Neuroscience Letters, 142 (1992) 237–240

© 1992 Elsevier Scientific Publishers Ireland Ltd. All rights reserved 0304-3940/92/$ 05.00

NSL 08834

Origins of parasympathetic postganglionic vasodilator fibers supplying the lips and gingivae; an WGA–HRP study in the cat

Satoshi Kuchiiwa[a], Hiroshi Izumi[b], Keishiro Karita[b] and Shiro Nakagawa[a]

[a]*Department of Anatomy, Faculty of Medicine, Kagoshima University, Kagoshima (Japan) and* [b]*Department of Physiology, Tohoku University School of Dentistry, Sendai (Japan)*

(Received 31 March 1992; Revised version received 12 May 1992; Accepted 12 May 1992)

Key words: Parasympathetic vasodilatation; Otic ganglion; Pterygopalatine ganglion; Lip; Gingiva; Cat

Application of WGA-HRP to the mandibular lip and buccal gingiva of the cat resulted in retrograde labeling in the ipsilateral otic ganglion (OG), whereas labeled neurons appeared in the pterygopalatine ganglion (PPG) as well as in the OG when the tracer was injected into the maxillary lip and buccal gingiva. The results suggest that both the facial and the glossopharyngeal preganglionic vasodilator fibers supplying the mandibular lip and buccal gingiva mediated in the OG, and those innervating the maxillary lip and buccal gingiva are mediated in the PPG and the OG.

We found in previous investigations that the vasodilator responses in both lips and buccal gingivae were elicited by stimulation of peripheral cut ends of the facial or the glossopharyngeal nerve [3, 5]. However, the pathways and the way-stations have not yet been anatomically clarified. The purpose of the present study is to examine the origins of the postganglionic parasympathetic fibers innervating the blood vessels in the lips and buccal gingivae by means of the horseradish peroxidase-conjugated wheat germ agglutinin (WGA–HRP) method.

Experiments were carried out on 10 adult cats weighing 2.0–4.0 kg. The animals were anesthetized with intramuscular injections of ketamine hydrochloride (30–50 mg/kg), and 5 μl of 5% WGA–HRP (Toyobo) dissolved in saline were injected into the mandibular lip and buccal gingiva (4 cats) or the maxillary lip and buccal gingiva (5 cats; one of them was injected within the upper lip restrictedly) or the oral angle (one cat) using a Hamilton microsyringe. The sites of the syringe needle penetrations were the portions on which we have so far measured the vasodilatations induced by stimulations of the facial or glossopharyngeal nerve branches or roots [3–5]. After a 45–51 h post-injection survival period, the cats were re-anesthetized and perfused through the ascending aorta with 1 liter of saline followed by 2 liters of 2.5% formalin and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer at pH 7.4. The otic ganglion (OG), pterygopalatine ganglion (PPG), superior cervical ganglion (SCG) and trigeminal ganglion (TG) ipsilateral to the injections and the injected tissue were removed and immersed in cold buffer containing 30% sucrose, and 50-μm-thick sections were cut on a freezing microtome. All sections of the ganglia and every 5th section of the injected tissues were prepared for peroxidase histochemistry by the tetramethylbenzidine reaction method [8]. A part of sections of the ganglia were counterstained with neutral red and the neighboring sections of the lips and buccal gingivae were stained with hematoxylin–eosin.

In the cat, there were no sharp boundaries between the lip and the buccal gingiva except in the vibrissal portion.

In all cases receiving an injection into the maxillary lip and buccal gingiva, the syringe needle was penetrated into the swelling of the lip just latero-caudal to the canine, and the enzyme infiltrated into the gingiva as well as the lip. The points of the syringe needle penetrations and the extent of the injection site in one of the cases are shown in Fig. 1A (solid arrow), B and C. Examination of the hematoxylin-eosin preparations revealed high vascularity and presence of a molar gland. The gland was well developed and extended caudally beneath the oral angle. No other structures know to be innervated by the parasympathetic nervous system were observed in the injection site [1]. The vessel walls, perivascular areas and the molar gland were infiltrated with the tracer. In these cases, many retrogradely labeled ganglion cells were observed in the OG. The total number of labeled cells ranged from 267 to 463, with an average of 380.5 (Table

Correspondence: S. Kuchiiwa, Department of Anatomy, Kagoshima University, Sakuragaoka 8-35-1, Kagoshima, 890, Japan. Fax: (81) 992-64-9321.

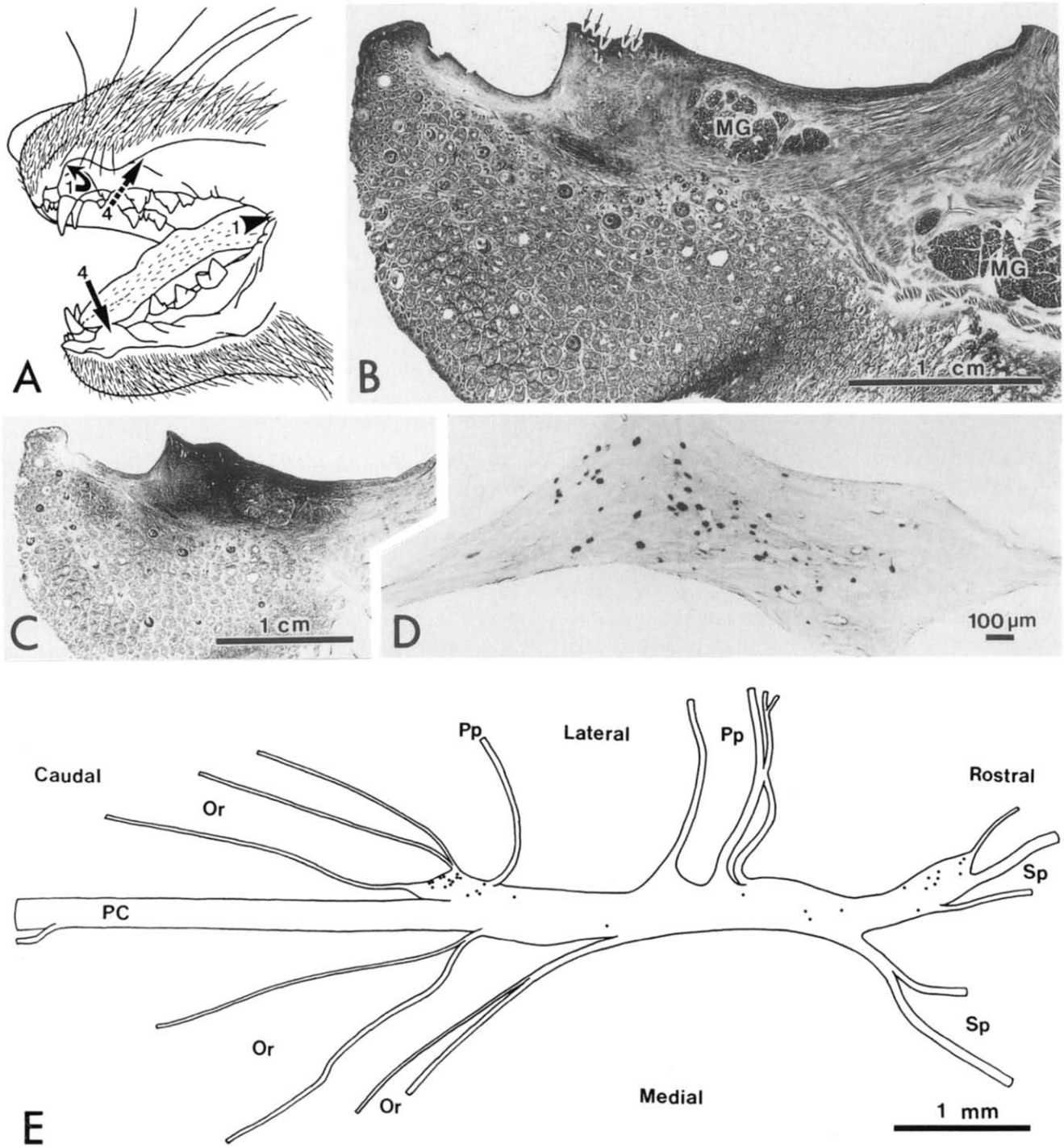

Fig. 1. A: illustration showing the points of the syringe needle penetrations, with the number of animals injected in each case. B: photomicrograph of the hematoxylin-eosin preparation of the mandibular lip and buccal gingiva. Arrows indicate the blood vessels. MG, molar gland. 50-μm-thick slice. C: photomicrograph showing the site of maximum extent of an injection of WGA–HRP in the mandibular lip and buccal gingiva (solid arrow in A). The neighboring section of B, TMB method, 50-μm-thick slice. D: labeled neurons in the otic ganglion after a WGA–HRP injection into the mandibular lip and buccal gingiva, the same animal as B and C. TMB method, 50-μm-thick slice. E: diagram showing the distribution of retrogradely labeled neurons in the pterygopalatine ganglion after an injection of WGA–HRP into the maxillary lip and buccal gingiva (broken arrow in A). Or, rami orbitales to the retro-orbital plexus; Or*, rami orbitales to the ethmoidal or infratrochlear nerve; Pp, pterygopalatine nerve; PC, nerve of pterygoid canal; Sp, sphenopalatine nerve.

I). The labeled neurons were distributed evenly in the ganglion (Fig. 1D). No labeled neurons were found in the PPG in 3 cases, however, in one case in which the injected enzyme infiltrated slightly into the vicinity of the

TABLE I

NUMBERS OF LABELED NEURONS IN THE OTIC GANGLION (OG) AND THE PTERYGOPALATINE GANGLION (PPG) AFTER APPLICATION OF WGA-HRP TO THE LIPS AND GINGIVAE

Cat No.	Site of injection of the tracer	No. of labeled cells	
		OG	PPG
1	mandibular lip and gingiva	445	4
2		347	0
3		267	0
4		463	0
5	maxillary lip and gingiva	18	257
6		17	99
7		22	54
8		16	105
9	maxillary lip	12	31
10	oral angle	223	40

oral angle, only a few weakly labeled cells appeared. In the SCG, abundant labeled neurons were evenly located in the ganglion without an apparent pattern. Labeled cells were also distributed in a localized fashion in the mandibular division of the TG.

In the second group of animals, the syringe needle was penetrated into the upper lip just dorsal to the second premolar in 4 of 5 cases (Fig. 1A; broken arrow), and into the upper lip mucosa covering the canine in the other one case (Fig. 1A; curved arrow). In the former 4 cases, the injected enzyme infiltrated into the maxillary buccal gingiva as well as the upper lip, while in the latter, it was restricted within the vibrissal portion of the upper lip. Examination of the hematoxylin-eosin preparations revealed an absence of small salivary glands in the maxillary lip and buccal gingiva, and no structures other than the blood vessels which had been known to receive parasympathetic innervation were found in the injection site. In all 5 cases, applications of WGA-HRP resulted in retrograde labeling of cells of the OG and the PPG. In the former 4 cases, the total number of labeled cells in the OG ranged from 16 to 22, with an average of 18.3 (Table I). In the PPG, labeled neurons were distributed not only in the rostral division but also in the caudal (Fig. 1E), and a few labeled cells were found in the accessory PPG. The total number of labeled cells in the main and the accessory PPG was larger than that in the OG, and ranged from 54 to 257, with an average of 128.8. The ratio of the total number of labeled neurons in the OG to that in the PPG ranged from 1:2.5 to 1:14.3, with an average of 1:7.3. In the latter one case, in which the enzyme was limited within the vibrissal portion of the upper lip, 12 labeled cels were found in the OG and 31 cells in the PPG. In all these cases, numerous labeled neurons were distributed in the SCG without an apparent pattern, and many WGA-HRP positive cells were detected in the maxillary division of the TG, and a small number also in the mandibular division.

When the WGA-HRP was applied to the oral angle (Fig. 1A; arrowhead), the tracer infiltrated into the caudal portion of the molar gland, and WGA-HRP positive neurons were observed in both the OG and the PPG. The numbers of labeled cells were 223 in the OG and 40 in the PPG (Table I). Numerous labeled cells also appeared in the SCG and the TG.

The results obtained in the present investigation demonstrate that in the cat the blood vessels and molar gland in the mandibular lip and buccal gingiva are supplied exclusively by the postganglionic parasympathetic fibers originating in the OG, and the blood vessels in the maxillary lip and buccal gingiva and in the oral angle receive dual parasympathetic innervation by the OG and the PPG.

We have previously reported that the vasodilatation occurs in the cat lower lip and the mandibular buccal gingiva following stimulation of peripheral cut ends of the facial nerve in a manner similar to that observed in the glossopharyngeal nerve [3, 5]. Both vasodilator responses were markedly reduced by pretreatment with autonomic ganglionic blocker, hexamethonium (C6), but never affected by lesion of the ipsilateral PPG. These findings and the present anatomical studies demonstrate that the facial nerve- and the glossopharyngeal nerve-induced vasodilatations in the mandibular lip and buccal gingiva are mediated via the OG but not the PPG. The great superficial petrosal nerve of man has been reported anatomically to reach the OG [11] as well as the PPG [9]. It seems likely that the facial vasodilator fibers leave by way of the greater superficial petrosal nerve to reach the OG, and then supply the blood vessels in the mandibular lip and buccal gingiva.

Moreover, we have recently suggested the occurrence of parasympathetic vasodilator fibers which seem to be involved in trigeminally or vagally mediated reflex vasodilatation in the cat mandibular lip and buccal gingiva [3, 5]. These fibers emerge from the brain stem with the glossopharyngeal nerve and reach the blood vessels in the mandibular lip and buccal gingiva by way of the inferior alveolar nerve [4, 5]. Unilateral extirpation of the PPG is without effect on these reflex vasodilatation, suggesting that the OG is a possible way-station on the path to the mandibular lip and buccal gingiva [3, 6]. The present anatomical study confirms this deduction.

After applying the tracer to the maxillary lip and buccal gingiva, we observed labeled cells in the mandibular

division of the TG, suggesting the possibilities that some mandibular branches, presumably twigs of the buccal nerve, supply these oral regions, and that the postganglionic fibers of the OG run by way of these twigs.

When WGA-HRP was applied to the upper lip and the maxillary buccal gingiva, labeled PPG neurons were observed not only in the rostral but also in the caudal division. The labeling in the rostral division was to be expected given its parasympathetic innervation in the oral regions [7, 9]. However, particular attention should be drawn to the relatively large number of labeled cells in the caudal division. It is known that the blood vessels in the eyeball and the cranial cavity are innervated by the postganglionic fibers of the PPG [2, 7, 10], and that the fibers originate in the caudal division of the PPG and the accessory ganglia, and some of them course by way of the microganglia in the retro-orbital plexus (ref. 7 and unpublished results). The present study indicates that a large number of vasodilator fibers innervating the maxillary lip and buccal gingiva also originate in the same caudal regions of the PPG. Moreover, it is possible that some fibers might also travel by way of the microganglia in the retro-orbital plexus.

We are most grateful to Dr. Toshiko Kuchiiwa for her helpful advice throughout the course of this work. We also thank Mr. K. Yamakuchi for his expert technical assistance. This research was supported by Kodama Foundation for Research of Medical Science and Hirosaki University Special Research Grant, 1990, No. 20.

1 Gabella, G., Structure of the Autonomic Nervous System, Chapman and Hall, London, 1976, pp. 150–161.
2 Gibbins, I.L., Brayden, J.E. and Bevan, J.A., Perivascular nerves with immunoreactivity to vasoactive intestinal polypeptide in cephalic arteries of the cat: distribution, possible origins and functional implications, Neuroscience, 13 (1984)1327–1346.
3 Izumi, H. and Karita, K., Vasodilator responses following intracranial stimulation of the trigeminal, facial and glossopharyngeal nerves in the cat gingiva, Brain Res., 560 (1991) 71–75.
4 Izumi, H. and Karita, K., Selective excitation of parasympathetic nerve fibers to elicit the vasodilatation in cat lip, J. Auton. Nerv. Syst., 37 (1992) 99–108.
5 Izumi, H. and Karita, K., Somatosensory stimulation causes autonomic vasodilatation in cat lip, J. Physiol., 450 (1992) 191–202.
6 Izumi, H., Kuriwada, S., Karita, K., Sasano, T. and Sanjo, D., The nervous control of gingival blood flow in cats, Microvasc. Res., 39 (1990) 94–104.
7 Kuchiiwa, S., Intraocular projections from the pterygopalatine ganglion in the cat, J. Comp. Neurol., 300 (1990) 301–308.
8 Mesulam, M.-M., Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents, J. Histochem. Cytochem., 26 (1978) 106–117.
9 Mitchell, G.A.G. and Learmonth, J., Anatomy of the Autonomic Nervous System, Livingstone, Edinburgh, 1953, pp. 160–175.
10 Uddman, R., Alumets, J., Ehinger, B., Håkanson, R., Lorén, I. and Sundler, F., Vasoactive intestinal peptide nerves in ocular and orbital structures of the cat, Invest. Ophthalmol. Vis. Sci., 19 (1980) 878–885.
11 Vidić, B. and Young, P.A., Gross and microscopic observations on the communicating branch of the facial nerve to the lesser petrosal nerve, Anat. Rec., 158 (1967) 257–262.