

Research article - Histology and cell biology

Distribution and roles of substance P in human parotid duct

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Abstract

Sialadenitis occurs with greatest frequency in the parotid glands because infection and inflammation arise easily from the oral cavity. Since patients often experience severe swelling and pain during inflammation, the distribution of sensory nerves in these ducts may have clinical significance. We used antibodies to the known neuropeptide substance P and to tyrosine hydroxylase - a marker of adrenergic fibres - to observe their distribution and gain insight on their functional role in adult human parotid duct. After excising the parotid duct along with the gland, specimens were divided into three regions: the tract adjacent to the parotid gland, the route along the anterior surface of the masseter, and the area where the duct penetrates the buccinator muscle and opens into the oral cavity. Specimens were prepared and examined under a fluorescence microscope following immunostaining. Substance P positivity was observed in all three regions of the duct, whereas tyrosine hydroxylase was distributed mainly in the vascular walls and surrounding areas. The distribution of substance P candidates this molecule to assist in tissue defense in conjunction with the blood and lymph vessels of this area. Tyrosine hydroxylase in the blood vessel wall likely contributes to regulation of blood flow in concert with substance P positive nerves surrounding the blood vessels.

Key words

Parotid duct, human adult, substance P, tyrosine hydroxylase, immunohistochemistry.

Introduction

Many studies in rats have focused on the presence and actions of neuropeptides distributed in the salivary glands and their main ducts (Goedrt et al., 1982; Gallacher, 1983; Sharkey and Templeton, 1984; Ekström, 1987; Yamamoto and Kondo, 1988; Soinila et al., 1989; Ekström et al., 1996; Ekström and Ekström, 2001). Many have examined the distribution of substance P (SP), a neuropeptide of the tachykinin family, in animal salivary glands, and several detailed reports have involved the human salivary gland ducts (Hauser-Kronberger et al., 1992). A number of reports have used SP antibodies to investigate the distribution of SP in regions that are highly sensitive to SP, such as the skin, dental pulp, tongue, and periodontal areas that reportedly show high density to SP (Nagy et al., 1982; Wakisaka et al., 1985; Jacobsen et al., 1998; Ruocco et al., 2002; Nolano et al., 2013). SP-responsive regions are widely distributed

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throughout the central and peripheral nervous systems. SP may also have an effect on the blood flow within glands (Antonio, 2003).

There is enough evidence to support that SP participates in the parasympathetic regulation of salivary secretion in concert with a variety of other sialogogic messengers (Goedrt et al., 1982; Gallacher, 1983; Sharkey and Templeton, 1984; Ekström, 1987; Ekström et al., 1996; Yamamoto and Kondo, 1988; Soinila et al., 1989; Ekström and Ekström, 2001), and some reports involve humans (Hauser-Kronberger et al., 1992). Furthermore, reports indicate that SP affects gland perfusion and participates in the regulation of salivary secretory function (Ekström, 1987). Additional reports suggest a correlation between SP and tyrosine hydroxylase (TH), which is instrumental to the synthesis of catecholamines and is distributed throughout human vascular smooth muscle (Mignini, 2012). SP is also reported to act in various ways on inflammation in humans (Weglicki et al., 1996; Collins et al., 1997; Beurman and Stern, 2005). Inflammatory diseases in the salivary glands most commonly occur in the parotid glands; for example, viral infections such as mumps in children are particularly common (Iro, 2014).

Virta (1992) observed the SP distributions of parotid and submandibular glands in fetal rats at different developmental stages. He found that SP nerve fibers were rich around ductal branches of salivary glands in rat fetuses between 19th day in utero and 16th day postnatal, but the numbers slowly decreased to adult levels thereafter. Based on the abundance of SP and neurokinin A positive nerve fibers around the ductal branches and secretory structure in the developing salivary glands, that author suggested their role in the functional maturation of salivary glands.

Tyrosine hydroxylase immunohistochemistry was used in order to observe the distribution of sympathetic noradrenergic nerve fibers throughout the parotid duct wall, known to have an abundance of blood vessels. In this study, we mainly examined and compared the distributions of both SP and TH in the human parotid duct for a correlation and further clarification of their possible roles in the parotid duct.

Material and methods

This study was approved by the Ethics Committee of the Department of Anatomy, Kyorin University School of Medicine (H21-0197), and conforms to accepted ethical standards formulated in the Helsinki Declaration of 2013. Specimens were prepared using 30 adult cadavers (56–86 years) donated to the Department of Anatomy. After the skin was dissected, the parotid glands and main parotid ducts were separated and part of the parotid gland and the parotid duct up to its opening into the oral cavity (parotid papilla) were removed en bloc. The specimens were further divided into three sections: the area of the duct adjacent to the parotid gland, the section of the duct traversing over the masseter, and the section from where the duct penetrates the buccinator muscle up to the parotid papilla. These materials were fixed for 24 hours at 4°C using 4% paraformaldehyde in 0.01 mol/L phosphate buffer (PBS), pH 7.35 (Wako). After washing with PBS the specimens were immersed in 20% to 30% sucrose solution at 4°C for 36–48 hours then frozen by liquid nitrogen to prepare standard 20 µm frozen sections.

For immunohistochemistry the sections were processed using 0.1% Triton solution for 10 minutes and three washes with PBS for 5 minutes. The sections were then

treated with 5% bovine serum albumin at room temperature for 1 hour. Primary rat monoclonal-antibody to SP (Abcam; 1:100) and rabbit polyclonal-antibody to TH (Millipore; 1:100) were applied at 4°C for 24 hours. After washing with PBS, they were immersed in the secondary antibodies; FITC-conjugated goat anti-rat IgG antibody (Abcam; 1:400) for SP, and Cy3-conjugated goat anti-rabbit IgG for TH (Abcam; 1:400), at room temperature for 2 hours. Subsequently, the sections were washed with PBS three times for 5 minutes each. The observation and imaging were performed using a fluorescence microscope (BZ-X700; KEYENCE, Osaka, Japan).

Results

Expression of SP

SP was distributed abundantly throughout the parotid glands (Fig. 1A left). SP distribution was observed with low reaction in the connective tissue immediately under the epithelium in the region of the duct adjacent to the parotid gland (Fig. 1B left). Intense SP reaction was observed under the epithelium in the wall of the parotid duct where it traverses over the masseter, as well as around the blood vessels (Fig. 1C left). In the area where the duct penetrates the buccinator muscle, the highest reaction was observed in the connective tissue under the epithelium (Fig. 1D left). There was no reaction of SP among the buccinator muscle fibers invading the duct wall in this area, and low reaction was seen immediately beneath the parotid papilla compared to other regions.

Expression of tyrosine hydroxylase

The highest reaction for TH was observed in the parotid gland and around the walls of intra-glandular blood vessels (Fig. 1A right). In the region of the duct adjacent to the parotid glands, TH reactivity was mainly concentrated in the vascular walls and was relatively low, similar to SP reaction in the same site (Fig. 1B right). TH distribution was also limited to the vascular walls and surrounding areas in the section of the duct traversing over the masseter (Fig. 1C right). In the area of the duct penetrating the buccinator muscle there was reduced TH distribution as compared with other regions (Fig. 1D right). Overall, TH reactivity was seen mainly in the smooth muscle layer of the blood vessel wall, whereas SP reaction was seen both in the blood vessel wall and in the connective tissue surrounding the walls.

Discussion

It is clinically well known that many inflammatory conditions of the parotid gland are caused by infections from the oral cavity (Manrique and Sato, 2009) and that the morphological features of the parotid gland duct connecting the gland to the oral cavity is a factor favoring inflammation. Because the parotid duct opening is on the buccal mucosa of the second maxillary molar, bacteria and viruses can directly and easily infiltrate the duct from the oral cavity. In addition, this is the area where food

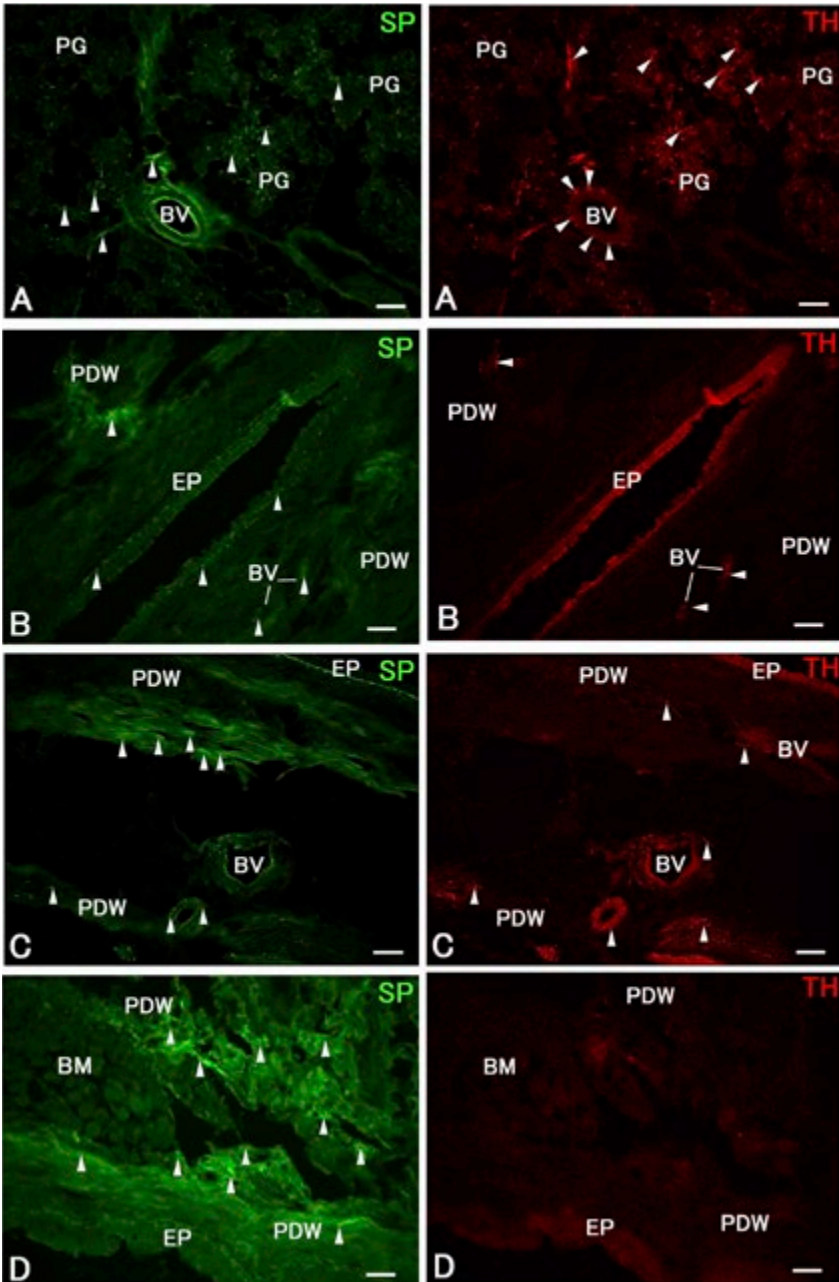


Figure 1 – Substance P (left panels) and tyrosine hydroxylase (right panels) expression in the human adult parotid gland and duct. A: Section of the parotid gland. B: Section of the parotid duct adjacent to the parotid gland. C: Section of the parotid duct traversing over the masseter. D: Section of the parotid duct penetrating the buccinator muscle. PG: parotid gland; PDW: parotid duct wall; BM: buccinator muscle; BV: blood vessels; EP: epithelium. Arrow heads: nerve fibers. All scale bars: 100 μ m.

particles in the posterior region of the oral vestibule are most likely to be trapped. The morphological characteristics of the papilla through which saliva is released into the oral cavity involve several individual differences. Since the diameter of the papilla can vary from microscopic to needle size, we cannot exclude the possibility that individual differences in morphological aperture may be a contributing factor to infection.

In the opening area or the papilla of the parotid duct where it penetrates through the buccinator muscle, part of the surrounding muscle fibers invade the duct wall. We suggested that these embedded muscle fibers in the parotid duct wall act similarly to a sphincter to help regulate the salivary flow in the parotid duct (Amano, 2013). Based on clinical considerations, Harrison (2009) hypothesized that if some type of dysfunction occurred in the surrounding buccinator muscle, then its role as a sphincter would be impaired. In this case, reflux salivation would take place in addition to infiltration by bacteria from the oral cavity. In other words, such a dysfunction could be associated with a state in which bacteria easily infiltrate the parotid gland. In this way, some patients may have a buccal environment with morphological characteristics that facilitate viral infection, although we presume that the parotid duct is also provided with a preventive mechanism against exogenous viral infiltration.

In addition to a steady secretion from the parotid gland, Kutta et al. (2006) noted the presence of granulocytes, T-lymphocytes, and macrophages in the mucosal lamina propria of the parotid duct wall and the formation of a complex epithelial layer composed of secreted and synthesized mucins and trefoil factor family peptides. This demonstrates an immune protection system that plays an important role in protecting the parotid duct and gland against ascending viral infections.

Many studies have reported SP to play various roles during inflammation and wound healing. The wide range of physiological functions of SP include being a mediator, a trigger, or inducing repair (Scardina et al., 2004). In addition, some reports have suggested that SP participates in the regulation of the inflammatory process and acts like a neuropeptide that plays a role as a modulator during allergic inflammation (El-Shazly, 1996). For instance, when tissues are injured, SP is released and may contribute in the initiation of a healing reaction in the injured connective tissues (Nilsson et al., 1985). Further, SP also acts as a sensory peptide, together with calcitonin gene-related peptide (CGRP), to accelerate wound healing (Khalil and Helme, 1996).

During our immunohistological examination of the human parotid duct, we observed the entire region of the duct from the papilla to the parotid gland for SP and TH reactivity. SP distribution was observed along the entire length of the main duct except in the papilla. TH distribution was observed within the gland, in the duct wall, as well as in and around the blood vessel walls. Our results show high reaction to both SP and TH in the parotid gland and its main duct wall, confirming an abundance of blood vessels in these regions. We suggested from TH reaction in our previous study that vascular walls of the parotid gland and main duct wall play a role in the regulation of salivary flow (Amano 2015). Based on the reactions of both SP and TH in the blood vessel walls, it is likely that the two work together to control the flow of blood stream in the blood vessels of the parotid gland and main duct wall. Furthermore, SP distribution in the connective tissue layer immediately beneath the parotid duct opens up possibilities of SP roles in the immune defense system of the

parotid duct and gland to protect them from viral infections, as well as in the regulation of the inflammatory process in the injured connective tissues.

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