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Identification of autonomic neuronal chains innervating gingiva and lip

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ABSTRACT

The major goals of this present study were 1) to further clarify which parasympathetic ganglion sends postganglionic fibers to the lower gingiva and lip that may be involved in the inflammatory processes besides the local factors; 2) to separately examine the central pathways regulating sympathetic and parasympathetic innervation; and 3) to examine the distribution of central premotor neurons on both sides. A retrogradely transported green fluorescent protein conjugated pseudorabies virus was injected into the lower gingiva and lip of intact and sympathectomized adult female rats. Some animals received virus in the adrenal medulla which receive only preganglionic sympathetic fibers to separately clarify the sympathetic nature of premotor neurons. After 72–120 h of survival and perfusion, the corresponding thoracic part of the spinal cord, brainstem, hypothalamus, cervical, otic, submandibular and trigeminal ganglia were harvested. Frozen sections were investigated under a confocal microscope. Green fluorescence indicated the presence of the virus. The postganglionic sympathetic neurons related to both organs are located in the three cervical ganglia, the preganglionic neurons in the lateral horn of the spinal cord on ipsilateral side; premotor neurons were found in the ventrolateral medulla, locus ceruleus, gigantocellular and paraventricular nucleus and perifornical region in nearly the same number on both sides. The parasympathetic postganglionic neurons related to the gingiva are present in the otic and related to the lip are present in the otic and submandibular ganglia and the preganglionic neurons are in the salivatory nuclei. Third order neurons were found in the gigantocellular reticular and hypothalamic paraventricular nuclei and perifornical area.

1. Introduction

It is generally accepted that the postganglionic parasympathetic fibers innervating the gingiva or lip tend to join the sensory or motor nerves (Izumi and Karita, 1991). The postganglionic sympathetic fibers associate with the vessels (Jacobsen et al., 1998). Clinically normal, sound gingiva was shown to contain nerve fibers exhibiting immunoreactivity for varying substances. Calcitonin gene-related peptide (CGRP), substance-P (SP), vasoactive intestinal peptide (VIP) and neuropeptide-Y (NPY) are the most common neuropeptides in the gingiva. The presence of NPY and VIP fibers occasionally observed around blood vessels of the gingiva suggests that they are autonomic in nature (see Lundy and Linden, 2004). In human attached gingiva of lower molars nerve fibers immunoreactive for CGRP, SP, NPY, tyrosine hydroxylase (TH), VIP, and peptide histidine isoleucine (PHI) were observed around blood vessels (Luthman et al., 1988). Another research group also demonstrated noradrenergic fibers in the lamina propria and in the wall of the vessels (Urbanovich et al., 1999). It was also demonstrated that following surgical sympathectomy, a marked increase of CGRP-containing fibers was seen in tongue, epiglottis and pharynx while TH-immunoreactive fibers were totally depleted. Conversely, after capsaicin treatment, an increase of TH-immunoreactive nerves was found in the same tissues (iris, cornea, tongue, palate and epiglottis) concomitant with a sharp decrease of CGRP-immunoreactive nerves (Terenghi et al., 1986). Horseradish peroxidase/wheat germ agglutinin (HRP/WGA) administration into the molar gland and the lower lip–gingiva of cat showed retrogradely labeled neurons in the otic (OG), superior cervical (SCG) and mandibular subdivision of the trigeminal ganglia (TriG) (Kuchiiwa and Kuchiiwa, 1996). This observation suggests that the nerve fibers found in the gingiva and lip derive from these sources. It was also demonstrated that the mucous membrane of the lip contains fibers immunoreactive for VIP, SP, NPY and nitric oxide

Abbreviations: CB, calbindin; CGRP, calcitonin gene-related peptide; DBH, dopamine β-hydroxylase; GFP, green fluorescent protein; Gi, gigantocellular reticular nucleus; HRP/WGA, horseradish peroxidase/wheat germ agglutinin; Il-1β, interleukin-1β; ISN, inferior salivatory nucleus; KPB, potassium phosphate buffer; LC, locus ceruleus; LH, lateral horn; L-ENK, leu-enkephalin; MCG, middle cervical ganglion; NOS, nitric oxide synthase; NPY, neuropeptide Y; OG, otic ganglion; Pf, perifornical region; PFU, plaque forming unit; PHI, peptide histidine isoleucine; PRV, pseudorabies virus; PVN, paraventricular nucleus; SCG, superior cervical ganglion; SMG, submandibular ganglion; SP, substance P; SSN, superior salivatory nucleus; STG, stellate ganglion; TH, tyrosine hydroxylase; TriG, trigeminal ganglion; VIP, vasoactive intestinal polypeptide; VLM, ventrolateral medulla.

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synthase (NOS) (Fehér et al., 1999). Lohinai et al. (1997) described NOS immunoreactive fibers in cat and dog gingiva; however, their origin was not clarified.

Numerous data accumulated in the literature on the location of neurons in the central nervous system which are involved in the autonomic innervation of many viscera including the suprarenal gland (Strack et al., 1989a; Geerling et al., 2003; Tóth et al., 2008), the ovary (Tóth et al., 2008), the kidney (Li et al., 1992; Schramm et al., 1993; Reichart et al., 2000; Valentino et al., 2000), the colon (Levatte et al., 1998; Vizzard et al., 2000), the penis, the prostate gland and the perineal muscles (Marson and Carson, 1999), the urinary bladder (Rouzade-Dominguez et al., 2003), the salivary (Jansen et al., 1992; Rezek et al., 2008) and mammary glands (Gerendai et al., 2001; Köves et al., 2012). As it was predicted by Strack and his co-workers (1989b), who injected a retrograde pseudorabies virus into various sympathetic ganglia and the adrenal gland, the premotor sympathetic neurons are generally found in several cell groups that regulate the entire sympathetic outflow: the hypothalamic paraventricular nucleus (PVN), perifornical region (Pf), A5 noradrenergic cell group, caudal raphe region, rostral ventrolateral medulla (VLM), the locus ceruleus (LC), and the Barrington's nucleus. Additionally, local interneurons in laminae VII and X of the spinal cord are also involved in the innervation of the preganglionic neurons in the lateral horn (LH). They also showed that the retrograde transneuronal viral labeling method can be used simultaneously with either neuropeptide transmitter or transmitter synthetic enzyme immunohistochemistry.

Only a few data are available in the literature about the detailed autonomic innervation of the structures of the oral cavity and associated salivary glands. Rezek and his co-workers (2008) mapped the neurons of the inferior salivatory nucleus (ISN) which supply the parasympathetic preganglionic innervation of rat parotid gland. Wojtkiewicz and his co-workers (2011) identified the chemical coding of the SCG which gives the postganglionic sympathetic innervation of the porcine parotid gland. They utilized the retrograde transportation of the fast blue from the parotid gland. The fast blue labeled neurons of SCG exhibited TH, calbindin (CB), NPY, leu-enkephalin (L-ENK) and galanin immunoreactivities. Morris and her co-workers (1997) demonstrated various peptides such as VIP, NPY, L-ENK, dynorphin and TH in sympathetic and parasympathetic nerve fibers which innervated the sublingual, submandibular, parotid, lacrimal and zygomatic glands of guinea pigs. They concluded that the different peptides made variable contributions to autonomic neurotransmission in different arterial segments and in different cranial exocrine glands.

Physiological data indicate that the blood supply of the gingiva and lip is regulated by both autonomic nervous system and local factors (Izumi et al., 1990; Izumi and Karita, 1991; 1993; Gyurkovics et al., 2009). The stimulation of the sympathetic nerves induced arterial constriction decreasing the blood flow (Izumi et al., 1990), the parasympathetic stimulation induced arterial dilation increasing the blood flow in the lower gingiva measured by a laser Doppler flowmeter. It was also suggested that the parasympathetic fibers may reach the lower gingiva not only along the trigeminal, but also along the facial and glossopharyngeal nerves (Izumi and Karita, 1991). It was also shown that local factors such as nitric oxide and endothelial growth factor (Gyurkovics et al., 2009) or CGRP released at sensory nerve endings (Györfi et al., 1992; Helyes et al., 1997) may induce vasodilation in the gingiva. Although, the occurrence of parasympathetic fibers was demonstrated (Kuchiiwa and Kuchiiwa, 1996) but not confirmed in the gingiva, their presence is still a matter of debate. The fact, that CGRP immunoreactive neurons were found in the cranial parasympathetic ganglia, complicates the situation (Hardebo et al., 1992).

The major goals of this present study were 1) to further clarify which sympathetic and parasympathetic ganglia send postganglionic fibers to the lower gingiva and lip of rat that may be involved in the inflammatory processes besides the local factors; 2) to examine separately the central pathways regulating the sympathetic and the parasympathetic innervation; and 3) to examine the distribution of central premotor neurons on ipsi- and contralateral sides. We have used transneuronal retrograde tracing technique and dopamine β-hydroxylase (DBH) immunohistochemistry. In some of the animals sympathectomy was carried out. This intervention gave us the possibility to examine the parasympathetic innervation of the lower gingiva and lip separately from the sympathetic innervation. Some animals received virus into the adrenal medulla (this organ is innervated only by preganglionic sympathetic fibers and does not receive parasympathetic innervation) to find out whether there is some overlap of the brainstem sympathetic and parasympathetic premotor areas as it was demonstrated in the hypothalamus (Hettigoda et al., 2014).

2. Materials and methods

2.1. Animals

Adult Wistar female rats (3–4 month-old) were used for the experiments. The animals were kept in a light/dark cycle (lights on at 5:00 and lights off at 19:00) and temperature controlled vivarium (22 ± 2 °C). The treatment of the animals was in accordance with the rules of the "European convention for the protection of vertebrate animals used for experimental and other scientific purposes", Strasbourg, 1986 and Hungarian Government Directive 243/98. Our protocol was approved by the Local Animal Care and Use Committee (Permission No: 22.1/1158/3/2010).

2.2. Preparing viruses

A genetically modified pseudorabies virus strain termed memGreen-PRV was used for the tracing experiments. Boldogkői and his coworkers (2000, 2002) modified a wild-type of strain Kaplan of PRV (which is able to spread in both ante- and retrograde directions) by the elimination of the gE and the gI genes of the virus. These are responsible for anterograde spreading of the virus. The gE and the gI genes were replaced by a gene expression cassette encoding a membrane-bound green fluorescent protein (GFP), which makes the identification of the infected cells easy by fluorescence microscope. This modified virus was able to spread only in a retrograde direction. After replication in a neuronal cell body, the virus could enter the next member of the neuronal chain. The concentration of the virus was 8×10^8/mL plaque forming unit (PFU). The spreading speed of the virus in a retrograde direction was 1.5 mm/h.

2.3. Virus inoculation

2.3.1. Intact animals

Under general anesthesia (chloralhydrate 35 mg/100 g bw) with the use of 10 μL Hamilton syringe 4 or 6 μL of virus containing buffer was injected into the gingiva lateral to the lower right incisive tooth or in the lower lip opposite the gingival injection. The needle was left inside the structure for 1 min to prevent the leakage of the solution. To test whether 4 μL remains in the gingiva and shows no penetration into the lower lip, the same amount of methylene blue of 0.01% was injected in the gingiva. 4 μL of injected solution remained *in loco*. We accepted that in this group the virus labeling derived from the gingiva. When 6 μL was injected, it could penetrate the lower lip. In the Group 11 6 μL of virus was injected in the right adrenal medulla.

2.3.2. Sympathectomized animals

Prior to the virus inoculation (2–4 days) the SCG was removed. Animals exhibiting ptosis and myosis (signs of sympathectomy) were used for inoculation.