IMMUNOCYTOCHEMISTRY OF KERATINS IN THE RAT LINGUAL MUCOSA

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UDC 616.313-018.73-008.939.629-078.3-092.9

KEY WORDS: taste bud; keratin; immunocytochemistry

The role of intermediate filaments in specialized cells of sensory receptors and their distribution and chemical composition have not been adequately studied. The exception is Merkel's cells in man, for which it has been shown that their intermediate filaments consist of cytokeratins 8, 18, and 19 [3]. Investigations into the organization of the cytoskeleton and its role in chemoreceptor cells are virtually only just beginning. It has been shown [4] that intermediate filaments in taste bud cells from the foliate papillae of the rabbit tongue consist of keratin. It has been suggested that the keratin of the intermediate filaments of taste bud cells and keratin of the tonofilaments of the surrounding epithelium differ in their antigenic properties. Taste bud cells of rodents have been shown to 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 has shown that chemoreceptor cells of type III contain the largest numbers. Monoclonal antibodies to human and porcine keratin with molecular weight of between 40 and 66 kD from normal and transformed epithelia in different parts of the body did not give a selective immunocytochemical reaction with taste buds.

In the present investigation monoclonal antibodies to rat cytokeratins, giving a positive immunocytochemical reaction with taste bud cells from the tongue of the same species, and not interacting with cytokeratins of the surrounding epithelium of the lingual mucosa, were chosen. Meanwhile, the same monoclonal antibodies to cytokeratins were used for the first time to undertake an immunocytochemical study of the exocrine glands connected with taste buds. Interest in these structures is due to the view that they are the source of precursors for the taste bud cells [1].

EXPERIMENTAL METHOD

Albino rats aged 3-4 weeks were killed under ether anesthesia and the foliate papillae of the tongue were isolated. Serial frozen sections through the papillae, 5-6 μ thick, were cut. The sections were dried and fixed in acetone for 10 min. They were then thoroughly washed in phosphate-salt buffer, pH 7.2, and an immunocytochemical reaction was carried out by the PAP method. For this purpose the following monoclonal antibodies were used: C12 to rat cytokeratin (49 kD), giving a weak crossed reaction with nonkeratin proteins of striated muscles; E2 to rat cytokeratin (55 kD), and H4 to human cytokeratin, giving a crossed reaction with cytokeratins of rat stratified epithelia. A monoclonal set for immunodiagnosis by the PAP method (USSR origin) was used. The substrate was 3,3'-diaminobenzidine (from "Sigma").

EXPERIMENTAL RESULTS

An intensive immunocytochemical reaction with C12 antibodies was recorded in cells of the taste buds and exocrine glands connected with the filiate papillae, and located both in the secretory portions of these glands and in their efferent ducts (Fig. 1). The epithelium surrounding the taste buds and the underlying connective tissue gave an almost indistinguishable and uniform color.

Department of Histology, S. V. Kurashov Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 313-314, September, 1991. Original article submitted December 28, 1990.

 0007-4888/91/0009-1340$12.50

Fig. 1. Immunocytochemical reaction with C12 monoclonal antibodies to rat cytokeratin in lingual foliate papillae. Residue present in cells of taste buds (filled arrows) and glands (empty arrows) connected with taste buds. 200×.

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

An intense brown color was observed in the epithelial layer in the experiments with H4 antibodies. The stratum corneum stained less strongly. 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 by cytokeratins. It can be tentatively suggested that taste bud cells and cells of the surrounding epithelium of the lingual mucosa express different forms of cytokeratins. Our findings agree in this respect with the results of an immunocytochemical investigation conducted with monoclonal antibodies against one of the cytoskeletal proteins of transformed cells of the PtkI line, which gave a positive reaction with taste buds cells but not with cells of the surrounding epithelium in rats and mice [2].

The data on immunocytochemistry of keratins in glands connected with the foliate papillae are particularly interesting. A positive immunocytochemical reaction similar to that in the taste bud cells was found in the secretory portion and efferent ducts of these glands, indicating that the chemical composition of the intermediate filaments of the cells of the taste buds and glands is identical. Experiments have shown that Ebner's gland, which is connected with a different chemosensory papilla, namely the vallate papilla of the tongue, is a source for regeneration of the epithelial layer in which taste buds appear [1]. The result confirmed the view that related cells are present in taste buds and glands connected with the taste papillae.

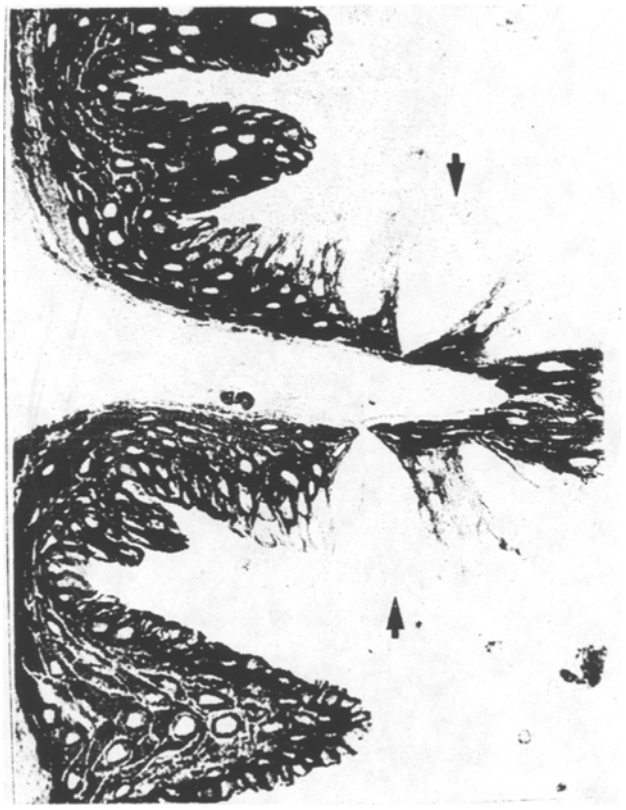

Fig. 2. Presence of residue of immunocytochemical reaction with H4 monoclonal antibodies in cells of stratified epithelium of foliate papillae of the tongue. Cells of taste buds (arrows) do not react with these antibodies. 200×.

The authors are grateful to S.M. Troyanovskii, Head of the Laboratory of Mechanisms of Carcinogenesis, Research Institute of Carcinogenesis, Academy of Medical Sciences of the USSR, for generously providing the monoclonal antibodies.

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