INNERVATION OF THE LINGUAL PAPILLOAE IN THE SHEEP AND CATTLE

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ABSTRACT

The distribution of nerve fibres was investigated in tongue papillae of the cattle and sheep with regard to the localization of protein S-100. Nerve fibres are concentrated in the connective tissue of mucosa from where they enter the connective tissue of lingual papillae. Positive nerve fibres for S-100 protein were found to enter the filiform papillae in reduced number and were concentrated at the top of the papillae. Dense accumulation of nerve fibres was found in the fungiform, lenticular and vallate papillae where nerve fibres were distributed centrally in the core of connective tissue of the papillae. Only a few fine nerve fibres were seen at the base of the taste buds in contact with the gustatory cells of taste buds. No nerve fibres were seen to penetrate into the taste buds though these displayed mild positive S-100 protein reactivity. Inside the lining epithelium of lingual papillae nerve fibres were not observed.

Key words: tongue; papillae; innervation; immunohistochemistry; sheep; cattle

INTRODUCTION

In the animals the various lingual papillae are present primarily on the tongue where they are generally divided into mechanical and sensory types (5). Their gross anatomy and structure was studied using histological, histochemical and electron microscopical methods (2, 3, 8, 11, 15, 16, 17, 19, 22, 23). By them the presence of ganglionic structures containing numerous cellular bodies and bundles of fibres, myelinic and amyelinic fibres and nerve corpuscles were found in various organs (12, 13). In the papillary stroma along stromal myelinic fibres the presence of the S-100 protein was demonstrated (4).

The taste buds, which are composed mainly of clusters of sensory cells, are located in the stratified squamous epithelium of lingual papillae. Mack et al. (11) studied number and distribution of the taste buds in the pig which is the best model for human as both are omnivorous (12). The aim of the present study was to localize nerve structures in the sheep and cattle lingual papillae with special reference to taste buds using the immunohistochemical method.

MATERIAL AND METHODS

Five adult sheep of both sexes weighing between 35–43 kg, 2–4 years old, and five clinically healthy cattle were used in the investigation. The samples of mandibular glands were dissected out of cattle and sheep in a slaughterhouse. The tissue samples were fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections of thickness 5 μm were deparaffinized with xylene and dehydrated in decreasing ethanol gradient. The sections were pretreated with 3% H2O2 in methanol to block endogenous peroxidase activity and pre-incubated with 2% goat serum to mask unspecific binding sites. They were incubated with the first antibody (polyclonal S-100 protein, Sigma) and washed in phosphate-balanced salt solution (PBS). Afterwards, the sections were incubated with biotinylated secondary antibody for 45 min. washed in PBS, and finely incubated with avidin-biotin-peroxidase complex according to Hsu et al. (6) (ABC kits, Vector Laboratories, USA). After that the sections
were washed with PBS and finally reaction product formation was achieved by incubating for 10 minutes at room temperature using a mixture of an equal volume of 0.02% hydrogen peroxide and 0.1% 3,3'-diaminobenzidine tetrahydrochloride made in Tris buffer. For negative controls, the first antibody was substituted by PBS or normal rabbit serum.

RESULTS

The thick bundles of nerves run among the muscle bands of the tongue from which smaller nerve bundles reach the connective tissue of the mucosa layer. Single nerve fibres and small bands enter into primary and secondary connective tissue papillae and reach the basal membrane of the basal surface of the lining epithelium (Figs. 1, 2). No nerve fibres were found to enter into the epithelium of filiform papillae. In fungiform papillae numerous nerve fibres positive for S-100 protein were seen, concentrated inside the top of primary papillae (Fig. 3). The most dense accumulation of the S-100 protein nerve fibres in both species studied were seen in the vallate papillae. Numerous nerve fibres from the connective tissue of the mucosa layer entered into the vallate papillae and ran directly to the apical surface of the papillae (Fig. 3). Despite the presence of numerous nerve fibres in the corresponding lamina propria of fungiform and vallate papillae of the tongue (Fig. 4) only a few fine nerve fibres were seen at the base of the sensory cells of the taste buds. No nerve fibres were seen to enter.

Fig. 1. Section of the cattle tongue in the filiform papillae. Small bands of nerve fibres are seen in the connective tissue. Fine nerve fibres reach the top of the connective tissue (arrows). Magn. x 200

Fig. 3. Section of the sheep fungiform papilla. Nerve fibres are found centrally in reduced number in the papillae (arrow). Magn. x 200

Fig. 2. Section of the sheep tongue in the interpapillary area. From the small bands of nerve fibres in the connective tissue branches of nerve fibres run at the basal surface of the epithelium (arrow). Magn. x 200

Fig. 4. Section of the cattle vallata papilla. Many S-100 protein positive nerve fibres are found in the base of the papilla. Fine nerve fibres run directly to the apical surface of the papilla. Magn. x 10
into taste buds and to have visible contact with gustatory cells. It is worth to mention that whole taste buds displayed positive reaction to S-100 protein. Outside the lingual papillae, nerve fibres of various thickness and positive for S-100 protein were distributed horizontally in the connective tissue of the mucosa.

DISCUSSION

In the tongue of the cattle and sheep numerous protein S-100-containing structures were found. The nerve fibres were found at several locations, i.e. in the glandular tissue, connective tissue, and around blood vessels. The density and distribution of the nerve fibres in these locations varied. Less abundant nerve fibres were found in the filiform and fungiform papillae. The occurrence of nerve fibres in the filiform papillae was studied electronmicroscopically by Böck (2, 3). The orientation of the nerve fibres in the observed species resembled that in sheep and cattle, and ran towards the tip of the papilla in parallel to the capillary loops. The entrance of the nerve fibres into the epithelial cells, described by Böck (3) and Sato et al. (18), could not be confirmed with S-100 protein.

Inside the vallate papillae the nerve fibres have been most numerous and thus were found in various animals species. Richly innervated vallate papillae were described by Vittoria et al. (20) in buffalo and in human tongue by Astbäck (1). According to Budetta et al. (4) the papillary stroma of the buffalo lingual papillae contains myelinic and amyelinated nerve fibres. We observed high density of nerve fibres in the vallate papillae in cattle and sheep. The presence of terminal nerve corpuscles, reported by the authors, could not be confirmed in the species studied after immunostaining with S-100 protein.

Studies made on the lingual papillae in various animal species (9, 10) detected presence of nerve fibres inside taste buds. These studies concluded that the taste buds in the lingual papillae are innervated by sensory nerve fibres which make contact with certain types of gustatory cells. Our observations made in the sheep and cattle tongue correspond with findings obtained by Yoshiie et al. (21) who described neuron-specific proteins in the taste bud which make contact with certain types of gustatory cells. The authors mentioned did not find any nerve fibres positive for S-100 protein in none of the cells of the taste bud, but exclusively in the subepithelial elements of the connective tissue. Within the epithelium of the vallate papillae no peptidergic fibres were found in the mentioned study. On the other hand, Kusakabe et al. (10) investigated human circumvallate papillae and observed immunoreactive peptidergic fibre fibres densely distributed in the connective tissue core of the circumvallate papillae and some fibres associated with the taste buds.

Though previous immunohistochemical studies have revealed that immunoreactivity for neurofilament protein is not contained in every kind of neuron (7), later publications reported that sensory neurons were intensely immunoreactive to anti-neurofilament protein sera. Sato et al. (18) noted a dense distribution of neurofilament protein and S-100 protein in the human taste buds of the fungiform and vallate papillae. Our observations of sheep and cattle tongue showed a fine nerve fibres densely distributed in the connective tissue core of the vallate papillae, but no fibres were seen inside the taste buds after S-100 protein immunostaining.

The sensory lingual papillae are rich in sensory nerves because of their dual innervation with general somatic and gustatory nerves. These findings extend the knowledge about both the general and neurochemical messenger-based innervation of fungiform papillae which can serve as a solid basis for functional investigations of normal, experimental and clinical materials.

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REFERENCES


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