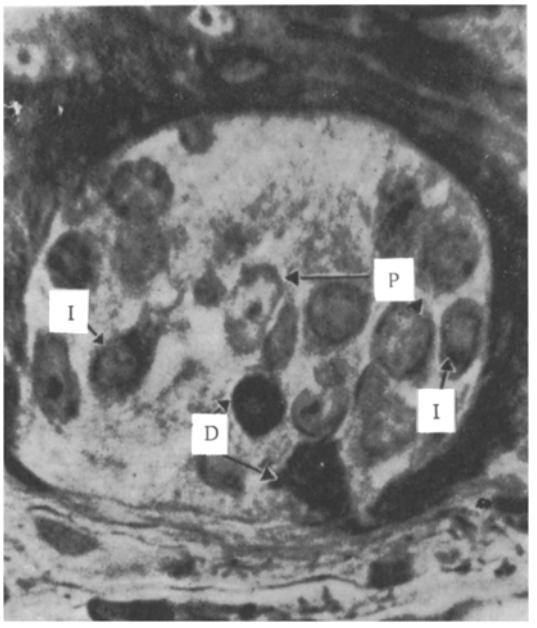

Fig. 1

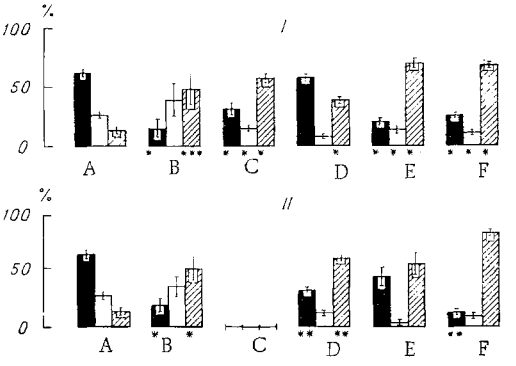

Fig. 2

Fig. 1. Taste bud of foliate papillae of rat tongue. D) Dark cells, I) intermediate cells, P) pale cell. Methylene blue. 342 ×.

Fig. 2. Cell composition of taste buds of rat tongue after division of glossopharyngeal nerve and application of colchicine to it. I) Contralateral, II) ipsilateral side. A) Intact animals, B and C) 3 and 7 days respectively after division of nerve, D, E, and F) 5, 7, and 10 days respectively after application of colchicine to nerve. Black columns denote number of dark cells, unshaded columns — number of pale cells, obliquely shaded columns — number of intermediate cells. *) $P < 0.001$ compared with intact animals, **) $P < 0.001$ for comparison of values on contralateral and ipsilateral sides, ***) $P < 0.05$ for comparison with intact animals.

chicine to the nerve showed a decrease in the relative number of dark cells (5 and 10 days) and an increase in the number of intermediate cells (5 days).

Changes in the distribution of cell types on the contralateral side after division of the nerve or application of colchicine to it may be connected both with the crossed innervation of the foliate papillae of the tongue and with a change in the character of the centrifugal effect on taste buds on the contralateral side as a result of action directed toward the nerve on the ipsilateral side. In the latter case, evidently, it must be accepted that the character of the discharge in the effector fibers has a controlling influence on the state of the cell types in the taste buds. A similar type of control has been demonstrated for skeletal muscle [11, 12]. It is therefore not accidental that the character of the discharge of motoneurons is regarded as an important factor controlling muscle activity *in vivo* [9]. The decrease in the number of dark and increase in the relative number of intermediate cells on the ipsilateral side after application of colchicine to the nerve may also depend on a disturbance of the character of impulsation in the efferent fibers. However, the effect of application of colchicine to the nerve was manifested more especially on the ipsilateral side. On the basis of this fact, and also ruling out any direct effect of colchicine in this concentration on the target tissue under similar experimental conditions [1], it can be concluded that changes in the relative numbers of cell types in the taste buds of foliate papillae of the rat tongue following application of colchicine to the nerve are connected with a disturbance of axon transport in sensory nerve fibers. Support for the specificity of this effect is given by the fact that changes in the number of dark and intermediate cells were in the same direction after division of the nerve and at all times after application of colchicine to it.

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A NEW APPROACH TO THE STUDY OF ERYTHROCYTE AGGREGATION

G. Ya. Levin, Yu. A. Sheremet'ev, and V. G. Yakhno

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The study of the mechanism of interaction (aggregation) of cells is an important problem in modern biology and medicine. Aggregation of blood cells (erythrocytes), a fundamental stage in microcirculatory disturbances in many pathological states [7], plays a special role. To study the mechanism of erythrocyte aggregation in model experiments *in vitro*, investigators have used dextran, a neutral polymer of glucose, and high-molecular-weight blood plasma proteins, namely fibrinogen and γ-globulin, as inducers of aggregation [1, 8]. According to some workers [5], erythrocyte aggregation stimulated by dextran is similar in its general features to aggregation taking place in various pathological states, and it provides a convenient model with which to study cellular interaction. The mechanism of the aggregating action of dextran is the formation of "bridges" of polymer macromolecules adsorbed on their membrane between neighboring cells [4]. However, no attention is paid in the mechanism of erythrocyte aggregation suggested by the authors cited to changes in cell shape (discoid—spherical transformation), which take place in many diseases in which intravascular erythrocyte aggregation is observed [11].

It was accordingly decided to study the possibility of inducing aggregation of erythrocytes by substances causing changes in their shape.

EXPERIMENTAL METHOD

Human erythrocytes, washed and resuspended (1:200) twice in buffered physiological saline (20 mM Tris-HCl and 146 mM NaCl, pH 7.4) were used. Erythrocyte aggregation was studied in a highly sensitive aggregometer of our own design, by a photometric method [3]. The viscosity of the erythrocyte suspension (77%) was studied by means of a VIR-75M rotatory viscosimeter (designed by A. N. Sundukov) with shearing velocities of 2.2 to 85.0 sec^{-1}. Erythrocyte morphology was studied in a phase-contrast microscope. The erythrocytes were first fixed in 1% glutaraldehyde solution. The known crenating agents 2,4,6-trinitrophenol (TNP) and 2,4-dinitrophenol (DNP) were used.

EXPERIMENTAL RESULTS

It was found that TNP (1 mM) induced sharp changes in shape of the erythrocytes: total disappearance of the discoid forms and the appearance of crenocytes and spherocrenocytes (Fig. 1b). It was also found that TNP, in the above dose, stimulated marked erythrocyte aggrega-

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