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Cutaneous distribution of sympathetic postganglionic fibers from stellate ganglion: A retrograde axonal tracing study using wheat germ agglutinin conjugated with horseradish peroxidase

TAEKO TANIGUCHI, MASATOSHI MORIMOTO, YOSHIO TANIGUCHI, MITSUHIRO TAKASAKI, and TADAHIDE TOTOKI

Department of Anesthesiology, Saga Medical School, 5-1-1 Nabeshima, Saga, 849 Japan

Abstract: Sympathetic postganglionic cells of the stellate ganglion (SG) projecting to the skin in young dogs were labeled retrogradely with wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP). After application of WGA-HRP into the dermis and/or subcutaneous tissue of the upper extremity, numerous labeled cells were observed in the SG. The sympathetic postganglionic fibers from the SG were distributed in the skin area between the third cervical vertebra and the level of the thirteenth rib. These fibers decreased in number toward the rostral and caudal skin area with a peak on the upper extremity. Sympathetic postganglionic cells in the SG projecting to these skin areas were found ipsilaterally to the injection side. No labeled cells were observed in the SG after injecting WGA-HRP into the epidermis and/or subcutaneous tissue of the face, the level of the second cervical vertebra, and the lower extremity.

Sympathetic postganglionic neurons of the SG which project in the cervical and thoracic sympathetic trunks were labeled retrogradely with unconjugated horseradish peroxidase (HRP) applied to the cut end of the sympathetic trunks proximal to the SG. The application was made in the cervical sympathetic trunk (CST) caudal to the superior cervical ganglion (SCG). In the thoracic sympathetic trunk (TST), the application was made between the fourth and fifth thoracic sympathetic ganglia (T4 and T5), and between T10 and T11. Labeled cell bodies were observed in SG bilaterally.

Key words: Stellate ganglion block—Stellate ganglion—Postganglionic fibers—Cutaneous distribution—Wheat germ agglutinin conjugated horseradish peroxidase—Unconjugated horseradish peroxidase

Introduction

Stellate ganglion block (SGB) is one of the most widely used and highly effective diagnostic and therapeutic procedures for the management of certain acute and chronic pain syndromes or other disorders of the upper limbs, thoracic viscera, head, face, and neck. This procedure can be accomplished by the interruption of sympathetic pathways to these areas and also interruption of the afferent (sensory or nociceptive) fibers supplying these organs [1–4]. The success of this block is indicated by the development within a few minutes of the signs of Bernard-Horner syndrome, characterized by myosis and ptosis of the upper eye-lid with narrowing of the palpebral fissure. Dryness and warmth of the hand, arm, and side of the face may be noted. The sympatholytic effects by SGB have been studied by various methods (sweating tests, thermocouples, skin resistance tests, oscillometric readings, and the psychogalvanic reflex test) [3]. Recently, thermography has been used to test the effectiveness of SGB in head, face, forearm, and hand [5–11]. SGB is usually performed in patients with skin lesions above the third or fourth thoracic (T3 or T4) spinal segment, but it is still uncertain to which spinal segment the sympatholytic effects of SGB extend.

In the present studies, retrograde axonal transport of wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) was employed to determine the distribution of sympathetic nerve fibers from the stellate ganglion (SG) on the skin area in young dogs. Unconjugated horseradish peroxidase (HRP) was also used to determine whether the postganglionic fibers from the SG run rostrally in the cervical sympathetic trunk (CST) and caudally in the thoracic sympathetic trunk (TST).

Materials and methods

Twenty-two young nongrel dogs (supplied from Yamato Animal Center, Kumamoto) over 2 months of age of both sexes weighing 1.5–3 kg were used for this study. The animals were fully anesthetized by intramuscular injection of ketamine (25 mg·kg^{-1} body weight)

Address correspondence to: T. Taniguchi
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and xylazine (3 mg·kg^{-1}) throughout the experimental procedure.

Application to the skin (n = *19*)

A 5–10 μl aliquot of 3–4% WGA-HRP solution was injected into the dermis and/or subcutaneous tissue of the following areas that contact the part of bone described below using a microsyringe (Fig. 1). All points were obtained from different dogs.

Face
F-1; frontal bone (orbital part)
F-2; maxilla (upper lip)
F-3; mandible (lower lip)
Neck
N-1; atlas
N-2; transverse process of the fifth cervical vertebra

Forelimb
N-3; scapula (acromion)
N-4; 5th digital pad
Chest wall
T-1; middle part of the fourth rib
T-2; middle part of the seventh rib
T-3; distal part of the seventh rib
T-4; middle part of the tenth rib
T-5; proximal part of the 13th rib
Abdominal wall
A-1; 1.5 cm caudal from the distal part of the 13th rib
A-2; 3 cm caudal from the distal part of the 13th rib
A-3; 4.5 cm ventral from the distal part of the 13th rib
Navel and hindlimb
H-1; navel

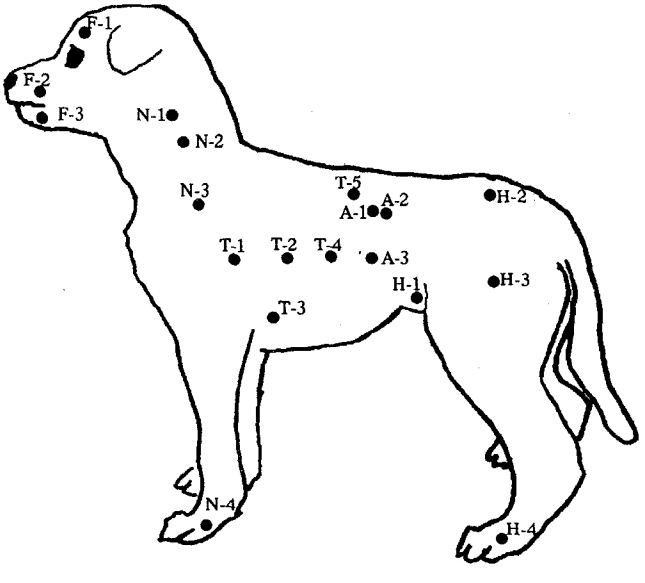


Fig. 1. Drawing of the lateral surface of the dog showing the locations of the injection sites

H-2; crest of ilium
H-3; middle part of the femoral bone
H-4; 5th digital pad

Application to the cut end of the sympathetic trunk (n = *3*)

After the unilateral CST was exposed by blunt dissection of the neck tissues and cut between the superior cervical sympathetic ganglion (SCG) and the middle cervical sympathetic ganglion (MCG), crystalline HRP was applied to the cut end proximal to the SG. Care was taken to avoid leakage of the tracer. Similary, TST was exposed and cut between either the fourth and fifth thoracic ganglia or the tenth and 11th thoracic ganglia. Crystalline HRP was applied to the cut end proximal to the SG. At the end of these procedures, the incision was closed in layers.

After survival for 24–72 h, animals were injected 1000 IU of heparin intravenously under anesthesia as previously described. They were perfused through the left ventricle with 500 ml of normal saline followed by 4000 ml of 0.1 M phosphate buffer containing 2.5% formalin and 1.25% glutaraldehyde (pH 7.4) and 400 ml of the phosphate buffer containing 5% sucrose at room temperature.

After perfusion, bilateral SCGs, MCGs, SGs and paravertebral ganglia were dissected. These ganglia were stored in the phosphate buffer containing 30% sucrose (pH 7.4 at 4°C) for several days. Serial 50-μm horizontal sections were cut using a cryostat and were mounted on gelatinized slides. These sections were reacted for HRP activity according to the tetramethylbenzidine (TMB) technique of Mesulam [12], and were stored in ammonium molybdate solution to increase the stability [13]. After completing the reaction, all the sections were air-dried and counterstained with 0.1% neutral red. The sections were then examined using an optical microscope with a polarized dark field illumination at a magnification of 200–400. The labeled cells were counted in every other section.

Results

The stellate ganglion, which is fused with the first, second, and third thoracic ganglia, and sometimes with the fourth ganglia, is the largest autonomic ganglion in the dog. This ganglion is located on the lateral surface of the longus colli muscle at the level of the first intercostal space. In these studies, considerable variation was noted between animals in the anatomy of the paravertebral ganglia at the thoracic, lumbar, and sacral levels. We observed several instances where ganglia were either absent or where adjacent ganglia were

fused. The number of fused ganglia were determined according to the level of the spinal vertebra where they were located.

WGA-HRP application to the skin

The labeled cells were observed on the same side of injection, but none on the opposite. Table 1 presents the percentage of HRP-labeled cells observed in each sympathetic ganglion. The distribution of labeled cells in the sympathetic ganglia for all animals examined are shown in Fig. 2.

Face (F-1, F-2, F-3) In these preparations, labeled cells were observed only in the ipsilateral SCGs (Fig. 2a).

Neck (N-1, N-2) Two types of distributional patterns of labeled cells were observed. In one case (N-1), with injected WGA-HRP into the rostral part of the cervical skin, all labeled cells were located in the SCG. In another case (N-2), with injected WGA-HRP into the middle part of the cervical skin, 76.3% of all labeled cells located in MCG and 23.7% in SG (Fig. 2b).

Forelimb (N-3, N-4) In two cases, labeled sympathetic postganglionic cells were distributed at one or two ganglia, although most cells were contained in the SG. Following the application of WGA-HRP to the proximal areas of the forelimb skin (N-3), which is closely related to the acromion, all labeled cells were found in the SG. Injection to the dermis and/or subcutaneous tissue of 5th digital pad (N-4) resulted in the small number of labeled cells (1.8%) in the MCG, and 98.2% of all labeled cells in the SG (Figs. 2b and 3a).

Chest wall (T-1, T-2, T-3, T-4, T-5) In order to determine the topographic organization of the sympathetic postganglionic cells innervating to the chest skin, five points were selected for injection of WGA-HRP. The number of sympathetic postganglionic fibers from the SG decreased according to the caudal level. The population of sympathetic postganglionic cells in the SG were 21.3% (T-1), 12.8% (T-2), 13.4% (T-4), and 1.5% (T-5), respectively (Fig. 3b). In addition, the number of sympathetic postganglionic fibers from the SG in the distal skin area at the level of seventh rib (T-3) was smaller than those in the middle skin area at the same level (T-2). These results showed that the sympathetic postganglionic fibers from the SG decreased according to the caudal and ventral level (Fig. 2c).

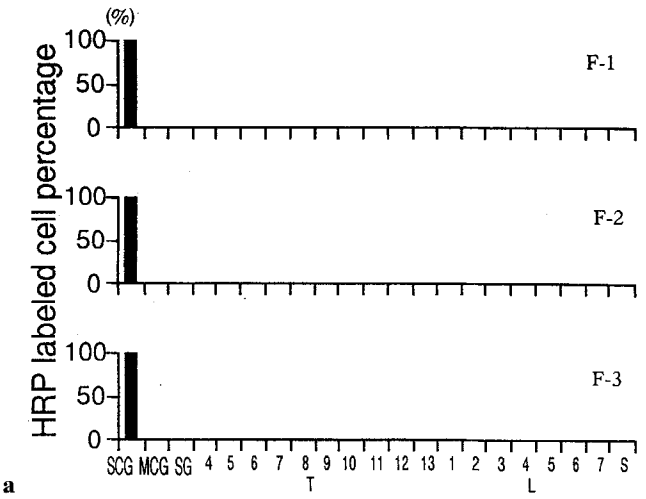
Abdominal wall (A-1, A-2, A-3) Labeled cells in the SG were observed in the following cases, which injected points were 1.5 cm (A-1) and 3 cm (A-2) caudal from the distal part of 13th rib. Sympathetic efferent fibers from the SG were markedly reduced. After the application of WGA-HRP to the ventral skin area (A-3), no labeled cells were observed in the SG (Fig. 2d).

Navel and Hindlimb (H-1, H-2, H-3, H-4) Following injection into the skin of the navel and hindlimb, no labeled cells were found in the SG (Fig. 2e).

Table 1. Percentage distribution of labeled cells in the sympathetic ganglia after application of wheat germ agglutinin conjugated horse-radish peroxidase (WGA-HRP) to various skin areas

	F-1	F-2	F-3	N-1	N-2	N-3	N-4	T-1	T-2	T-3	T-4	T-5	A-1	A-2	A-3	H-1	H-2	H-3	H-4
SCG	100.0	100.0	100.0	100.0															
MCG	0.0	0.0	0.0	0.0	76.3	0.0	1.8												
SG	0.0	0.0	0.0	0.0	23.7	100.0	98.2	21.3	[12.8	[7.1	[13.4	[1.5	8.9	[2.2					
T4								52.0					1.2			1.0			
T5								0.5	1.4		1.0								
T6								[25.7	3.1	[21.4	1.4	[0.1	[1.5	[2.2		[2.4			
T7									31.1	42.9	0.9	0.5	[0.1						
T8								0.5	13.3	25.0	21.2	7.7		[0.7		[1.9			
T9									35.8	3.6	14.4	5.5		7.5		2.1			
T10									2.4		[24.8	34.6	[1.0	8.2		37.1			
T11												[47.4		9.7		25.6			
T12											23.0		[35.9	[64.2	[42.1	[23.8			
T13												2.0	49.3						
L1												0.6		5.2	46.9	2.5		0.2	
L2													[2.1			3.0	14.6		
L3															[10.4		61.0	[24.3	1.8
L4																[0.6			
L5															[0.6		[5.6	[25.4	[1.6
L6																	17.6	48.7	6.6
L7																	1.4	1.1	66.4
S																		0.2	23.5

[shows the location of the fused ganglia.
SCG, superior cervical sympathetic ganglion; MCG, middle cervical sympathetic ganglion; SG, stellate ganglion.



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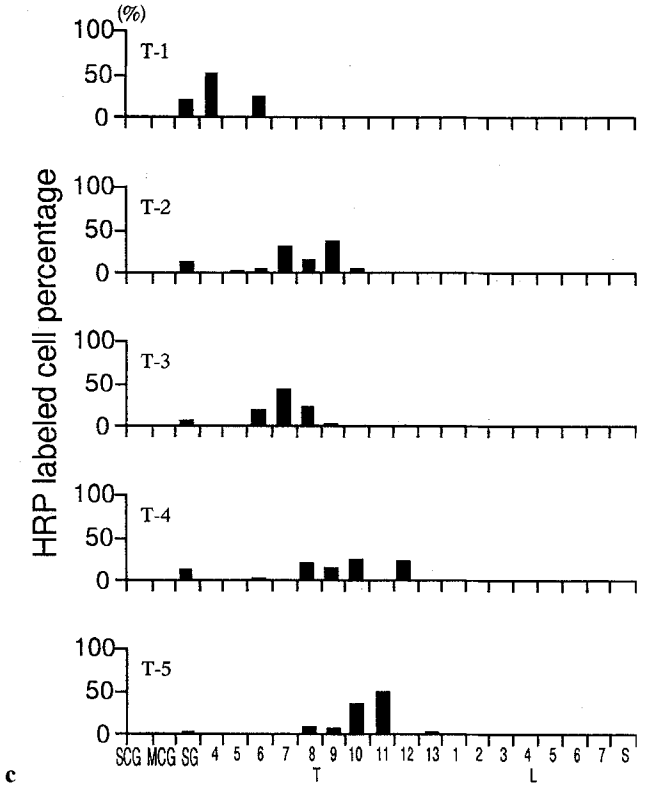

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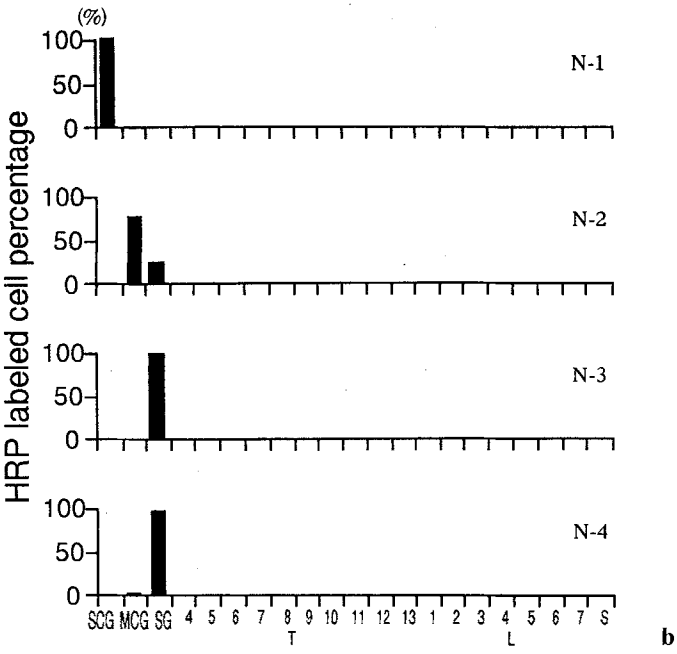

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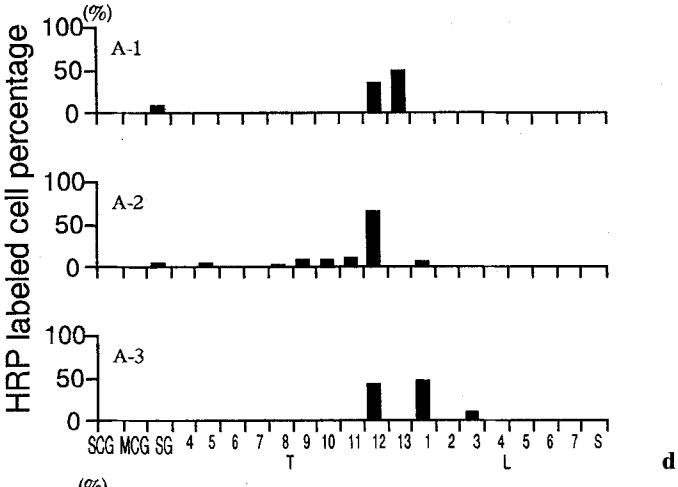

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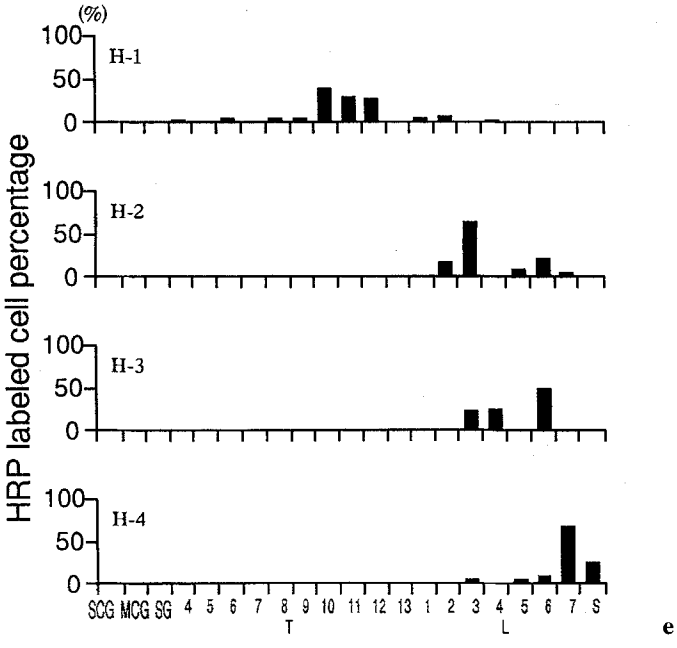

e

Fig. 2a–e. Segmental distribution (%) of wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP)-labeled cells in the ganglia of the sympathetic trunk. Injection sites were **a** the face, **b** the neck and forelimb, **c** the chest wall, **d** the abdominal wall, and **e** the navel and hindlimb. *SCG*, superior cervical sympathetic ganglion; *MCG*, middle cervical sympathetic ganglion; *SG*, stellate ganglion

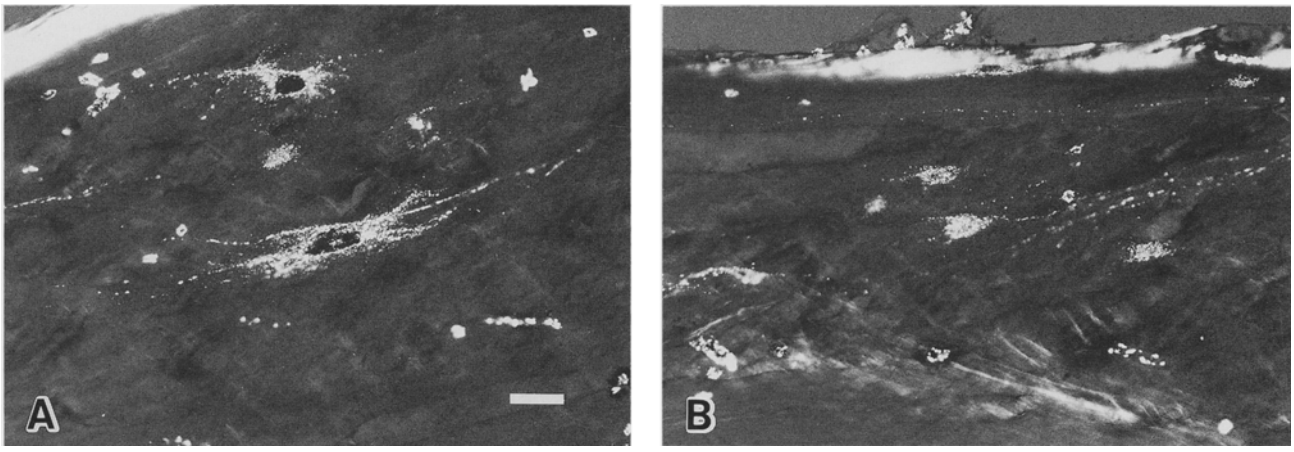

Fig. 3. Polarized dark field photomicrographs showing wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP)-labeled cells in SG. A: N-4, B: T-5. *Scale bar* in A (also valid for B), 100 μm

HRP application to the cut end of the sympathetic trunk

After application of HRP to the CST and TST, labeled cells were found in the SG. The number of labeled cells observed in the MCG and SG after each injection are given in Table 2. When HRP was applied to the cut end of the right CST between the SCG and MCG, the large number of labeled cells (n = 2001) were seen in right SG, and observed also in left SG (n = 189). Following application of HRP to the cut end of TST between the fourth and fifth thoracic sympathetic ganglia, labeled cells were observed in right SG (n = 291) and left SG (n = 18). After application of HRP to the cut end of the TST between the 10th and 11th thoracic sympathetic ganglia, labeled cells were seen in both the right SG (n = 66) and left SG (n = 29).

Discussion

Small sympathetic nerve branches are generally distributed in a segmental arrangement to the hypodermis in all body areas in dogs. Examination by light microscopy has revealed that large nerve trunks enter the dermis from the hypodermis, where they branch and give rise to nerves which travel alongside the blood vessels, forming a branching plexus which supplies the blood vessels, hair follicles, skin glands, and epidermis [14–16]. In this study, WGA-HRP was injected into the dermis and/or subcutaneous tissue expecting epidermal or dermal dilatation and uptake to nerves.

Broadwell and Brightman reported that vascular infusion of peroxidase resulted in retrograde neuronal labeling of the neurosecretory, motor, sensory, and autonomic systems [17]. They injected 30–50 mg HRP into the tail vein of the mouse, and found HRP labeling in cranial motor nerve nuclei III, IV, V, VI, VII, XII, the ambiguus nucleus, and so on. In the present study, no HRP labeling was found in these areas. This result suggested that vascular transfusion of HRP could be ruled out.

The SG gives off many branches. One of them is a gray ramus which communicates to the brachial plexus via the seventh and eighth cervical and the first thoracic nerves, and sometimes via the fifth and sixth cervical nerves. Other branches are to the cardiac plexus, to the vertebral artery, to the inferior thyroid plexus, to the subclavian plexus and to the phrenic nerve [3,4,18,19].

Experimental study showed the origin of sympathetic innervation of the elbow joint in the rat. After injection of WGA-HRP into the elbow joint, labeled neurons were found in the SG and the T2–T4 ganglia of the

Table 2. Number of labeled cells in bilateral MCG and SG after application of HRP to the right cervical or thoracic sympathetic trunk

	Between SCG and MCG		Between T4 and T5		Between T10 and T11	
	Right	Left	Right	Left	Right	Left
MCG	#	75	734	74	275	216
SG	2001	189	291	18	66	29

[#] The labeled cells could not be identified due to the presence of numerous red cells.

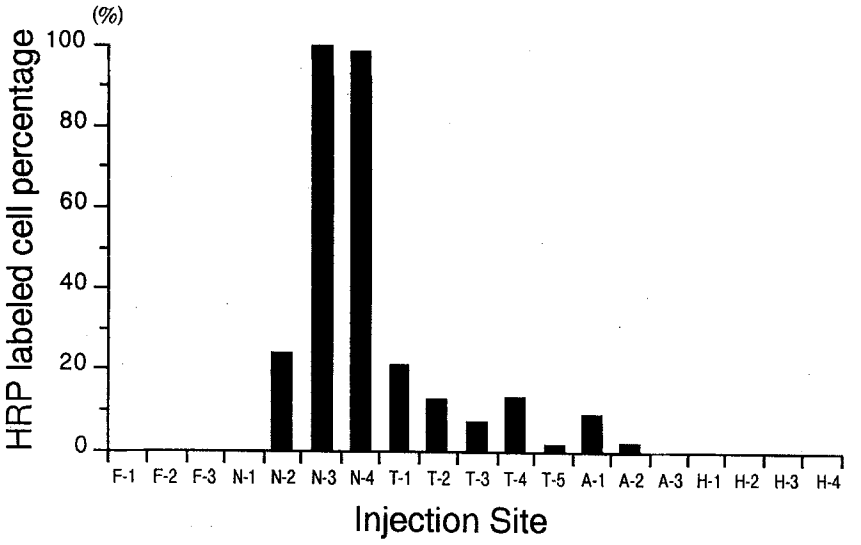

Fig. 4. Distribution of labeled cells at SG

ipsilateral sympathetic trunk, and 90% or more were located in the SG [20].

After injection of WGA-HRP into various cutaneous areas, the labeled cells in the sympathetic ganglia were observed only ipsilaterally to the injection site, indicating that no tracer had leaked into the systemic circulation and reached the neurons through the vasculature. The segmental distribution of labeled cells in the ganglia of the sympathetic chain were virtually identical on the injection side of each animals (Table 1). The percentage of labeled cells in the SG with each injection, summarized in Fig. 4, suggests that the postganglionic fibers from the SG distribute on the skin area between the third or fourth cervical (C3 or C4) vertebra and 3 cm caudal from the 13th rib. They distribute predominantly on the skin area of the forelimb, and the number decreases according to the rostral, caudal, and ventral level. The results are summarized in Fig. 5.

The postganglionic fibers from the SCG distribute directly to the facial skin while those from the SG do not. Marfurt et al. showed that a large number of labeled fibers from SCG were traced into the facial skin using a WGA-HRP tracing technique [21]. Nevertheless, SGB is usually performed for the diagnosis and treatment of clinical entities in the head and face, and increased blood flow in these areas has been reported.

Why is SGB an effective diagnostic and therapeutic procedure for clinical entities in head and facial area? The following two explanations can be considered.

First, in the SCG, the CST is thought to be the exclusive route of input from preganglionic sympathetic neurons [22], and some ascending preganglionic sympathetic fibers in the CST are suspected to go through the SCG [23]. The CST contain the axons of preganglionic

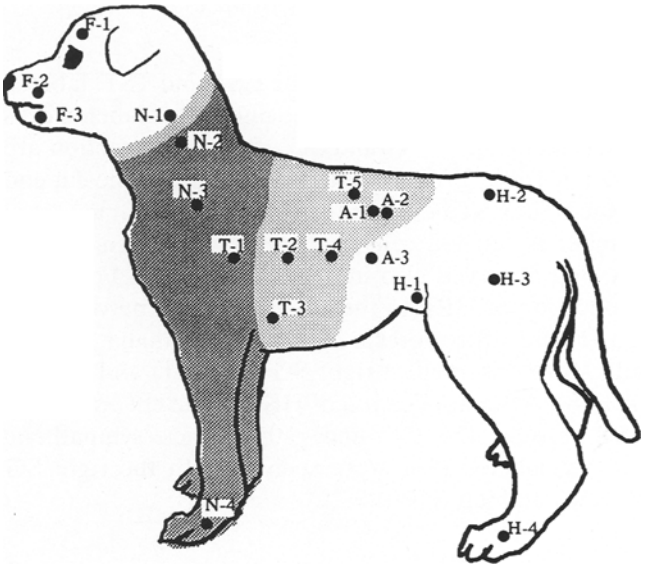

Fig. 5. Distribution of the postganglionic fibers from SG on the cutaneous area is located mainly on the skin of the forelimb (*darkly shaded area*); the number of these fibers decreases according to the rostral, caudal, and ventral level (*lightly shaded area*)

neurons that innervate the ipsilateral SCG [24]. Pilowsky et al. reported that sympathetic preganglionic neurons projecting to the SCG were found in spinal segments T1 to T8, and almost 95% of these neurons had axons that passed through the SG [25]. These results suggest the preganglionic fibers reach the MCG and SCG through the SG. Hence the same effect as blockade of the MCG and SCG is obtained when these preganglionic fibers to the SCG are blocked by SGB.

Second, local anesthetics are considered to diffuse

along the CST to MCG or SCG and block these ganglia. Moore [3] and Alexander and Lovell [26] reported that when 10 ml to 15 ml of a 35% Diodrast solution containing the blocking agent is injected for SGB, the solution diffuses cephalad to the region of the C5 vertebra and caudad to the region of the T4 vertebra. Studies by Bonica [4] with contrast media have shown that 5 ml of solution injected into the proper fascial plane spreads to involve the T2 thoracic and intermediate cervical ganglia. Injection of 10–12 ml of solution spreads from the level of the upper part of the C5 vertebra down to T3 or T4. Injection of 20 ml of solution blocks the chain from the level of the C3 vertebra to the T5 vertebra. Yamamuro reported that when SGB is executed in the region of C7 vertebra using 8 ml of contrast medium with local anesthetics, the solution diffuse from upper edge of the C4 vertebra to lower edge of T3 [5]. The diffusion pattern is considered to vary between contrast media and local anesthetics. According to the position of the SCG, which is lying in front of the transverse process of C2 and C3, it would be paralyzed directly. As the sympathetic nervous system above the T4 sympathetic ganglion are all blocked by SGB, it has been considered that SGB is effective for the skin region above T3 or T4.

There were some reports that postganglionic sympathetic fibers run in the lumbar or cervical sympathetic trunk. Jänig and McLachlan showed that after application of HRP to the proximal end of the cut lumbar sympathetic trunk (LST) just proximal to ganglion L6, postganglionic cell bodies were labeled in ganglia L2–L5 (maximum labelling at L5) [27]. It revealed postganglionic fibers run caudally in LST. Studies in the cat [28–30], the rabbit [31], the rat [24,32], and in the guinea pig [33] demonstrated the existence of postganglionic fibers in the CST, which ascends from cells located in the lower cervical, stellate, or upper thoracic sympathetic ganglia, or descends from cells in the SCG.

Some experiments were performed to prove that the postganglionic fibers from SG run rostral and caudal in the sympathetic trunk. One procedure was to expose CST and to apply HRP between the SCG and MCG. The other was to expose TST and to apply HRP between ganglia T4 and T5, or T10 and T11. Labeled cells were observed in both the SG and MCG bilaterally in all cases. We revealed that the postganglionic fibers from the SG run rostrally in the CST and caudally in the TST to T10. These results show the postganglionic fibers from SG run rostrally and caudally in the sympathetic trunk. Other postganglionic fibers from SG run to the periphery with the vessels. Investigations of the effect of SGB on the arterial blood flow have shown an increase in blood flow in the common carotid artery, radial artery, and brachial artery, but no change in the posterior tibial artery or the dorsal pedal artery [34–36].

Regarding the superficial arteries of the trunk in dogs [16], the brachiocephalic trunk and the left subclavian artery are arising from the aortic arch, and the brachiocephalic trunk terminates in the common carotid arteries (left and right) and the right subclavian artery. The superficial cervical artery arises from the subclavian artery, courses cranially, and supplies the cervical skin area. The internal thoracic artery leaves the subclavian artery and runs caudally under the transversus thoracis and supplies the chest and abdominal skin area. The subclavian artery is continued by the axillary artery. The cranial circumflex humeral artery, the caudal circumflex humeral artery and the collateral radial artery of the humerus are arising from the axillary artery and supply the shoulder and thoracic limb. The lateral thoracic artery, the subscapular artery and the thoracodorsal artery are from the axillary artery and supply an area of shoulder and upper back skin. Twelve pairs of the intercostal arteries arise from the thoracic vertebral artery and the aorta, and supply a skin area of the chest.

The skin area supplied by the superficial arteries arising from subclavian artery and intercostal arteries are coincident with the skin area in which the postganglionic fibers from the SG distribute in this study. We inferred that the postganglionic fibers from the SG run to the periphery with these vessels described above and distribute to the skin area. We therefore speculate that there are two routes by which the postganglionic fibers run from the SG to the skin. Firstly, some of the postganglionic fibers from the SG run rostrally in the CST and caudally in TST (as to T10) and join the adjacent spinal nerves or travel along the vessels. Secondly, other fibers run to the periphery with the branches arising from subclavian artery and intercostal arteries.

We found that the postganglionic fibers from the SG were distributed much more widely on the skin area than had previously been thought. Supposing the distribution of the postganglionic fibers from the SG in human is similar to that in dogs, the sympatholytic effect by SGB could be obtained more caudal on the skin. Nevertheless, SGB is usually performed for the patients with skin lesions above T3 or T4. One possible explanation for this is that the number of postganglionic fibers from the SG decreases according to the caudal level; thus, the sympatholytic effect by SGB is not satisfactory. When WGA-HRP was injected to the chest or abdominal skin, more labeled cells were observed in the thoracic sympathetic ganglia than in the SG. These results indicate that a more intense sympatholytic effect in these areas could be obtained from epidural block or thoracic sympathetic ganglion block than from SGB.

McLachlan and Jänig [37] and Baron et al. [38] showed that the sympathetic somata which projects to

the skin and skeletal muscle of the rat hindlimb were more widely distributed rostrocaudally (four to six segments) than the sensory outflow.

For each injection, the labeled cells observed were most numerous in the sympathetic ganglion, corresponding to the spinal segment innervated by the injection area. Incidentally, the experimental counting of the labeled cells in the dorsal root ganglia revealed the appearance of the most numerous labeled cells at the same level as the sympathetic ganglia (unpublished data). In addition, many labeled cells were also seen in the adjacent rostral and caudal sympathetic ganglia (Table 1, Fig. 2). It revealed the distribution of the sympathetic postganglionic cells that innervate the skin spread widely compared to the dorsal root ganglion (DRG) cells.

The sympathetic ganglion innervates the skin area more widely than the dorsal root ganglion. This suggests that it is necessary to block the sympathetic ganglia widely to obtain satisfactory sympatholytic effect on the cutaneous region.

This study neuroanatomically proved that SGB is essentially a block of the cervicothoracic portion of the sympathetic nervous system as it has been known clinically, although the term "stellate ganglion block" refers to chemical paralysis of the SG by local anesthetic agents.

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