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Morphological distinction between vasodilator and secretomotor neurons in the pterygopalatine ganglion of the cat

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Abstract

We investigated whether vasodilator and secretomotor ganglion neurons are morphologically distinguishable from each other in the parasympathetic ganglion of the cat. When Cholera toxin B subunit, a retrograde tracer, was injected into the palatine gland, both large and small ganglion neurons were retrogradely labeled in the pterygopalatine ganglion. On the other hand, when the tracer was injected into gland-free areas (the upper gingiva or epidural space), all neurons labeled in the ganglion were small in size. Thus, it was assumed that small and large neurons labeled in the ganglion represented, respectively, secretomotor and vasomotor neurons. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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It has been considered that the peripheral parasympathetic vasodilative regulation and secretomotor regulation are independent of each other [4,14]. However, the data concerning morphological differences between vasodilator and secretomotor neurons in parasympathetic ganglia are scanty. Thus, in the present study, attempts were made to distinguish morphologically secretomotor neurons from vasodilator ones in the pterygopalatine ganglion (PPG) of the cat. Firstly, the cell size analysis of PPG neurons was performed. Secondly, a retrograde tracer was injected into the palatine gland or non-glandular area (upper gingiva or epidural space on the basal surface of the brain), and then cell size analysis of the labeled PPG cells was performed.

Animal care and the experiments were carried out in accordance with the guidelines of the Ethical Committee of The Institute of Laboratory Animal Science, Kagoshima University, Kagoshima, Japan. For cell size analysis, four cats weighing 2.0–3.5 kg were anesthetized with an intramuscular injection of ketamine hydrochloride (30–50 mg/kg) and an intraperitoneal injection of sodium pentobarbital (20–40 mg/kg). They were perfused transcardially with 10% buffered formalin. The PPGs of one side were removed and cut 25 μm-thick into serial longitudinal sections on a freezing microtome. The sections were mounted on slides and stained by the Holmes' method [10]. In alternative sections, projection drawings of all neuronal profiles with a nucleolus were prepared at 650× by using a microscope equipped with a camera lucida. The major diameter and the cross-sectional area of each neuronal profile with a nucleolus were measured on a computer by using the public domain NIH Image program software.

For the tracer study, 12 adult cats weighing 2.0–4.0 kg were used. The cats were anesthetized with an intramuscular injection of ketamine hydrochloride (30–50 mg/kg) and an intraperitoneal injection of sodium pentobarbital (20–40 mg/kg), and 20 μl of 0.25% Cholera toxin B subunit (CTB) solution was injected into the palatine gland, the upper gingiva on both sides or the epidural space on the basal surface of the brain; four cats were used in each experiment. The cats were allowed to survive for 3 days and then re-anesthetized. The anesthetized cats were perfused through the aorta with 800 ml of sodium phosphate-buffered physiological saline (pH 7.4), followed by 2000 ml of 4% buffered paraformaldehyde containing 0.2% picric acid. After the perfusion, the PPGs on both sides were removed and cut 40-μm thick into serial longitudinal sections on a freezing microtome. CTB was visualized immunohistochemically by using the peroxidase-antiperoxidase detection system, as described elsewhere

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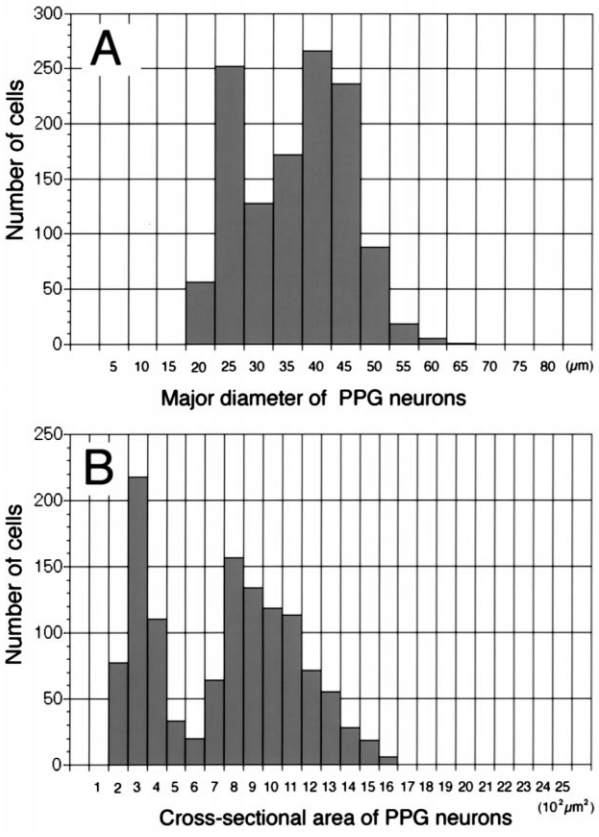

Fig. 1. Histograms showing the distribution of the major cell body diameters (B) and the cross-sectional areas (A) of PPG neurons that were stained by the Holmes' method.

[9]. The major diameter and cross-sectional area of CTB-labeled neurons were also measured in the same manner as in the PPGs that were stained by the Holmes' method.

In the Holmes' silver impregnation method, PPG neurons

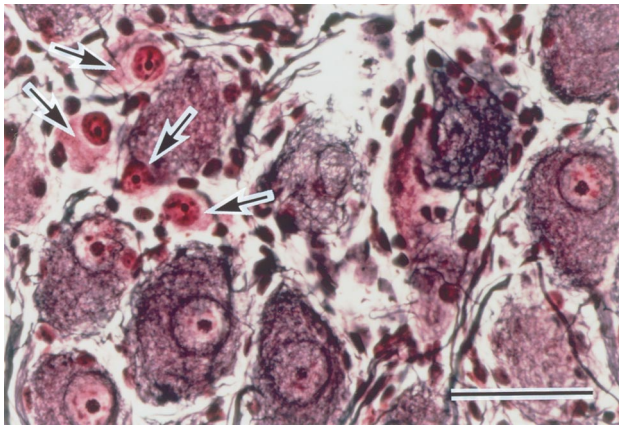

Fig. 2. Photomicrograph of a section through the PPG, stained by the Holmes' method. Arrows point to small neurons. Scale bar, 50 μm.

varied in size. In four PPGs from four cats, the major diameters and cross-sectional areas of neuronal cell bodies ranged from 18.6 to 66.0 (mean ± SD = 36.3 ± 9.1 μm) and from 177.8 to 1616.4 μm^2 (mean ± SD = 754.9 ± 368.6 μm^2), respectively ($n = 1222$). The histograms of the size distributions of the PPG cells displayed a pattern of distribution with two main peaks at 25 and 40 μm in major diameters and 300 and 800 μm^2 in cross-sectional areas (Fig. 1). The small and large PPG neurons showed striking differences in their morphological features in the Holmes' staining (Fig. 2). The small PPG neurons (about 40%) had dark red cytoplasm, a few thin processes and intensely stained eccentric nuclei (Fig. 2); the major diameters and the cross-sectional areas ranged from 18.6 to 38.3 μm (mean ± SD = 26.6 ± 3.8 μm) and from 177.8 to 691.0 μm^2 (mean ± SD = 334.6 ± 98.0 μm^2), respectively. On the other hand, the large PPG neurons (about 60%) had

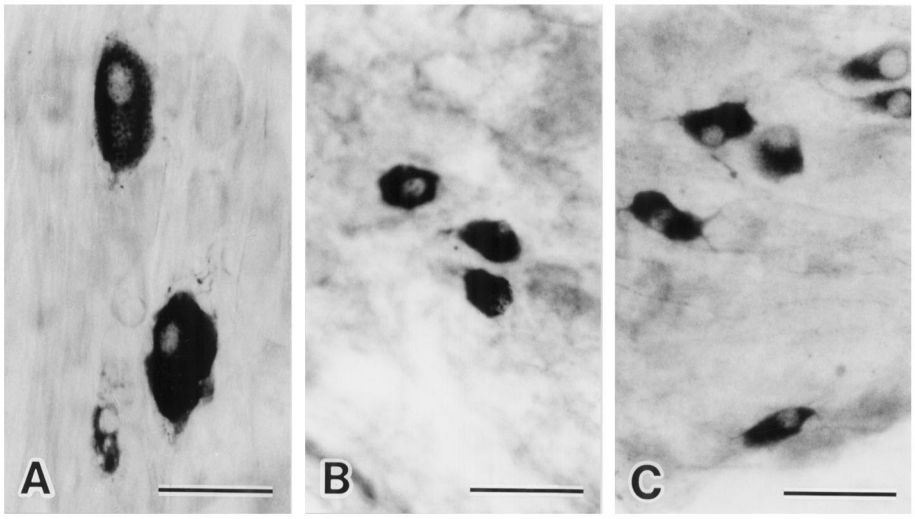

Fig. 3. Photomicrographs of sections through the PPG, after CTB injections into the palatine gland (A), into the upper gingiva (B) and into the epidural space on the basal surface of the brain (C). 40 μm-thick. Scale bar, 50 μm.

blackish cytoplasm with many neurofibrils, a few blackish thick processes, and lightly stained nuclei (Fig. 2); the major diameters and the cross-sectional areas of them ranged from 31.5 to 66.0 μm (mean ± SD = 42.0 ± 5.2 μm) and from 624.1 to 1616.4 μm^2 (mean ± SD = 1016.4 ± 215.5 μm^2), respectively.

In four cats injected with CTB into the palatine gland, many ganglion neurons were labeled in the PPG (Fig. 3A). In eight PPGs from four cats, the major diameters and the cross-sectional areas of CTB-labeled neuronal cell bodies ranged from 15.7 to 59.1 μm (mean ± SD = 34.7 ± 8.5 μm^2) and from 171.4 to 1490.4 (mean ± SD = 711.7 ± 296.0 μm^2), respectively ($n = 561$). When the tracer was injected into the upper gingiva or the epidural space on the basal surface of the brain, many retrogradely labeled ganglion cells were found in the PPG (Fig. 3B,C). In eight PPGs from four cats injected with CTB into the gingiva, the major diameters and the cross-sectional areas of CTB-labeled neuronal cell bodies ranged from 16.6 to 37.4 μm (mean ± SD = 26.2 ± 4.2 μm) and from 141.7 to 619.8 μm^2 (mean ± SD = 348.1 ± 88.8 μm^2), respectively ($n = 465$). In eight PPGs from four cats injected with CTB into the epidural space on the basal surface

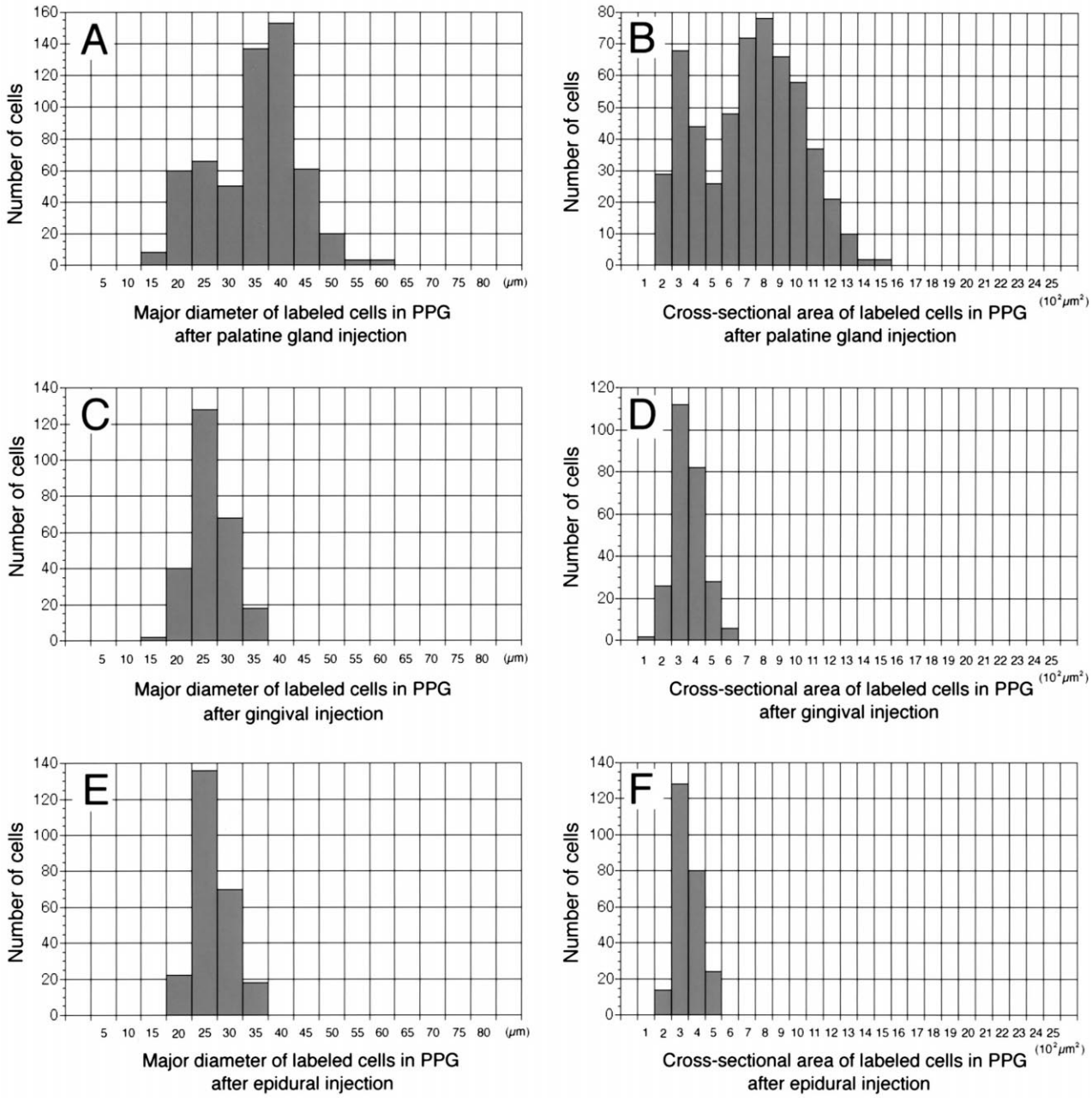

Fig. 4. Histograms showing the distribution of the major cell body diameters and the cross-sectional areas of labeled PPG neurons, after injections of CTB into the palatine gland (A,B), the upper gingiva (C,D) and the epidural space on the basal surface of the brain (E,F).

of the brain, the major diameters and the cross-sectional areas of labeled neuronal cell bodies ranged from 19.1 to 35.1 μm (mean ± SD = 26.6 ± 3.3 μm) and from 181.1 to 531.4 μm^2 (mean ± SD = 345.4 ± 70.1 μm^2), respectively ($n = 591$). In the latter two experiments, the CTB-labeled ganglion cells were morphologically similar. They were small, irregular in shape and had scant cytoplasm with eccentric nuclei (Fig. 3B,C). On the other hand, after palatine gland injections, ellipsoid or oblong ellipsoid ganglion neurons with abundant cytoplasm were labeled besides the above- described small neurons (Fig. 3A).

The histograms of the size distributions of the CTB-labeled ganglion cells after injections into the palatine gland displayed a similar pattern of those of the PPG neurons stained by the Homes' method (Fig. 4A,B). On the other hand, the histograms of the distributions of the major diameters and the cross-sectional areas of the CTB-labeled cells after injections into the upper gingiva and the epidural space displayed only one peak at 25 μm and at 300 μm^2, respectively (Fig. 4C–F). These peaks were in accord with those of the major diameter and the cross-sectional area of the small Holmes-stained neurons (Fig. 2A,B).

It is generally accepted that the PPG and the otic ganglion (OG) contain both vasomotor and secretomotor neurons. [2,3,11,12], and that they are independent of each other [4,14]. In the present study, striking differences in staining properties were observed between small and large PPG neurons in the Holmes' preparations. The results of the tracer experiments indicated that small PPG neurons innervated both gland and gland-free areas, but that large PPG neurons sent no axons to gland-free areas. Thus, it was assumed that labeled large and small PPG neurons represented, respectively, secretomotor and vasomotor neurons.

The mouse submandibular ganglion neurons and the rat OG neurons have been classified roughly into large and small neurons [5,13]. The cat OG and oromandibular ganglia were also reported to contain both large and small neurons [6–8]. It was suggested further that large and small neurons in the rat OG innervated the salivary gland and blood vessels separately [5]. Our preliminary experiments in the cat PPG and OG suggested that the gland free area of the tongue was innervated by small neurons, and that the lingual and lacrimal glands were supplied by both large and small neurons [1,8]. The present results were in good accordance with these previously reported ones.

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