KERATIN IMMUNOCYTOCHEMISTRY IN THE LINGUAL MUCOSA OF RATS

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The role of the intermediate filaments in the specialized cells of sensory receptors, and their distribution and chemical composition have not been adequately studied. The exception to this rule is the human Merkel's cells, for which it has been shown that the intermediate filaments consist of cytokeratins 8, 18, and 19 [3]. By contrast with the mechanoreceptors, special investigations devoted to the problem of organization of the cytoskeleton and its role in chemoreceptor cells are virtually only just beginning. It was shown in [4] that intermediate filaments in taste bud cells from foliate papillae of the rabbit tongue consist of keratin. Rashbass suggested that the keratin of the intermediate filaments of taste bud cells and of the tonofilaments of the surrounding epithelium differ in their antigenic properties. A detailed study of the distribution of intermediate filaments in different types of rodent taste bud cells was undertaken in [5]. It was shown that taste bud cells have less dense concentrations of intermediate filaments than cells of the surrounding epithelium. A comparative study of the content of intermediate filaments in different types of taste bud cells showed that type III chemoreceptor cells contain the largest number of them. Monoclonal antibodies against human and porcine keratin with mol.wt. of 40 to 65 kD from normal and transformed epithelia from different parts of the body did not give an immunocytochemical reaction selectively with taste buds. In the present investigation monoclonal antibodies were chosen against rat cytokeratins, giving a positive immunocytochemical reaction with lingual taste bud cells of the same species and not interacting with cytokeratins of the surrounding epithelium of the lingual mucosa. Meanwhile, an immunocytochemical study of the exocrine glands, connected with the taste buds, was undertaken for the first time with the aid of these same monoclonal antibodies against cytokeratins. Interest in these structures is due to the existing ideas regarding their role as the source of precursors for taste bud cells [1].

EXPERIMENTAL METHOD

Experiments were carried out on albino rats aged 3-4 weeks, killed under ether anesthesia; the lingual papillae were isolated. Serial frozen sections through the papillae, 5-6 thick, were prepared. The sections were dried and fixed in acetone for 10 min. They were then carefully washed in phosphate-buffered saline (pH 7.2) and tested in the immunocytochemical reaction by the PAP method. For this purpose monoclonal antibodies were used: C12 against rat cytokeratin with mol.wt. of 49 kD, giving a weak cross-reaction with nonkeratin proteins of striated muscles, E2 against rat cytokeratin with mol.wt. of 55 kD, and H4 against human cytokeratin, giving a cross-reaction with cytokeratins of stratified rat epithelia. A monoclonal kit for immunodiagnosis by the PAP method was used in the experiment (Gor'kii Research Institute of Experimental Medicine, Ministry of Health of the RSFSR, Gor'kii). 3′-3′-Diaminobenzidine (Sigma) was used as the substrate.

EXPERIMENTAL RESULTS

An intensive immunocytochemical reaction with C12 antibodies was recorded in cells of taste buds and exocrine glands connected with the foliate papillae, and located both in their secretory portions and in the efferent ducts (Fig. 1). The epithelium surrounding the taste buds and the underlying connective tissue gave a hardly preceptible and uniform stain.

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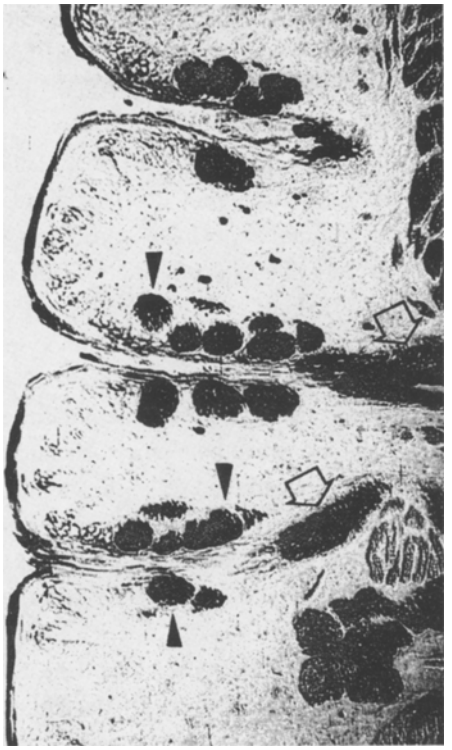

Fig. 1. Immunocytochemical reaction with C12 monoclonal antibodies against rat cytokeratin in foliate lingual papillae. 125×. Residue in taste bud cells (dark arrows) and gland cells (unshaded arrows) connected with taste buds.

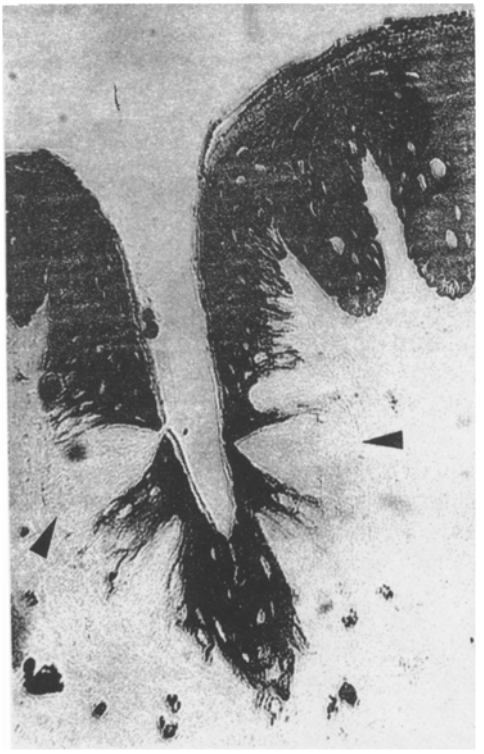

Fig. 2. Residue of immunocytochemical reaction with H4 monoclonal antibodies in cells of stratified epithelium of foliate lingual papillae. 400×. Taste bud cells (arrows) do not react with these antibodies.

When E2 antibodies were used the residue of the immunocytochemical reaction was found in the taste bud cells. Staining of similar intensity was found in the secretory portions of the glands connected with the papillae, and their efferent ducts, and also in the epithelium at the base of the papilla. The epithelium surrounding the taste buds and the underlying connective tissue did not react with these antibodies.

In the experiments with H4 antibodies, a deep brown stain of the epithelial layer was observed. The stratum corneum had a weaker color. Taste buds, secretory portions, and efferent ducts of glands connected with the papillae, and also the underlying connective tissue did not give a positive immunocytochemical reaction with these antibodies (Fig. 2).

The results confirm the view that intermediate filaments of taste bud cells are formed from cytokeratins. On the basis of the investigation it can be postulated that cells of taste buds and surrounding epithelium of the lingual mucosa express different forms of cytokeratins. In this respect our findings agree with those of immunocytochemical study using monoclonal antibodies against one protein of the cytoskeleton of transformed PLK1 cells, which give a positive reaction with taste bud cells but not with cells of the surrounding epithelium in rats and mice [2]. Data on immunocytochemistry of keratins in the glands connected with the foliate papillae are particularly interesting. A positive immunocytochemical reaction was found in the secretory portion and efferent duct of these glands, similar to that in the taste bud cells; this points to an identical chemical composition of the intermediate filaments of the taste bud cells and of the gland cells. It has been shown experimentally that Ebner's gland, connected with another chemosensory papilla, namely the circumvallate papilla of the tongue, is the source for regeneration of the epithelial layer, in which taste buds appear [1]. The results confirm the hypothesis that related cells are present in the taste buds and glands connected with the taste papillae.

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