Substance P immunoreactivity in rat von Ebner's gland

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Summary
The present immunohistochemical study revealed substance P-immunoreactive neuronal elements in the von Ebner's gland of rats. Immunoreactive ganglion cells were observed as single cells or groups of several immunoreactive ganglion cells among intra-lingual muscles, at the base of the vallate papillae and near the von Ebner's gland. Very numerous substance P-immunoreactive varicose nerve fibres ran closely associated with the serous cells and excretory duct cells, and were seen to run along blood vessels in the gland. Since substance P-immunoreactive ganglion cells were present near the glands, the immunoreactive varicose nerve fibres in the von Ebner's gland may be partly derived from the intra-lingual ganglion cells. These substance P-immunoreactive varicose nerve fibres may have an effect on the secretory activity of the serous cells and duct cells, and on the vasodilation of blood vessels of the von Ebner's gland. Actin immunoreactivity was seen in numerous myoepithelial cells embracing serous cells and duct cells, and in the smooth muscle cells of blood vessels of the gland. By using a double immunolabelling technique with anti-substance P and anti-actin sera, substance P-immunoreactive varicose nerve fibres were found to be in close contact with myoepithelial cells.

Introduction
Von Ebner's lingual glands are located under the vallate and the foliate papillae and secrete serous materials (Ellis, 1959). The secretory ducts open at the bottom of the vallum or in the grooves of the foliate papillae. It has been suggested that these glands could remove alimentary debris from the vallum to prepare the sensorial cells of the taste buds to receive new stimuli (Ellis, 1959). Moreover, it has been asserted that von Ebner's glands produce a powerful lipase activated by the low pH in the stomach (Hamosh & Burns, 1977), and carrier protein of small lipophilic molecules, lipocalin (Kock et al., 1992, 1994). The von Ebner's glands contain acini which empty into ductules similar to those found in other serous exocrine glands (Hand, 1970). Ultrastructural studies have shown nerve fibres containing a few dense-cored and numerous clear vesicles within the parenchyma of the glands (Hand, 1970; Azzali et al., 1989). Neuropeptides may have significant functions in von Ebner's gland as well as in other salivary glands. Recently it has been reported that substance P (SP) had an effect on the secretory activity in adult rat von Ebner's glands (Ueba & Uchihashi, 1991) and salivary glands (Gallacher, 1983; Ekström et al., 1984). However, the presence and distribution of SP-immunoreactive neuronal elements in the von Ebner's gland, and the functional significance of the close relationship between the SP-immunoreactive neuronal elements and parenchymal cells of this gland are unknown.

The present study was undertaken to demonstrate the appearance and localization of SP-immunoreactive neuronal elements in the von Ebner's glands. Furthermore, previous studies have shown the presence and functional significance of myoepithelial cells in the salivary glands (Garret & Emmelin, 1979; Satoh et al., 1994). This study also focuses on the relationship between SP-immunoreactive neuronal elements and myoepithelial cells in the glands.

Materials and methods
Ten male Sprague-Dawley rats (about 120 g body weight) were used in this study. The animals received commercial
food pellets and water freely. They were kept under constant conditions (temperature 22°C, relative humidity 45%, light–darkness ratio 14:10 with light from 0500 to 1900 h) in the Animal Laboratory for Medical Research of Asahikawa Medical College.

The animals were anaesthetized with ether and perfused through the heart with 200 ml physiological saline and then 200 ml 4% paraformaldehyde in 0.1 m phosphate-buffered saline (PBS), pH 7.4. The tongues were removed and immersed in the same fixative for 2 h at 4°C. After rinsing in PBS, the specimens were left overnight in PBS containing 30% sucrose at 4°C. The tongues were rapidly frozen in liquid nitrogen, sections cut at about 12 μm thick in a cryostat, and mounted on glass slides coated with poly-L-lysine (Sigma, St Louis, MO, USA).

As it is well known that the exocrine cells in salivary glands have strong peroxidase activity (Redman & Field, 1993), the cryostat sections were immersed for 1–2 h in a solution containing absolute methanol and 0.3% hydrogen peroxide in order to remove the endogenous peroxidase activity in the serous cells of the gland prior to immunohistochemistry. For immunohistochemistry, the avidin–biotin–peroxidase (ABC) method (Hsu et al., 1981) was used with a staining kit purchased from Vector Laboratories (Burlingame, CA, USA). The cryostat sections were incubated with rabbit anti-SP (20064; 1:10 000; Incastor Corp., Stillwater, MN, USA), or mouse anti-smooth muscle actin (A-2547; 1:7500; Sigma) antibody and fluorescein isothiocyanate (FITC)-conjugated goat biotinylated anti-rabbit IgG or horse biotinylated anti-mouse IgG and ABC complex for 1 h at room temperature. The antigen–antibody reaction sites were made visible by incubating sections with diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 25 mM Tris-HCl buffer (pH 7.6). For the double labelling technique, the same section was immunostained with monoclonal anti-actin antibody and fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG serum. These sections were observed and photographed with a Zeiss fluorescence microscope with simultaneous use of the epi-illumination system equipped with filter No. 9 selective for FITC and the transmitted bright-field illumination at its lowest intensity setting.

The specificity of the immunohistochemical staining was confirmed by replacing the primary antibody with normal serum, and by using diluted antisera pretreated with adequate antigen (50 μg ml⁻¹) for 24 h at 4°C. No immunostaining was observed under these conditions.

Results and discussion

Intense SP immunoreactivity was seen in the large ganglion cells (40–60 μm in diameter), which had large nuclei and long neuronal processes. These SP-immunoreactive ganglion cells were found as single cells or as a cluster of several cells near or at the bottom of the vallate papillae, among intra-lingual muscles and near or among von Ebner’s glands. SP immunoreactivity was observed in the cytoplasm and around the nucleus of the ganglion cells (Figs 1–3). The exact origin of SP-immunoreactive nerve fibres in the von Ebner’s gland is not well known. Since the present study revealed SP-immunoreactive ganglion cells in the tongue, SP-immunoreactive nerve fibres in the von Ebner’s gland may be partly derived from the intra-lingual ganglion cells. The SP-immunoreactive neurons were located in the trigeminal ganglion and otic ganglion (Sharkey & Templeton, 1984), and in the submandibular ganglion (Goedert et al., 1982; Ayer-Le Lievre & Seiger, 1984). However, the postganglionic sympathetic ganglion cells do not contain SP (Robinson et al., 1980). Thus, the SP-immunoreactive nerve fibres in the von Ebner’s gland may be coming from the intra-lingual ganglia and the above mentioned ganglia. However, no SP immunoreactivity was seen in the serous cells of the von Ebner’s glands.

Thick and thin bundles of SP-immunoreactive nerve fibres were seen under the epithelium of the vallate papillae and tongue, and among the epithelial cells of the taste buds. Numerous SP-immunoreactive varicose nerve fibres were found to encircle the serous cells of the von Ebner’s gland, and were in close contact with serous secretory cells in the gland (Figs 4 and 5), and with the excretory duct cells extending toward the base of vallate papillae (Fig. 6). Previous studies have shown that varicose nerve fibres containing peptide-like dense-cored vesicles and clear vesicles were in direct contact with serous secretory cells of the rat von Ebner’s glands (Hand, 1970; Azzali et al., 1989). Physiological studies have suggested that SP caused depletion of secretory granules in acinar cells of the rat von Ebner’s glands (Ueba & Uchihashi, 1991) and enhanced the secretory activity in other salivary glands (Gallacher, 1983; Ekström et al., 1984). Probably, SP-immunoreactive nerve fibres in the gland may stimulate the release of lipase and lipocalin from the serous cells and may also have similar effects on the secretory activity of the duct cells. However, relatively few SP-immunoreactive varicose nerve fibres were found in the mucous gland near the Ebner’s gland compared with the serous cells of the glands (Fig. 7).

In the present study, SP-immunoreactive varicose nerve fibres were seen to run around blood vessels in the von Ebner’s gland (Fig. 5). Previous physiological studies have shown that SP had a vasodilatory action in blood vessels (Lembeck & Gamse, 1982). This may be involved in the mediation of vasodilation associated with secretion in the gland.

Actin immunoreactivity was seen in myoepithelial cells of mucous and serous glands, and in smooth muscle cells of the blood vessels. In the von Ebner’s glands, actin immunoreactive myoepithelial cells formed networks of branched cells with each other and lay among the glandular and ductal epithelium. They were spindle-shaped and exhibited stellate body and numerous cytoplasmic processes (four to six processes) embracing the secretory portion or duct.