Preganglionic Neurons of the Sphenopalatine Ganglia Reside in the Dorsal Facial Area of the Medulla in Cats

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Abstract

Stimulation of the sphenopalatine ganglion (SPG), a parasympathetic ganglion of the facial nerve, or the dorsal facial area (DFA), an area in the lateral tegmental field just dorsal to the facial nucleus, induces an increase in blood flow of the common carotid artery (CCA). This study attempted to clarify the anatomical and functional relationships between the SPG and the DFA, and to demonstrate putative serotonergic (5-HT) and substance P (SP) innervations to the neurons of the DFA in regulation of the CCA blood flow in cats. Horseradish peroxidase (HRP), a retrograde tracer, was injected in the SPG. All HRP-labeled neurons were distributed in the reticular areas dorsal and lateral to the superior olivary nucleus and the facial nucleus, extending from the caudal half of the superior olivary nucleus to the rostral 3/4 of the facial nucleus on the HRP-injected side. They were grouped into five clusters, namely lateral circumference of the superior olivary nucleus, dorsal circumference of the superior olivary nucleus, lateral circumference of the facial nucleus, dorsal circumference of the facial nucleus, and the DFA. The percentage of HRP-neurons in each cluster was 0.5±0.1% (mean±S.E., n=6), 15.2±1.9%, 23.7±0.9%, 52.5±1.7%, and 8.3±0.7%, respectively. Glutamate stimulation of the DFA (at 5.0 to 7.0 mm rostral to the obex, 2.8 to 4.0 mm lateral to the midline, and 2.5 to 3.5 mm ventral to the dorsal surface of the medulla), but not other areas, resulted in the increased CCA blood flow. The 5HT- and SP-immunoreactive nerve terminals abutted on the ChAT-immunoreactive cell body (preganglionic neurons) in the DFA. In conclusion, parasympathetic preganglionic neurons in the DFA project fibers to the SPG, are innervated by 5HT- and SP-like nerve terminals, and are responsible for regulation of the CCA blood flow. They may be also important in regulation of the cerebral blood flow.

Key Words: carotid artery, cerebral vessel, immunohistochemistry, facial nerve, glossopharyngeal nerve, lateral tegmental field, parasympathetic, sphenopalatine ganglion

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Introduction

The postganglionic parasympathetic fibers arising from the sphenopalatine ganglion (SPG) innervate lachrymal glands, nasal and palatal membranes (29, 40), basilar artery (15), middle cerebral artery (10, 41) and intracranial segment of the internal carotid artery (38) in different species. The SPG serves as the major source of fibers containing vasoactive intestinal peptide and choline acetyltransferase (ChAT) that innervate vascular beds of the cerebral hemispheres (9, 34, 35, 37). Electrical stimulations of either the SPG in cats (7), the greater superficial petrosal nerve, or postganglionic fibers from the SPG in rats (37, 39) and dogs (5) increase cortical blood flow. The increased cortical blood flow is mediated primarily by non-cholinergic fibers (5, 19, 36, 37) and is independent of glucose utilization (7). Therefore, the SPG is involved in regulation of the cortical and/or carotid arterial blood flows.

The central location of preganglionic neurons that project to the SPG in cats (14, 15) and rats (33) has been demonstrated in the superior salivary nucleus (SSN). A substantial literature indicates the existence of the serotonergic (5HT) (2, 13, 25) and substance P (SP) nerves (6, 24, 25) in the medulla oblongata. More specifically in the SSN, various neuropeptide- and amine-containing axons, including SP- and 5HT-containing axons, make synaptic contact with parasympathetic preganglionic neurons (24). Based on these findings together with the fact that the SPG regulates the cortical and/or carotid arterial blood flow, it is not surprising that the SP- and 5HT-axons (24) and preganglionic neurons (14, 15) in the SSN were suggested to regulate the cerebral blood flow.

The SSN, however, is a nucleus of multifunction, including autonomic regulation of the nasal and palatal mucosa, the lacrimal glands and cerebral blood vessels (24). The central location of which portion of the SSN preganglionic neurons regulating the cerebral blood vessels (or the cortical and/or carotid arterial blood flow) is not yet clearly demonstrated. In fact, a reticular area just dorsolateral to the facial nucleus, so-called "the dorsal facial area (DFA)" by Kuo and his colleagues, regulates the cerebral (3) and common carotid arterial (CCA) blood flows (3, 16-20, 22). Because the DFA seems to be located or overlapped in the SSN, the DFA may contain the SP- and 5HT-nerves (24) that innervate the preganglionic neuron of the SPG (14, 15) to regulate the CCA blood flow. Nevertheless, detail mapping of the DFA is lacking, and whether DFA contains the SP- and 5HT-nerves that innervate the preganglionic neuron of the SPG is not known. Therefore, it is important to delineate the detail location of the DFA and to clarify the anatomical and functional relationships between the SPG and the DFA in regulation of the CCA blood flow. It is worth to determine the existence of the SP- and 5HT- nerve innervations to the DFA.

In the present study, we mapped the DFA that responded to glutamate stimulation to induce the increase in the CCA blood flow. We demonstrated that the DFA had preganglionic neurons projecting to the SPG, by retrograde tracing technique with application of horseradish peroxidase (HRP) on the SPG. We found that DFA had sparsely but definitely 5HT- and SP-immunoreactive fibers abutting on the preganglionic neurons. Findings indicate that parasympathetic preganglionic neurons in the DFA project fibers to the SPG, are innervated by 5HT- and SP-like nerve terminals, and are responsible for regulation of the CCA blood flow.

Materials and Methods

Animal Preparations

Animal care. The experiments were carried out in accordance with the guidelines of the Taichung Veterans General Hospital Ethical Committee for Animal Research and were approved by the committee.

General procedure. Nine cats of either sex, weighing 1.6 to 4.0 kg were anesthetized intraperitoneally with α-chloralose (35 mg/kg, Sigma, St. Louis, MO, USA) and urethane (350 mg/kg, Sigma), and paralyzed intravenously with Tracurium (initial dose, 0.08 mg/kg; subsequent dose, 0.02 mg/kg/40 min, Wellcome Co., Temple Hill, NY, USA). To avoid respiratory acidosis or alkalosis, the trachea was intubated for artificial ventilation (Harvard respirator 55-0798, Holliston, MA, USA) maintaining the end expiratory CO_2 concentration at 3.5 to 4.5% under continuous monitoring by a capnometer (Engstrom, UK). The rectal temperature, detected by a thermister probe, was automatically maintained constant at 38±1°C with an electrical heating pad. Femoral vein and artery were cannulated for chemical and fluid administrations and for monitoring of the systemic arterial pressure (SAP), respectively. The latter was measured through a Statham pressure transducer (Gould P23ID, Eastlake, OH, USA) connected to a pressure processor amplifier (Gould) by which the mean systemic arterial pressure (MAP) and heart rate (HR) were electronically computed.

Recording of CCA blood flow. Blood flows of bilateral common carotid arteries (CCA) were monitored by blood flow probes (1.5 to 2.0 mm inner diameter, Gould) coupled with electromagnetic flowmeters (Gould SP 2202). All recordings were made on a Gould 2800S polygraph.

Brain stimulation. The head of the cat was immobilized in a stereotaxic instrument (David-Kopf,

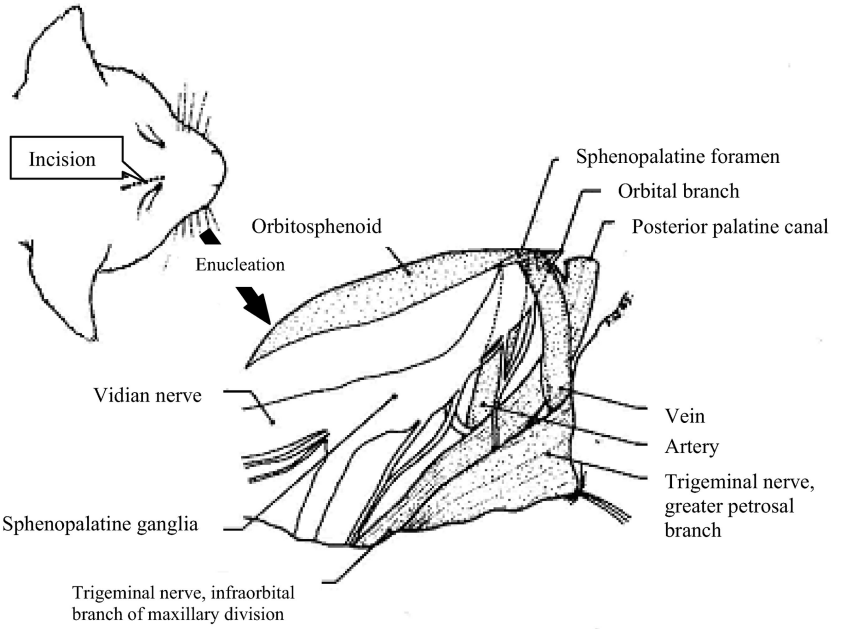

Fig. 1. Schematic diagram shows anatomical location of the right sphenopalatine ganglion (SPG) of the cat for reference of surgical approach.

Tujunga, CA, USA). After removal of the occipital bone, the cerebellum was removed to expose the dorsal surface of the medulla. Stimulation of the medulla was accomplished with a 33-G electrode-tubing fixed on an electrode carrier at an angle of 34° from the vertical axis of the stereotaxic frame. This electrode-tubing was made from a stainless tubing insulated except at the tip (0.3 mm). The other end was connected to a PE-50 tubing linked with a Hamilton syringe (10 μl). With such arrangement, the same locus of the medulla could be stimulated chemically or electrically. Chemical stimulation was accomplished with microinjection of 100 nl sodium glutamate (0.25 M, pH 7.4 in artificial cerebrospinal fluid) at each point. Electrical stimulation was accomplished with a 5-sec train of rectangular pulses of 100 μA, 20 Hz, and 0.5 ms, provided by a constant current through an isolation unit (Grass PSIU6, Braintree, MA, USA) connected with a Grass S88 stimulator (16). Pontamine blue sky (1% final concentration) was mixed in the glutamate solution or in the artificial cerebral spinal fluid in case of chemical stimulation or electrical stimulation, respectively, for identification of the stimulated points.

HRP Histochemistry

Application of HRP to the SPG. Six cats weighing 2.2 to 3.4 kg in either sex were anesthetized with α-chloralose (35 mg/kg, Sigma) and urethane (350 mg/kg) intraperitoneally. An incision of 1.5 to 2 cm was made over the supraorbital arch. Lidocaine, 4%, 0.05 to 0.1 ml, (Fujisawa Pharmac. Tokyo, Japan) was topically applied to the orbital cavity to paralyze the surrounding tissues and subsequently the eyeball was enucleated. Then all connective tissues from the orbital side of the frontal bone were removed until the sphenopalatine foramen on the palatine bone was exposed. The SPG was explored in the nearby adipose tissue by tracing the nerve trunk passing through the foramen. Locations of the SPG and other surrounding structures are illustrated in Fig. 1.

The capsule of the SPG was opened with a needle. By means of a forceps with fine tips, 4 to 10 μg crystals of HRP (Boehringer, Bracknell, UK) were directly applied at three to four sites in the middle part of the ganglion (36). Subsequently, a small piece (about the size of the SPG) of gelatin sponge, which had been soaked with 50% HRP in 0.05 M Tris · Cl buffer (pH 7.6), was put on the surface of this ganglion for one to two hours. Then the tissue around the operation field was rinsed with normal saline and the skin was sutured.

Demonstration of the HRP granules. Forty eight h (five animals) or 72 h (one animal) after the HRP treatment, the animal was reanesthetized intraperitoneally with pentobarbital, 50 mg/kg (Abbott). One thousand units of heparin was given intravenously, then the animal was perfused through the left ventricle with 2,000 ml normal saline followed by 2,000 ml mixed fixative of 3% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4, 4°C). After perfusion, the brain was taken out and transferred to fresh ice-cold fixative for 5 to 6 h. The brain was kept overnight at 4°C in 20% sucrose in phosphate buffer.

Continuous transverse sections at 40 μm thickness

from the cervical spinal cord (C_1) to the inferior coliculli of the brainstem were made on a freezing microtome 2800 Frigocut E (Reichert-Jung, Heidelberger, Germany). All sections were reacted with tetramethylbenzidine and subsequently stabilized with diaminobenzidine and $CoCl_2$ for the demonstration of black-colored HRP granules (30). Sections taken from every 80 μm were mounted onto gelatin-coated slides and counter stained with 0.3% neutral red. The rest of free-floating sections with positive HRP reaction were preserved for further immunohistochemical reactions.

Immunohistochemistry of ChAT, 5-HT, or SP

ChAT immunoreaction. The avidin-biotin-peroxidase complex (ABC) method was used for immunohistochemical visualization (12). The brain sections were treated with 0.4% Triton X-100 in 0.05 M Tris · Cl buffered saline (pH 7.4) with 30% normal goat serum (NGS, Vector) for 45 min, then incubated in the rat polyclonal anti-ChAT solution (diluted 1:1, 000 in Tris · Cl buffered saline with 1% NGS and 0.4% Triton X-100, Boehringer, Bracknell, UK) for 18 to 24 h at 4°C. After two rinses with Tris(Cl buffered saline, each for 5 min, the sections were incubated in a solution of goat-anti-rat-IgG (secondary antibody, Bioscience, San Jose, CA, USA) diluted 1:100 in the same anti-ChAT solution for 45 min at room temperature. Because the ChAT-immunostained cells appeared in brown color, they were differentiated from the cells colored with black HRP granules as described above.

5-HT or SP immunoreaction. The ABC method was also used for immunohistochemical visualization (12). The sections were treated with 0.4% Triton X-100 in phosphate buffered saline with 30% NGS for 45 min, then incubated in the rat polyclonal anti-5-HT, or anti-SP solution (diluted 1:20,000, 1:5,000 in phosphate buffered saline with 1% NGS and 0.4% Triton X-100, respectively) for 18 to 24 h at 4°C. The rest of procedures were the same as above.

Charting

The sections were examined with a light microscope (Leitz, Ladorlux D, Heerbrugg, Switzerland). The HRP-labeled soma with a clear nucleus was recorded. The distribution of these cell bodies and fibers was mapped by the aid of a HP X-Y plotter connected with a microscope. The total number of HRP-labeled soma was calculated as the sum in each section. The identification of brainstem structures was made according to Berman's atlas (1).

Results

Area Responsible for the Increase of CCA Blood Flow

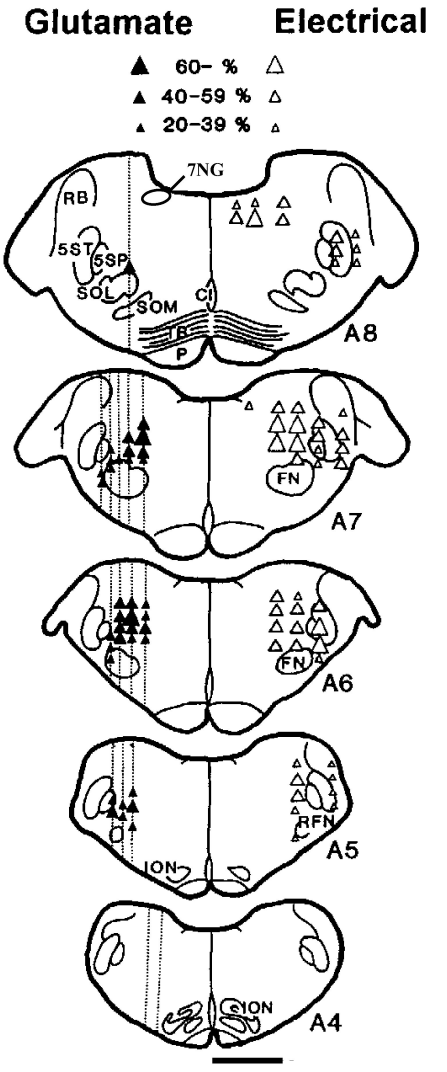

Fig. 2. Schematic drawings of brain stem sections show the points upon stimulation with either microinjection of sodium glutamate (Glu, 0.25 M, 100 nl) or electrical current (E, 100 μA, 20 Hz, 0.5 ms for 5 s) produced an increased blood flow in the common carotid artery (CCA). Calibration bar = 3 mm. Solid (left) and open (right) triangles of three different sizes indicate, respectively, the Glu- and E-induced increases of CCA blood flow from 20 to above 60% of the resting values. Dashed lines (...) indicate needle tracks for Glu-microinjection. Abbreviations: A8 to A4, 8 to 4 mm rostral to the obex; 5SP, alamina spinal trigeminal nucleus; 5ST, spinal trigeminal tract; CI, inferior central nucleus, i.e., raphe pallidus nucleus; FN, facial nucleus; RFN, retrofacial nucleus; 7NG, genu of the facial nerve; ION, inferior olivary nucleus; P, pyramidal tract; RB, restiform body; SOL, lateral nucleus of superior olive; SOM, medial nucleus of superior olive; TB, trapezoid body.

Glutamate was microinjected into 120 points on 15 tracks in the medulla of four cats, extending from the caudal pons to the middle medulla (8.5 to 4 mm rostral to the obex). Electrical stimulations were made to 260 points on 42 tracks in another four animals. Only the stimulated points that elicited more than 20% increase in the CCA blood flow are plotted in Fig 2.

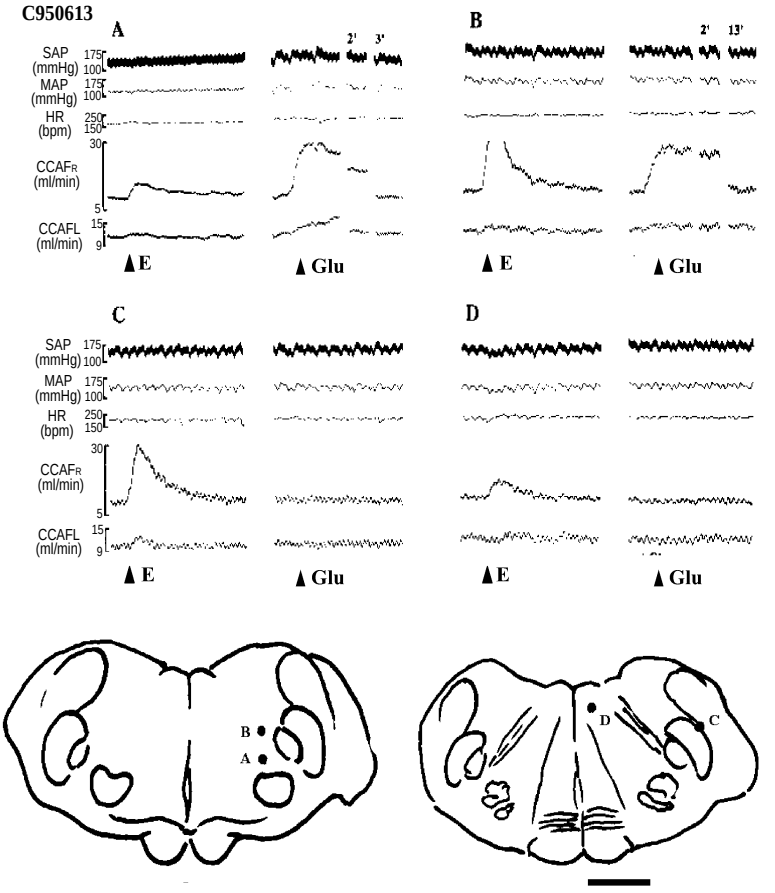

Fig. 3. Effects of electrical and sodium glutamate stimulations at different sites of the brainstem. Panels A, B, C, and D illustrate changes of systemic arterial pressure (SAP), mean systemic arterial pressure (MAP), heart rate (HR), right common carotid blood flow (CCAFR), and left common carotid blood flow (CCAFL) after electrical (E, 100 μA, 20 Hz, 0.5 ms for 5 sec) or sodium glutamate (Glu, 0.25 M, 100 nl) stimulation at points A, B, C, or D marked in the brain stem drawings. Both Glu- and E-stimulations of points A and B produced increases of the CCAFR. Points C and D, however, did not respond to Glu but responded to electrical stimulation to increase the CCAFR. The increase was particularly prominent at point C located in the dorsomedial area of the section. All abbreviations are the same as Fig. 2.

These points, in glutamate stimulation, were confined in a circumscribed area primarily in the parvocellular reticular nucleus (the lateral tegmental field) just dorsal to the facial nucleus, at 5.0 to 7.0 mm rostral to the obex, 2.8 to 4.0 mm lateral to the midline, and 2.5 to 3.5 mm ventral to the dorsal surface of the medulla (or 5.0 to 4.0 dorsal to the ventral surface of the medulla). The area was slightly smaller than that identified by electrical stimulations. Furthermore, points that only responded to electrical stimulation were found in the dorsomedial, dorsal, dorsolateral, and lateral portions of the medulla (Fig 2, A8-A5). This area extended from the genu of the facial nerve (7NG) to the spinal trigeminal tract, a band area that appeared to follow the course of the facial nerve tract at the caudal pontine level. One animal received electrical and chemical stimulations of the same sites. Both glutamate and electrical stimulations of points A and B were all able to increase CCA blood flow, while another two (C and D) responded to electrical stimulations only (Fig. 3).

Distribution of SPG Preganglionic Neurons in the Brainstem

Since there is no difference between 72 h (one animal) and 48 h (five animals) of HRP retrograde tracing studies, we pooled these data together. Fig. 4 maps the distribution of HRP-labeled neurons and fibers in one representative animal. The HRP-labeled neurons were morphologically multipolar or fusiform (Figs. 5 and 6). All HRP neurons were found ipsilaterally in the portions of the parvocellular reticular nucleus dorsal and lateral to caudal half of the superior olivary nucleus and rostral 3/4 of the facial nucleus (Fig. 4). For the convenience, distribution of the HRP-labeled neurons was grouped into five areas: 1) the lateral circumference of the superior olivary nucleus, 2) the dorsal circumference of the superior olivary nucleus, 3) the lateral circumference of the facial nucleus, 4) the dorsal circumference of the facial nucleus, and 5) the dorsal

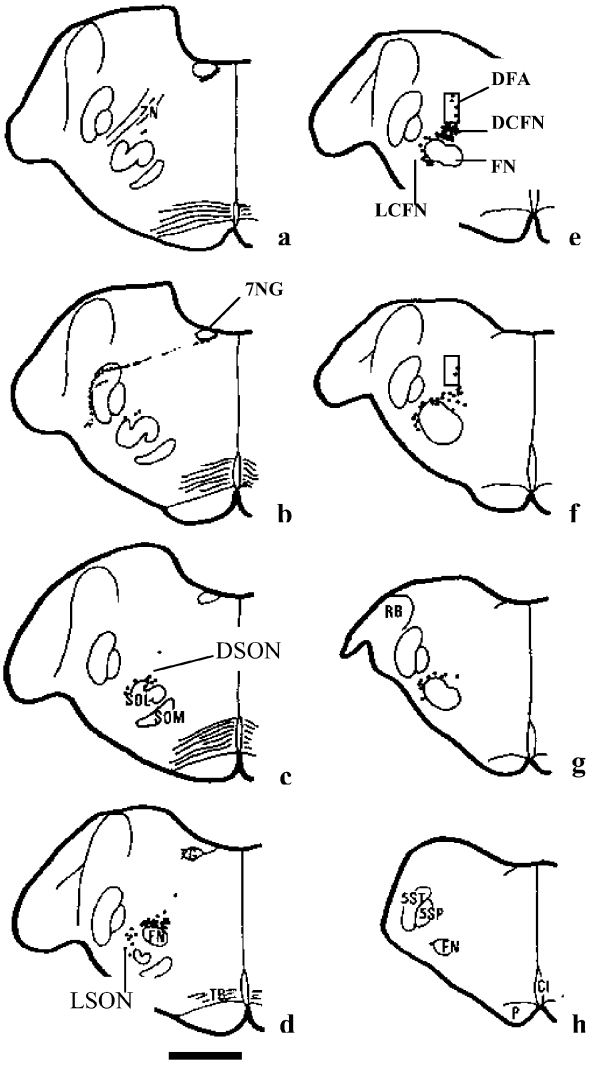

Fig. 4. Schematic drawings of brainstem sections (40 μm thickness) show the distribution of HRP-labeled neurons. HRP was applied to the left SPG of a cat for 48 h. Each large solid dot represents one HRP-labeled neuron. Fine dots on section b indicate HRP-labeled nerve fibers. Brain drawings from a to h indicate sections in a rostral-caudal sequence. Rectangles indicate the DFA. Calibration bar = 3 mm; DFA, dorsal facial area; DCFN, LCFN, dorsal and lateral circumference of FN; DSON and LSON, dorsal and lateral circumference of superior olivary nucleus; FN, facial nucleus; 7N, facial nerve; 7NG, genu of the facial nerve; TB, trapezoid body.

facial area (DFA) (16), a small area just dorsal to the dorsal circumference of the facial nucleus. On average, the total number of HRP neurons in each animal was 420±104 (mean±S.E., n=6). The percentage of HRP-neurons in each area was 0.5±0.1% (mean±S.E., n=6), 15.2±1.9, 23.7±0.9, 52.5±1.7, and 8.3±0.7, respectively. Although the total number of HRP neurons was different in each animal, the distributional pattern of these neurons in five areas was similar among six animals.

Fractions of bundle made up of HRP-labeled fine fibers ran from an area ventral to the genu of the facial nerve, passed through the dorsal and dorsolateral margins of the trigeminal spinal tract, and went ventrolaterally out of the medulla (Fig. 4a, b). This bundle appeared to run slightly caudal to the facial nerve tract (Fig. 4a, b). As glutamate activates soma and dendrites but not axons, findings from the responses of points C and D at A8.5 level (Fig. 3) that responded only to electrical stimulation illustrate the location of the HRP-labeled fibers.

Neurochemical Characteristics of the HRP Neurons

In study of combination of ChAT immuno-histochemical staining with HRP retrograde tracing, all HRP-labeled neurons appeared to be ChAT-IR (Figs. 5 and 6). This indicates that the preganglionic neurons projecting to SPG are cholinergic in nature. In the DFA, however, HRP-ChAT-IR neurons appeared less than ChAT-IR neurons (Fig. 5a). Nevertheless, all HRP-labeled soma were exclusively ChAT-IR, indicating the cholinergic nature of the DFA preganglionic neurons that project to the SPG.

The 5HT-IR (Fig. 7a) or SP-IR (Fig. 7b) terminals were also found sparsely but definitely around the HRP-labeled neurons (preganglionic neurons), including those in the DFA.

Discussion

The present studies substantiated the previous report by Kuo *et al*. (16) who first defined the DFA without studies on the functional and anatomical relations between the DFA and SPG. We delineated a detail map of the DFA responding to glutamate stimulation in increase in the CCA blood flow (Fig. 2). We found that the DFA had the HRP-labeled and ChAT-IR preganglionic neurons (Figs. 5, 6) projecting to the SPG, and the HRP-labeled but ChAT-IR-spared preganglionic neurons probably projecting to the otic ganglia. We demonstrated physiologically and anatomically the intra-medulla route of the preganglionic fibers projecting to the SPG. The DFA had sparsely but definitely 5HT- and SP-immunoreactive fibers abutting on the preganglionic neurons (Fig.7). Findings indicate that parasympathetic preganglionic neurons in the DFA project fibers to the SPG and possibly to the otic ganglion, are innervated by 5HT- and SP-like nerve terminals, and are responsible for regulation of the CCA blood flow.

The present experiment demonstrated that preganglionic neurons projecting to the SPG were distributed in five clusters (Fig. 4) including the DFA, supporting the other experiments (15, 28, 33,

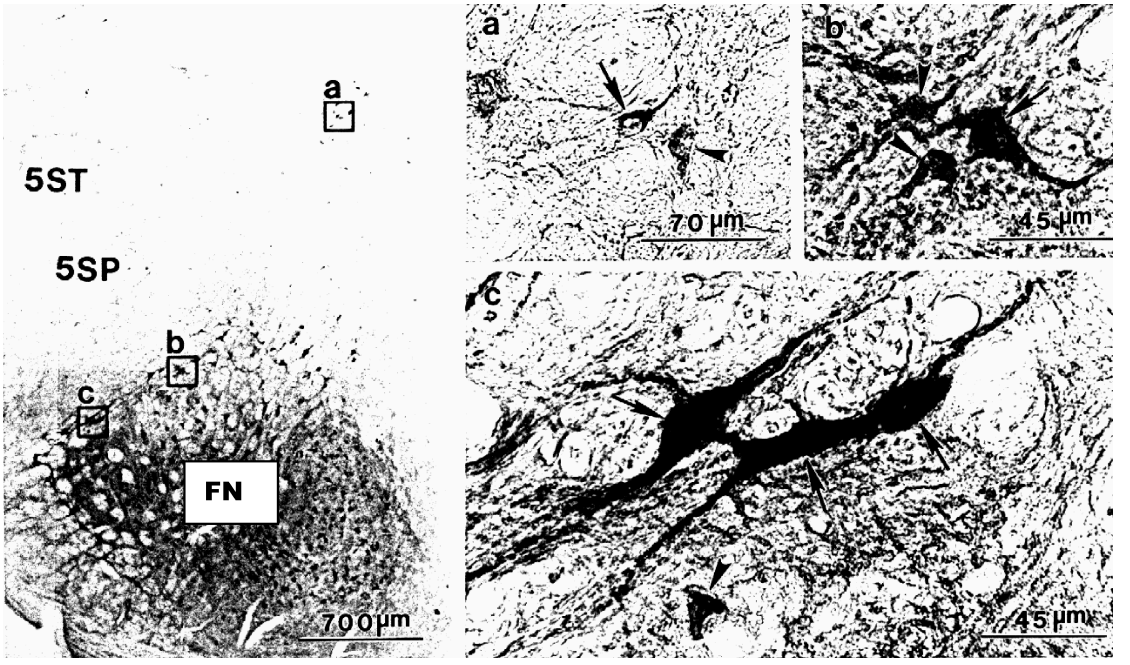

Fig. 5. Photomicrographs show ChAT-immunoreactive (ChAT-IR) and HRP-labeled cells on the histological section at the level of facial nucleus (7N). ChAT-immunostaining was combined with HRP-retrograde tracing. HRP was applied to the left SPG for 48 h. Photomicrographs a, b, and c on the right are the high power magnifications of the three outline squares a, b, and c marked in the low power magnification on the left side. The HRP-labeled neurons often appear in pairs around the lateral circumference of the facial nucleus (area c) but randomly in the dorsal circumference of the facial nucleus (area a and b). Tail-arrows, ChAT-IR and HRP-labeled cells; arrow heads, ChAT-IR cells. Abbreviations are the same as Fig. 2.

36). We, however, further found that the DFA was the only area capable of inducing increase of the CCA blood flow among the five clusters stimulated, and was only a small portion containing but 8.3±0.7% of total preganglionic neurons projecting to the SPG (Figs. 2, 3 and 4). These findings together with the findings that stimulation of the DFA induces the increase in the cerebral and the CCA blood flows (19) and that the SPG postganglionic fibers innervate the basilar artery (15), middle cerebral artery (10, 41) and intracranial segment of the internal carotid artery (38) indicate that the DFA is the preganglionic site responsible for regulation of these arteries. The functions of other four clusters may involve in regulation of the lachrymal glands, nasal and palatal membranes, because the postganglionic fibers arising from the SPG also innervate the lachrymal glands, and the nasal and palatal membranes (24, 29, 40).

Findings of the present as well as other experiments indicate that preganglionic fibers get out of the medulla through the parasympathetic divisions of both the facial and glossopharyngeal nerves and the preganglionic neurons overlap in the DFA. First, in our previous studies, the increase of the CCA blood flow upon stimulation of the DFA was completely abolished after both the facial and glossopharyngeal nerves were cut (16). Second, preganglionic neurons projecting to the chorda tympani and the otic ganglion which innervates the submandibular and sublingual glands (11, 26, 32), and parotid gland (4, 31), respectively, were similarly and exclusively located at the DFA. Third, the preganglionic neurons projecting to all parasympathetic divisions of the facial nerve, namely the SPG (15, 33, 36), chorda tympani (26, 31) and greater petrosal nerve (28), were located in the parvocellular reticular area at levels extending from the caudal half of the superior olivary nucleus to the rostral 3/4 of the facial nucleus. On the other hand, preganglionic neurons projecting to all parasympathetic components of the glossopharyngeal nerves, including the glossopharyngeal nerve root (31), the lesser petrosal nerve (27), or the otic ganglion (4), were distributed from the rostral pole of the facial nucleus to the area slightly caudal to the facial nucleus or the rostral levels of the solitary nucleus. Therefore, preganglionic neurons projecting to the parasympathetic division of the facial and glossopharyngeal nerves may overlap in the reticular area dorsal to the rostral 3/4 of the facial nucleus, most likely the DFA (Figs. 2 and 4). Fourth, in the present experiment, only portion of ChAT-IR neurons, namely the parasympathetic preganglionic neurons, are labeled with HRP in the DFA, indicating a projection to the SPG *via* the facial nerve. Others

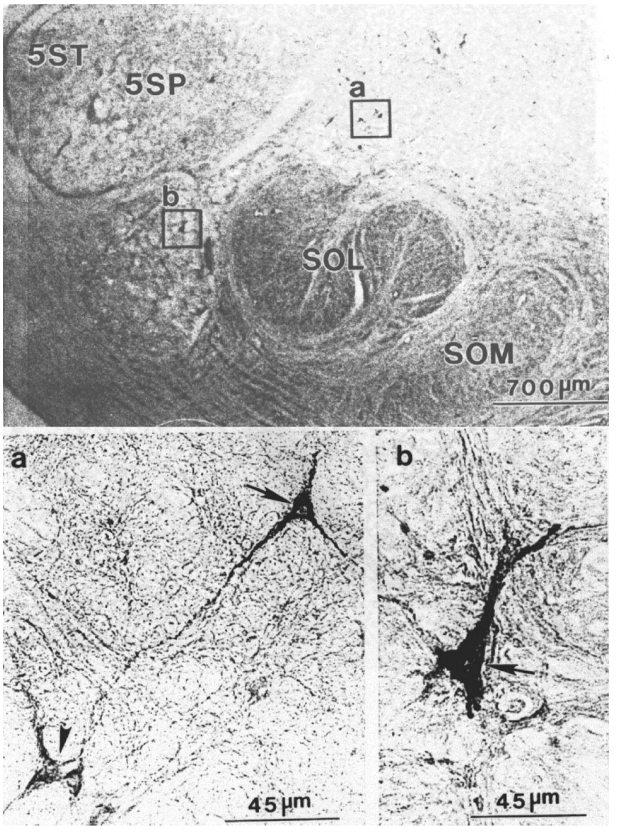

Fig. 6. Photomicrographs show ChAT-IR and HRP-labeled cells at the level of the superior olivary nucleus (SOL and SOM). ChAT-immunostaining was combined with HRP-retrograde tracing. HRP was applied to the left SPG for 48 h. Photographs a and b (bottom) are high power magnifications taken from the squares marked with a and b in low power magnification (top). Tail-arrows, ChAT-IR and HRP-labeled cells; arrow heads, ChAT-IR cells. All abbreviations are the same as in Fig. 2.

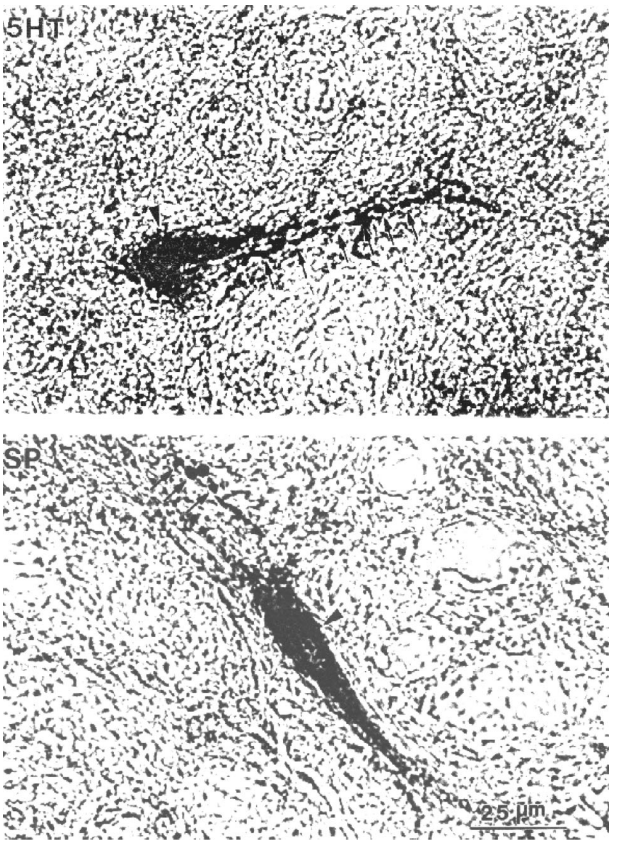

Fig. 7. Photomicrographs show the immunoreactive serotnin (5HT, top) or substance P (SP, bottom) terminals (tail-arrows) near by the HRP-labeled neuron (arrow heads). 5HT- or SP-immunostaining was combined with HRP-retrograde tracing. HRP was applied to the left SPG for 48 h.

spared from HRP-labeling may project to other parasympathetic ganglion (the otic ganglion) through the glossopharyngeal nerve. Some of the ChAT-IR but HRP-unlabeled neurons still might be the preganglionic neurons projecting to SPG that failed to take up HRP.

The intra-medulla route of the preganglionic fibers projecting to the SPG was physiologically and anatomically delineated in the present experiment. In studies by electrical stimulation that excites only soma but not fibers en passant, we found that the area that only responded to electrical current was in the dorsomedial, dorsal, dorsolateral, and lateral portions of the medulla (Fig. 2, A8-A5). These areas extended from the genu of the facial nerve (7NG) to the spinal trigeminal tract, a band area that follows the course of the facial nerve tract at the caudal pontine level. In consistent with this finding, fractions of bundle made up of HRP-labeled fine fibers were traced to run through the same area (Fig. 4a, b).

In the present experiment, the 5HT- or SP-IR nerves were traced abutting on the preganglionic neuron projecting to the SPG. The cell body of 5HT nerve that innervates the preganglionic neuron in the superior salivary nucleus is near by the raphe nucleus (23, 33). Those innervating in the DFA may be the same. The function of 5HT and glutamate has been disclosed in a series of experiment by placing the microdialysis probe in the DFA and analyzing changes in extracellular 5HT and glutamate concentrations with high performance liquid chromatograph technique (18, 20, 22). Findings indicate that both 5HT and glutamate nerves release their transmitters in tonic and result in decrease and increase in the CCA blood flow, respectively. 5HT can inhibit tonic release of glutamate *via* 5-HT_2 receptors (21), while glutamate can stimulate DFA *via* both NMDA and AMPA receptors (8). The origin of SP nerve and the function of SP of the DFA neurons have to be ascertained.

Based on the present and the finding in the

literature, the DFA can be considered as a parasympathetic nucleus that contains but a small portion of the preganglionic neurons projecting to the SPG and the otic ganglion, *via* the parasympathetic division of the facial and glossopharyngeal nerves, respectively; the preganglionic neurons are innervated by the SP- and 5HT-IR axons in the DFA and participate in regulation of the CCA blood flow and possibly the cerebral blood flow.

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