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# Facial nerve parasympathetic preganglionic afferents to the accessory otic ganglia by way of the chorda tympani nerve in the cat

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**Abstract** The distribution of accessory otic ganglia and connections between the ganglia and the chorda tympani nerve were investigated in the cat in order to determine the parasympathetic preganglionic facial nerve afferents to the otic ganglia using whole mount acetylthiocholinesterase (WATChE) histochemistry. The otic ganglia consist of a sigle main prominent ganglion and many small accessory ganglia lying on a plexus around the origins of the branches of the mandibular nerve and near the junction of the chorda tympani nerve and lingual nerve. In cell analysis of Nissl-stained preparations, the neurons composing the accessory otic ganglia were morphologically similar to the main otic ganglion neurons. Connecting branches from the chorda tympani nerve to the peripherally located acccessory otic ganglia were found and they were not stained by WATChE histochemistry. WATChE-positive connecting branches from the ganglia to the inferior alveolar, lingual, and mylohyoid nerves were also found in the same preparations. The WATChE histochemistry on various autonomic nervous tissues revealed that autonomic postganglionic nerve fibers are selectively stained darkly and that preganglionic fibers remain unstained. Therefore, it is considered that the WATChE-negative connections from the chordra tympani nerve consist chiefly of autonomic preganglionic fibers, whereas the WATChE-positive connections to the branches of the mandibular nerve are mainly postganglionic fibers. This suggests that some of the facial nerve parasympathetic preganglionic fibers in the chorda tympani nerve are mediated in the accessory otic ganglia and then join the branches of the mandibular nerve to supply the target mandibular tissues.

**Key words** Glossopharyngeal nerve · Otic ganglion · Salivatory nucleus · Blood flow · Cranial nerve · Pterygopalatine ganglion

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## Introduction

Recent evidence indicates that the cat lower lip is innervated by parasympathetic vasodilator fibers of the facial nerve as well as the glossopharyngeal parasympathetic system (Izumi and Karita 1991). It has been speculated that the lower lip vasodilative fibers pass along the chorda tympani nerve and the lingual nerve, and/or the inferior alveolar nerve (Izumi and Karita 1991, 1993). In our previous anatomical study, however, we could find no morphological evidence for the direct lingual nerve projection to the lower lip or the existence of direct connections between the lingual nerve and inferior alveolar nerve (Kuchiiwa and Kuchiiwa 1996).

Microdissection of cat mandibular tissue blocks has revealed the existence of several prominent parasympathetic microganglia associated with the chorda tympani nerve or the lingual nerve, but a relationship between the ganglia and the vasodilator pathway has not been detected (Kuchiiwa and Kuchiiwa 1996). Hence, the location of the ganglionic way station of this pathway has been unclear. It has been hypothesized that both facial and glossopharyngeal preganglionic vasodilator fibers to the lower lip are mediated in the otic ganglion (Kuchiiwa et al. 1992; Izumi and Karita 1992; Kuchiiwa and Kuchiiwa 1996). Until recently, however, no anatomical evidence to support this hypothesis was available.

In newly developed whole-mount acetylthiocholinesterase (WATChE) histochemistry, small clusters of autonomic ganglion cells and fine bundles of autonomic fibers can now be detected in three dimensions in whole-

mount preparations under a dissecting microscope. In this histochemistry, postganglionic autonomic fibers are selectively stained darkly but preganglionic fibers remain unstained (Kuchiiwa 1990; Kuchiiwa and Kuchiiwa 1996). It is therefore to be expected that postganglionic roots formed mainly by autonomic postganglionic fibers will be discernible in whole-mount preparations under a dissecting microscope.

The purpose of this study was to investigate the trajectories of the parasympathetic facial nerve fibers innervating the mandibular tissues, and to demonstrate the existence of connections from the chorda tympani nerve to the otic ganglia in order to present anatomical evidence for the hypothesis that both facial and glossopharyngeal preganglionic vasodilator fibers to the lower lip are mediated in the otic ganglia. We also wished to clarify the distribution of the accessory otic ganglia.

## Materials and methods

Animal care and experiments were carried out under the responsibility of the Ethical Committee of The Institute of Laboratory Animal Science, Kagoshima University.

A total of nine adult cats (body weight=1.6–3.4 kg) were used. The cats were deeply anesthetized with an intramuscular injection of ketamine hydrochloride (30–50 mg/kg) and an intraperitoneal injection of Nembutal (sodium pentobarbital: 20–40 mg/kg), and perfused through the ascending aorta with sodium phosphate-buffered physiological saline (pH 7.4) followed by 10% buffered formalin.

### WATChE histochemistry

A preliminary dissection was performed on the left side of the facial tissue block to expose appropriate tissues around the ramification site of the mandibular nerve and the point of union of the chorda tympani nerve and the lingual nerve. Connective tissue was dissected without damage to the underlying fine nerves and adipose tissue was removed under a binocular dissecting microscope.

The tissue was treated by WATChE histochemistry (modified from Kuchiiwa and Kuchiiwa 1996) to facilitate detection of microganglia and fine autonomic nerve connections and to demonstrate the postganglionic nerve roots.

The WATChE histochemistry was as follows. The tissue was soaked in 400 ml of a 20% phosphate-buffered sodium sulfate solution (pH 7.4) at 40° C and depressurized in a vacuum chamber using a vacuum pump until the temperature of the solution cooled to 35° C. Then the solution was taken out of the vacuum chamber and the specimen was exposed to microwave irradiation until the solution was warmed up to 40° C, and then depressurized again to 35° C. This depressurization and irradiation was repeated for 3 h to facilitate penetration of the solution into the deeper parts of the tissue. The specimen was then incubated in 400 ml of 0.05 M acetate buffer solution (pH 5.0) containing 4 mM of acetylthiocholine iodide, 75 mM of glycine, and 15 mM of copper sulfate for 180 min at 35–40° C with the depressurization and irradiation procedure. It was then rinsed in distilled water in a vacuum chamber (over five changes of 30 min each) and washed at 4° C over night. The tissue was transferred to 1.25% sodium sulfide solution (three changes of at least 30 min each), and again rinsed in distilled water (at least five changes of 15 min each, in the vacuum chamber at room temperature and washed in a refrigerator at 4° C overnight. The specimen was then placed in a solution of 1% silver nitrate for about 5–15 min at room temperature in the vacuum chamber depending upon the degree of staining desired. It was subsequently rinsed in distilled water (at least three changes of 10 min each) and treated in 1% sodium thiosulfate (three changes of 30 min each) in the vacuum chamber. Finally the preparation was thoroughly rinsed in distilled water (at least five changes) in the vacuum chamber. The stained preparation was stored in 0.2% phosphate buffered (pH 7.4) sodium azide solution at 4° C.

The selectively stained preparations were dissected under a binocular dissecting microscope with special attention to the distribution of the main and accessory otic ganglia, connections between the ganglia and the chorda tympani nerve, and the branches of the mandibular nerve. When necessary, fine nerves and microganglia were dissected out, dehydrated in graded alcohol, cleared in xylene, mounted on slides, and observed with a light microscope. Larger parts of the tissue were mounted on slides with 30–40% polyvinylpyrrolidone (Sigma) dissolved in 30% acetic acid without dehydration (Gienc 1977), and observed with a light microscope and a dissecting microscope.

### Cell analysis

Main and accessory otic ganglia were removed from the right side of the facial tissue block in three cats. For cell-size analysis, serial 40-μm-thick sections were cut on a freezing microtome, mounted on slides, stained with thionine and coverslipped. All the cells that were present in an individual alternative section that contained a nucleolus were drawn at ×400, using a microscope equipped with a drawing tube. The long and short axes and the cross sectional area of each neuron drawing were measured on a Power Macintosh 8500/180 using a public domain NIH Image program.

## Results

In the WATChE-preparations the following were stained intensely: the superior cervical ganglion, the postganglionic branches of the superior cervical ganglion, the main and the accessory pterygopalatine ganglia, most peripheral branches derived from the pterygopalatine ganglion, the main and the accessory submandibular ganglia, the postganglionic branches from these ganglia in the submandibular gland, the microganglia in the sphenotympanic fissure (sphenotympanic ganglia), the oromandibular ganglion (see Kuchiiwa and Kuchiiwa 1996), fine peripheral branches from the oromandibular ganglion, the perilingual ganglia (see Kuchiiwa and Kuchiiwa 1996), and the lingual ganglia. The following remained unstained: the optic nerve, the trunk of the oculomotor nerve, the trochlear nerve, the trigeminal ganglion, the root of the abducens nerve, the trunk of the facial nerve, the genicular ganglion, the vestigulocochlear nerve, vestibular ganglion, the superior and inferior glossopharyngeal ganglion, the superior and inferior vagal ganglion, and the accessory nerve.

The main and the accessory ciliary ganglion and the short ciliary nerves were stained darkly, but the inferior branch of the oculomotor nerve contained no stained fibers. The greater petrosal nerve was observed to be unstained, but the deeper petrosal nerve was stained intensely; the Vidian nerve to the pterygopalatine ganglion showed a striped pattern of stained and unstained fibers. In the tympanic plexus, the tympanic nerve was found to be unstained, but the rest of the nerve network from the superior cervical ganglion was stained darkly. The lesser petrosal nerve central to the sphenotympanic ganglia was found to be unstained, but that peripheral to the ganglia contained a few intensely stained WATChE-positive fibers.

Considered together, these findings showed that the nerves consisting chiefly of the postganglionic autonomic fibers were stained intensely but that those consisting chiefly of preganglionic, sensory and/or motor fibers remained unstained (Fig. 1A).

In the mandibular tissue, a single darkly stained main otic ganglion was observed on the lateral surface of the origin of the mandibular nerve (Fig. 1). It was fusiform or oval in shape and measured approximately 1.5–2.6 mm in long diameter and 0.7–1.2 mm in short diameter ($n$=9). The lesser petrosal nerve was observed to be divided into several to over ten branches near the main otic ganglion, and some of them were found to join the main otic ganglion. The main ganglion was discernible from the accessory otic ganglia because it was always the largest.

Many (15–28: $n$=9) intensely stained accessory otic ganglia were found around the main otic ganglion, the trunk of the mandibular nerve, and the origins of the branches of the mandibular nerve. Most accessory ganglia were located within 6 mm peripheral to the main otic ganglion. Mostly peripherally located accessory ganglia were found in the vicinity of the union of the chorda tympani nerve and the lingual nerve. On the other hand, only a few accessory ganglia were found on the lesser petrosal nerve just proximal to the main otic ganglion, located within 2 mm centrally. Each accessory otic ganglion was connected with WATChE-positive fine nerves and a nerve plexus around the branches of the mandibular nerve (Fig. 2).

Generally, accessory ganglia were very small; most of them were 0.1–1.0 mm in long diameter. On rare occasions, larger polygonal, horseshoe-shaped or stelliform ganglia, over 1.0 mm in long diameter, were found, and they were considered to be unions of several accessory otic ganglia. Occasionally, a single neuron or clusters of neurons were observed on the network of the postganglionic nerve branches from the main otic ganglion.

The accessory ganglion neurons were generally oval in shape and closely resembled neurons of the main otic ganglion (Fig. 3). The component cells of the both ganglia varied in size from small to large, as shown in the histograms in Fig. 4. Quantitative analysis of the dimensions of the neuronal somata observed in Nissl-stained sections indicated that the accessory otic ganglia consisted of groups of neurons that had the same range of cell sizes as neurons in the main otic ganglion. The neurons in the accessory otic ganglia had a mean cross-sectional area of 1029.4 μm$^2$ (Fig. 4A; SD=500.6 μm$^2$, range=216.3–2512.0 μm$^2$, $n$=691), with a mean long axis of 42.8 μm (Fig. 4B; SD=11.8 μm; range=19.3–77.1 μm). The main otic ganglion neurons had a mean cross sectional area of 1001.1 μm$^2$ (Fig. 4C; SD=492.8 μm$^2$, range=197.3–2551.7 μm$^2$, $n$=915), with a mean long axis of 41.2 μm (Fig. 4D; SD=11.5 μm, range=18.3–73.1). No distinction could be made between the accessory otic ganglia and the main otic ganglion on the basis of their component cells.

The chorda tympani nerve was observed to be unstained except for WATChE-positive microganglion neurons (chorda tympani ganglion; Fig. 1A) and the small bundles of axons on the surface of the nerve (Gibbins et al. 1984). In seven of the nine cases, two or three WATChE-negative fine nerves separated from the chorda tympani nerve were found to enter peripheral accessory otic ganglia (Fig. 1B, arrows). Several to over ten darkly stained postganglionic fine nerves derived from the accessory ganglia were also found to join the inferior alveolar, lingual or mylohyoid nerve. The former un-

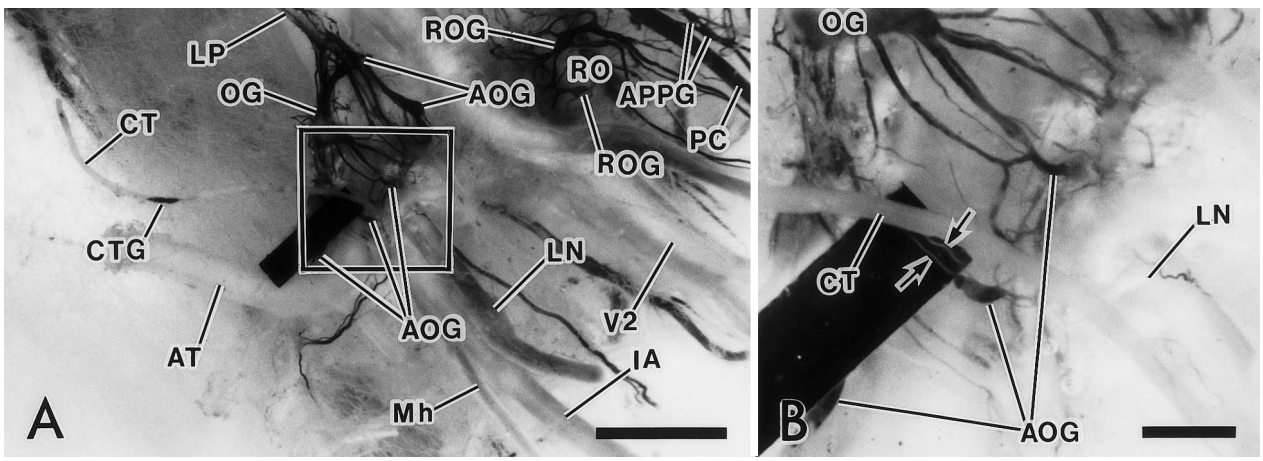


**Fig. 1** **A** Photomicrograph of the whole-mount acetylthiocholinesterase (WATChE) preparation showing the selectively stained postganglionic nerves in the submandibular and infraorbital regions of the cat. The main (*OG*) and accessory otic (*AOG*) ganglia, retroorbital plexus (*RO*), retroorbital ganglia (*ROG*), chorda tympani ganglion (*CTG*) and the autonomic postganglionic nerves are stained darkly, but the chorda tympani nerve (*CT*), lesser petrosal nerve (*LP*) and most branches of the submandibular nerve and the maxillary nerve (*V2*) remain unstained. A black film plate is inserted under the chorda tympani nerve in order to show the connecting branches between the chorda tympani nerve and the accessory otic ganglion (*APPG* accessory pterygopalatine ganglia, *AT* auriculotemporal nerve, *IA* inferior alveolar nerve, *LN* lingual nerve, *Mh* mylohyoid nerve, *PC* Vidian nerve). The rectangular frame indicates position of the magnified photomicrograph (**B**). *Bar* 5 mm. **B** Photomicrograph showing the WATChE-negative connecting branches from the chorda tympani nerve to the peripheral accessory otic ganglion (*arrows*). *Bar* 1 mm

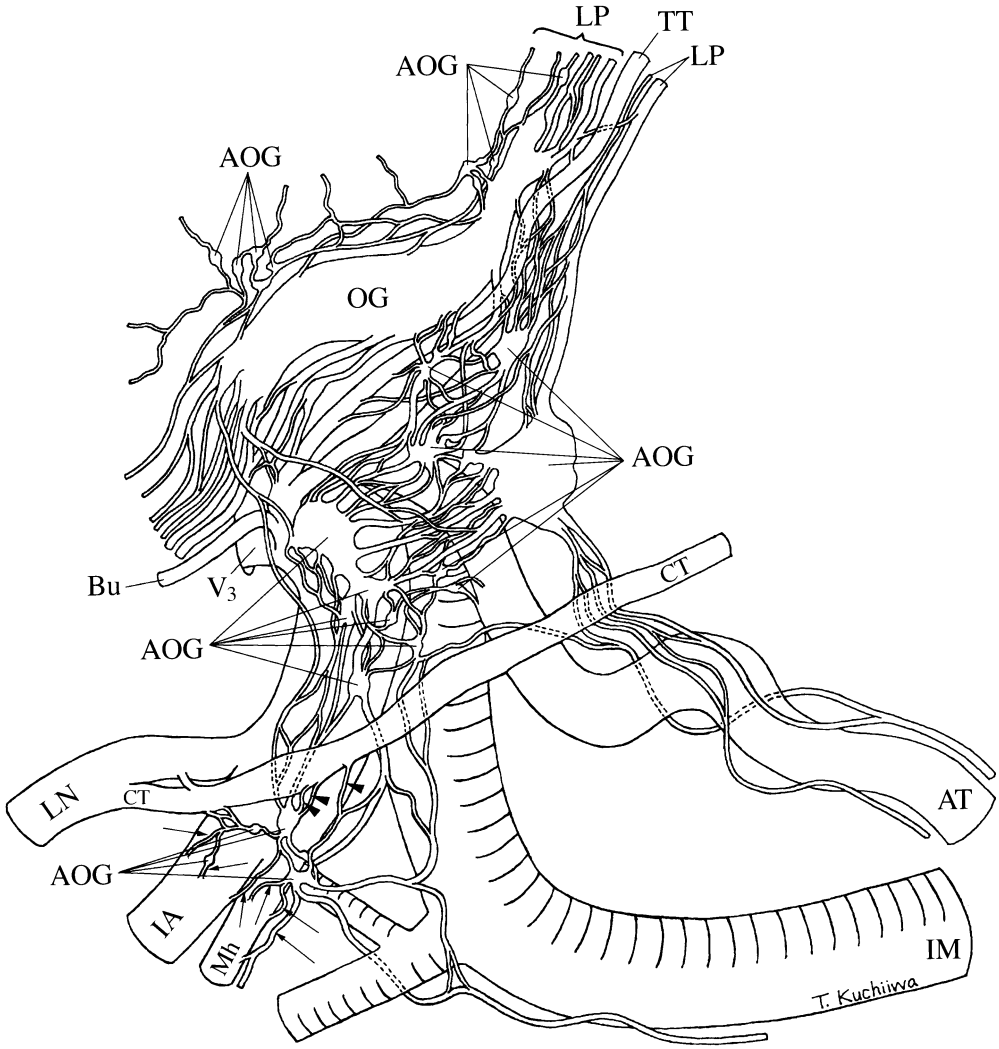


**Fig. 2** A sketch of the WATChE preparation showing the distribution of the main and accessory otic ganglia and connections between the ganglia and the chorda tympani nerve and the branches of the mandibular nerve. *Arrowheads* indicate the WATChE-negative connections from the chorda tympani nerve to the accessory otic ganglia. *Long arrows* indicate the WATChE-positive connections from the accessory otic ganglia to the inferior alveolar and mylohyoid nerves. Left submandibular tissue: ventral view with the central side to the top ($V_3$ mandibular nerve, *Bu* buccal nerve, *IM* internal maxillary artery, *TT* nerve of tensor tympani)

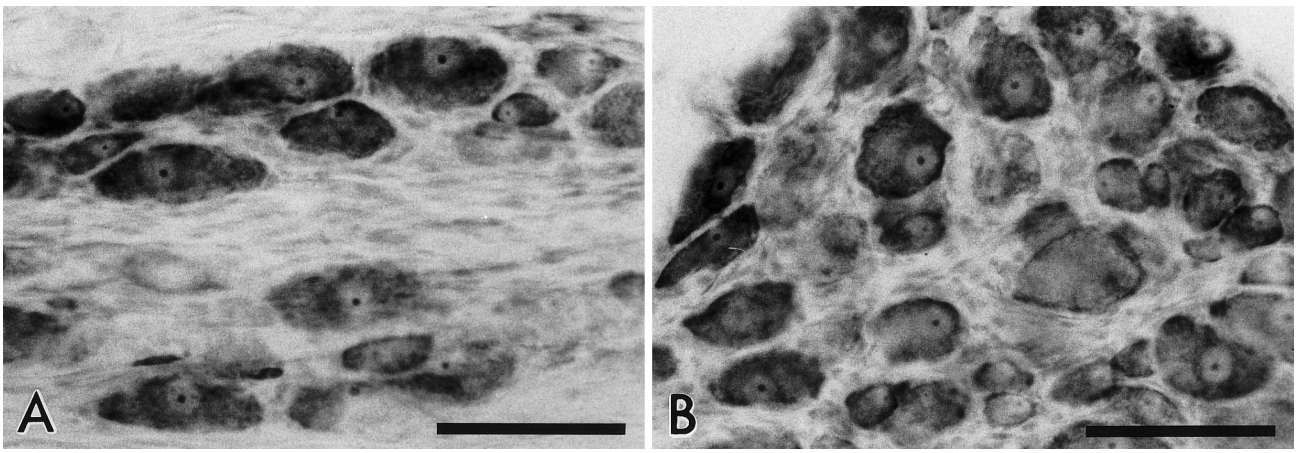


**Fig. 3** Photomicrograph of Nissl-stained sections of the accessory otic ganglion (**A**) and the main otic ganglion (**B**) showing that neurons in the accessory otic ganglia morphologically resemble those in the main otic ganglion. Stained with thionine, 40-µm-thick frozen section. *Bars* 100 µm

stained connections between the chorda tympani nerve and the accessory olic ganglia were usually so fine that they were difficult to detect in the unstained connective tissue. It is possible that the connective branches had been cut during the microdissection procedure in the two cases in which the connections were not detected.

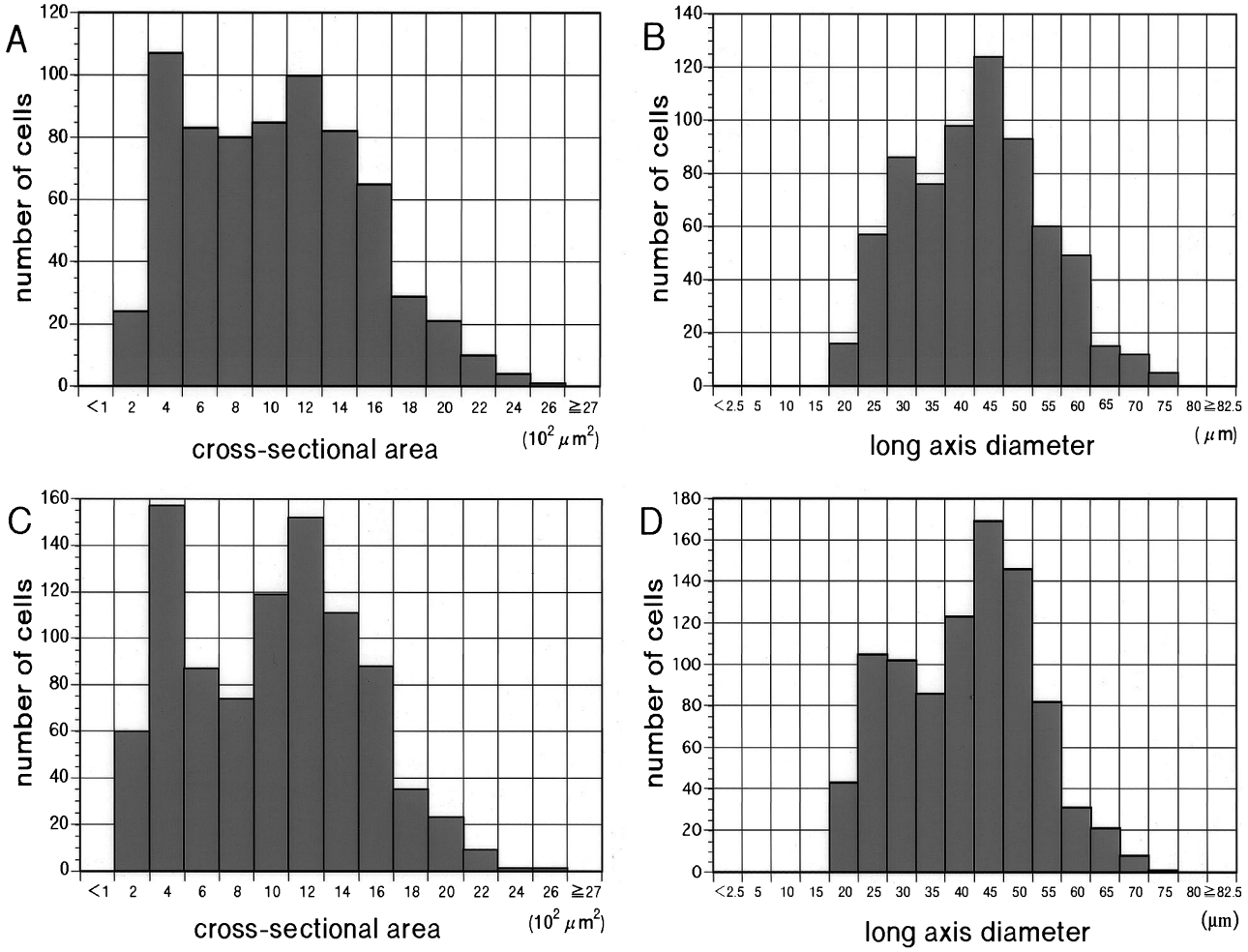


**Fig. 4** Histograms showing the distribution of somatic cross sectional areas (**A**) and long axis diameter of neurons in the accessory otic ganglia (**B**); and somatic cross sectional areas (**C**) and long axis diameter of the neurons in the main otic ganglion (**D**) drawn from 40-μm-thick Nissl-stained preparations. Note that the accessory otic ganglia consist of neurons that have the same morphological characteristics and the same range of cell size as those in the main otic ganglion

## Discussion

In the microdissection study of the WATChE-preparations, the main otic ganglion of the cat was distinguishable from the accessory otic ganglia through its size. In the cell analysis of the Nissl-stained preparations, however, the neurons composing the accessory otic ganglia were morphologically similar to neurons of the main otic ganglion. These findings agree with our histochemical and immunohistochemical experiments for neurotransmitters including nicotinamide adenine dinucleotide phosphate-diaphorase, vasoactive intestinal polypeptide, calcitonin gene related peptide, neuropeptide Y and substance P in both ganglia (unpublished). We conclude that the accessory otic ganglia consist of ganglion neurons displaced from the main otic ganglion, i.e., that the accessory otic ganglia form a part of the otic ganglionic system.

The present WATChE-preparations revealed that autonomic ganglia and nerves consisting chiefly of the postganglionic fibers were selectively stained darkly, but nerves consisting mainly of the preganglionic, sensory and/or motor fibers remained unstained, indicating that the postganglionic roots are discernible in the WATChE-preparations under a dissecting microscope.

The chorda tympani nerve was found to be WATChE-negative. This agrees with the view that the chorda tympani nerve consists chiefly of taste fibers from the tongue and parasympathetic preganglionic facial nerve fibers originating in the superior salivatory nucleus. The connections between the chorda tympani nerve and the accessory otic ganglia were found to be WATChE-negative; on the other hand, the connections from the accessory otic ganglia to the inferior alveolar, lingual and mylohyoid nerve were stained intensely, indicating that the former connections chiefly consist of autonomic preganglionic fibers and that the latter are mainly postganglionic. Because it is considered on the basis of staining intensity that the latter connections contain few if any sensory fibers, it is conceivable that the former also contain few or no sensory fibers of passage. This suggests that some of the preganglionic facial nerve fibers in the chorda tympani nerve are mediated in the accessory otic ganglia, and then pass to the mandibular

target tissues by way of the inferior alveolar, lingual or mylohyoid nerve.

It has been reported that electrical stimulation of the peripheral cut end of the facial trunk or the chorda tympani nerve elicits vasodilatation response in the lower lip (Izumi and Karita 1991, 1993). This indicates that the blood vessels in this portion are innervated by the parasympathetic facial nerve fibers as well as the glossopharyngeal nerve. Since the response was significantly reduced by pretreatment with hexamethonium, an autonomic ganglionic blocker, it is conceivable that the response is parasympathetic vasodilatation, but not the antidromic vasodilatation mediated by the sensory nerves (Izumi and Karita 1993). The response is never affected by lesion of the ipsilateral pterygopalatine ganglion (Izumi and Karita 1992), and no labeled neurons were observed in the pterygopalatine ganglion or in its accessory microganglia after tracer injections into the lower lip (Kuchiiwa et al. 1992; Kuchiiwa and Kuchiiwa 1996), indicating that the response is not mediated in the pterygopalatine ganglion. The vasodilative response was abolished by sectioning the inferior alveolar nerve (Izumi and Karita 1993). Hence, it is probable that the parasympathetic facial nerve vasodilator fibers passing to the lower lip pass through the chorda tympani nerve and the inferior alveolar nerve by way of the connective branches between the chorda tympani nerve and the accessory otic ganglia observed in the present study.

As mentioned above, the hypothesis that some preganglionic facial nerve fibers synapse in the otic ganglia has been presented in the previous reports (Kuchiiwa et al. 1992; Izumi and Karita 1992; Kuchiiwa and Kuchiiwa 1996). The present study provides the first significant anatomical evidence of this.

No other study has been reported on the preganglionic facial nerve termination in the otic ganglia in other mammalian species. In man, however, a communicating branch from the greater petrosal nerve to the lesser petrosal nerve was observed consistently in cadavers (Vidić and Young 1967). This raises the possibility that the preganglionic facial nerve afferents to the otic ganglia pass through this route in man.

It has been generally accepted that the superior salivatory nucleus sends preganglionic axons to the facial nerve and that the inferior salivatory nucleus sends preganglionic axons to the glossopharyngeal nerve, and that the two nuclei are independent of each other. However, the area of distribution of the preganglionic neurons sending their axons to the glossopharyngeal nerve considerably overlaps that of neurons sending their axons to the chorda tympani nerve (Nomura and Mizuno 1982). Moreover, the preganglionic neurons of the otic ganglia are cytologically indistinguishable from those of the ganglia associated with the facial nerve (Contreras et al. 1980). Histochemical and immunohistochemical experiments show that most neurotransmitters in the parasympathetic ganglia associated with the facial nerve are common with those associated with the glossopharyngeal nerve (Kuchiiwa et al. 1994). The present investigation suggests that some of the preganglionic facial nerve fibers in the chorda tympani nerve are mediated in the accessory otic ganglia as well as the glossopharyngeal preganglionic fibers. Therefore, there is a possibility that the superior and inferior salivatory nuclei form an inseparable nucleus, and that the nucleus sends vasodilator preganglionic fibers subserving the mandibular blood vessels to two different pathways, the facial nerve and the glossopharyngeal nerve, and that both pathways are mediated in the accessory otic ganglia observed in the present study.

The present study has revealed many accessory otic ganglia and complex networks of parasympathetic plexus near the junction of the chorda tympani nerve and the lingual nerve, and around the branches of the mandibular nerve. We conclude that great care must be taken in electrophysiological experiments that employ electrical stimulation of the nerves in the mandibular tissue, especially the lingual and chorda tympani nerves near the chorda tympani-lingual nerve junction.

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