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AN EXAMINATION OF THE CLOSE RELATIONSHIP BETWEEN LYMPHATIC VESSELS AND NERVE FIBERS CONTAINING CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P IN RAT SKIN

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ABSTRACT

The distribution of nerve fibers containing either calcitonin gene-related peptide (CGRP) and substance P (SP) was investigated in rat skin with special reference to their relationship to the lymphatic vessels. These nerve fibers exhibited a similar distribution pattern but the former were more numerous than the latter. In the dermis and subcutaneous layers, thin nerve fibers containing CGRP or SP were in abundance, and were observed running along the blood vessels as well as freely in the tissue. Nerve fibers with these peptides were often located close to lymphatic capillaries, and innervated lymphatic vessels in the subcutaneous layer, reaching smooth muscles of the tunica media. These findings suggest that some CGRP and SP may directly drain into lymphatic vessels when released under noxious stimulation from nerve fibers around the lymphatic vessels. When discharged from nerve fibers in the vicinity of blood vessels, both peptides may also drain into the lymphatic vessels after causing blood vascular dilation and an increase in permeability producing edema. These peptides may then be transported to the draining lymph nodes where they can modulate the functions of the immune system.

Key Words: Neuropeptide-containing nerve fibers, Lymphatic vessels, Blood vessels, Rat skin, Immunohistochemistory

INTRODUCTION

Calcitonin gene-related peptides (CGRP) and substance P (SP) have widespread distributions in the body, including a population of primary sensory neurons in the dorsal root ganglia.[1-8] These peptides, produced in the cell body of the sensory neuron, were transported to the afferent and the efferent in the central nervous tissues.[9,10]

These peptides are released non-synaptically from peripheral sensory axons by noxious stimulations, and produce various local regulatory effects.[10-12] Neurogenic inflammation in the skin, including vasodilation,[13,14] extravasation of blood plasma and histamine release from mast cells,[10,15,16] has been extensively studied. A close morphological relationship has been shown between the nerve fibers containing these peptides and blood vascular structures in the skin.[1-3,5-8] As for other local regulatory effects of these peptides, many experimental studies[17-29] suggest that they modulate the function of the immune system, primarily in the lymph nodes. The purpose of the present study is to obtain a morphological basis for the relationship between these peptide-containing nerve fibers and the lymphatic vascular structures in the skin.

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MATERIALS AND METHODS

Tissue preparation

Seven male Wistar rats 6 weeks old, weighing 170 ~ 180 g were used. The animals were anesthetized with sodium pentobarbital (40 mg/kg body weight). Skin fragments were excised from the fore and hind legs, cut into small pieces, and fixed with Zamboni's fixative overnight at 4°C. The specimens were rapidly frozen in liquid nitrogen and cut into sections 15 μm in thickness in a cryostat.

Immunohistochemistry

Sections were processed with the streptavidin-biotin-peroxidase complex (SAB) method. In brief, nonspecific endogenous peroxidase staining was reduced with 3% H_2O_2 in 100% methanol for 15 minutes. The sections were washed in PBS and incubated with normal goat serum 1:10 for 10 minutes, incubated in a 1:7000 dilution anti-CGRP rabbit serum (Affiniti, Co.) or 1:7500 anti-SP rabbbit serum (Affiniti, Co.) overnight at room temperature, and then biotinylated goat-antirabbit IgG (Nichirei, Co.) at a dilution of 1:100 for 10 minutes followed by peroxidase-streptoavidin (Nichirei, Co.) at a dilution of 1:10 for 5 minutes; labeling was visualized with 3,3′-diaminobenzidine · 4HCL in addition to 0.6% H_2O_2 as chromogen. Most of the sections were counterstained with hematoxylin.

RESULTS

Immunoreactive CGRP- or SP-containing nerve fibers in the rat skin were about 1 ~ 3 μm with or without many small varicosities. An abundant distribution of CGRP-containing fibers was observed in the skin of the fore and hind legs. SP-containing fibers exhibited a similar but lower distribution than that of CGRP-containing fibers. No difference was observed in the distribution pattern between the fore and hind legs. Both fibers occurred more frequently toward the distal, glabrous end portions of the legs. They were observed frequently around sweat glands and hair follicles, but rarely around sebaceous glands. They were also observed reaching the stratum spinosum of the epidermis.

In the dermal and subcutaneous layers, the peptide-containing nerve fibers were seen running close to the blood capillaries (Fig. 1) and venules. They formed a plexus around small arteries and veins. These nerve fibers ran close to the tunica media of larger blood vessels (Fig. 2). The nerve fibers were also located on the periphery of lymphatic capillaries in the dermis and subcutaneous layers (Fig. 3). The lymphatic capillaries often ran in the vicinity of blood vessels which were closely associated with the peptide-containing nerve fibers (Fig. 4). The lymphatic vessels having valves in the subcutaneous layer was innervated by the fibers containing either of these peptides in a manner similar to that of the blood vessel wall (Fig. 5). In the dermal layer, there were many clusters of mast cells, and these peptide-containing nerve fibers occasionally were distributed among these clusters (Fig. 6).

DISCUSSION

This study demonstrated a close morphological relationship between CGRP- and SP-containing thin nerve fibers and lymphatic vessels in the skin of rats. By heating rat's legs at 47°C, production of both CGRP- and SP-contents increased sharply in the perfused fluid from the local skin.[16] Jonsson et al.[30] reported an immediate increase of SP-levels in the lymph collected from

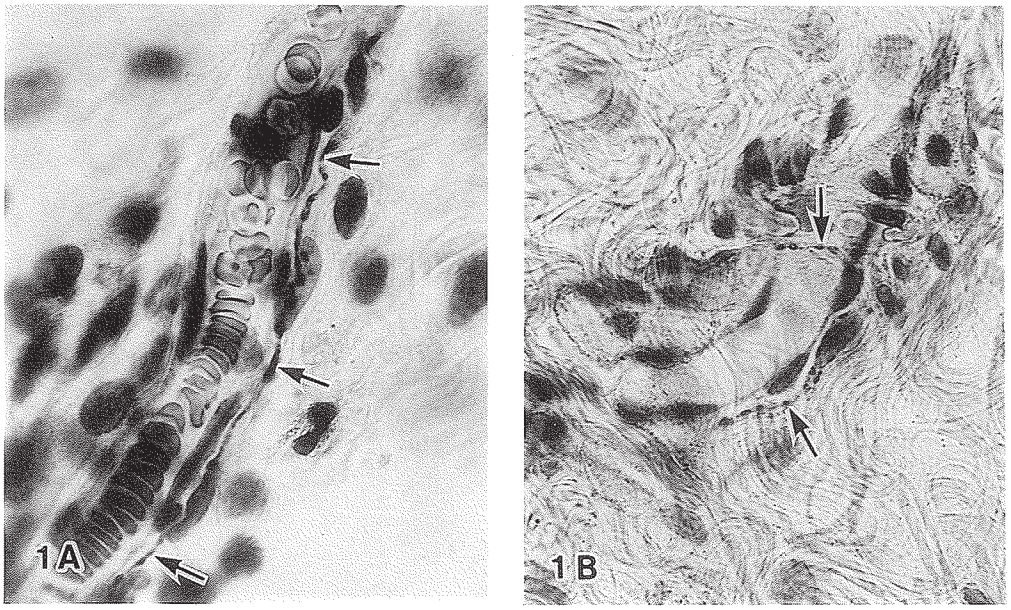

Fig. 1. Neuropeptide-containing nerve fibers with small varicosities. Arrows indicate CGRP immunopositive terminals in 1A, and SP immunopositive terminals in 1B. They run close to the blood capillary. × 1,100

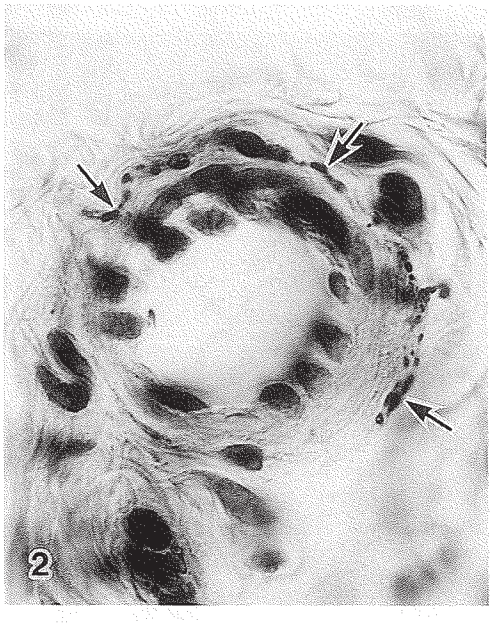

Fig. 2. Nerve fibers containing CGRP. Immunopositive fibers (arrows) run within the tunica media of a small artery. × 1,100

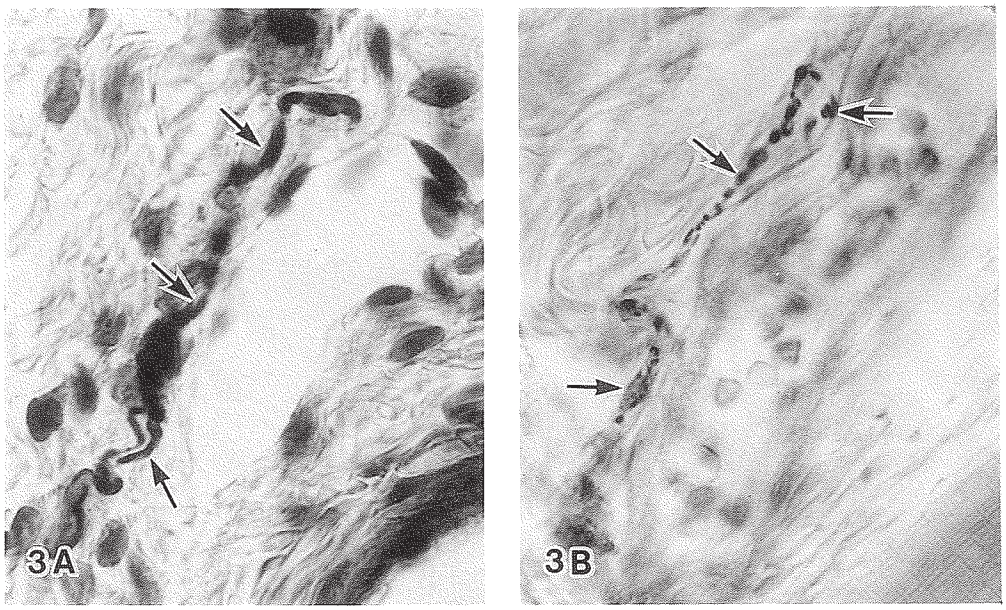

Fig. 3. Neuropeptide-containing nerve fibers in the hind leg just above the paw. CGRP-containing nerves (arrows in 3A) and SP-containing nerves (arrows in 3B) are present in the area around the lymphatic capillary. × 1,100

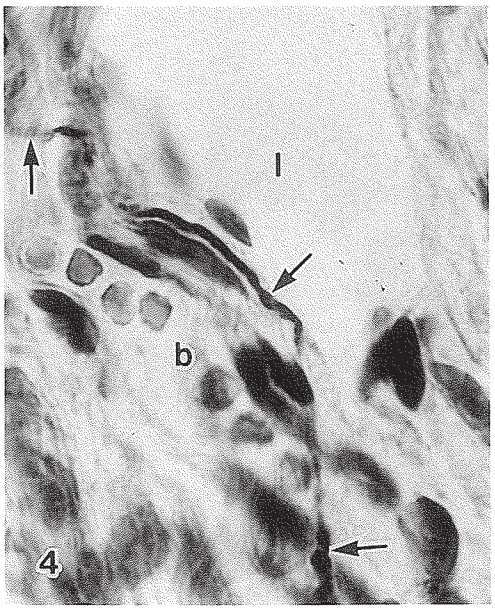

Fig. 4. A bundle of CGRP-containing nerve fibers. This bundle (arrows) runs between a lymphatic capillary (l) and a blood capillary (b). Specimen was obtained from the dorsal aspect of hind limb just above the paw. × 1,100

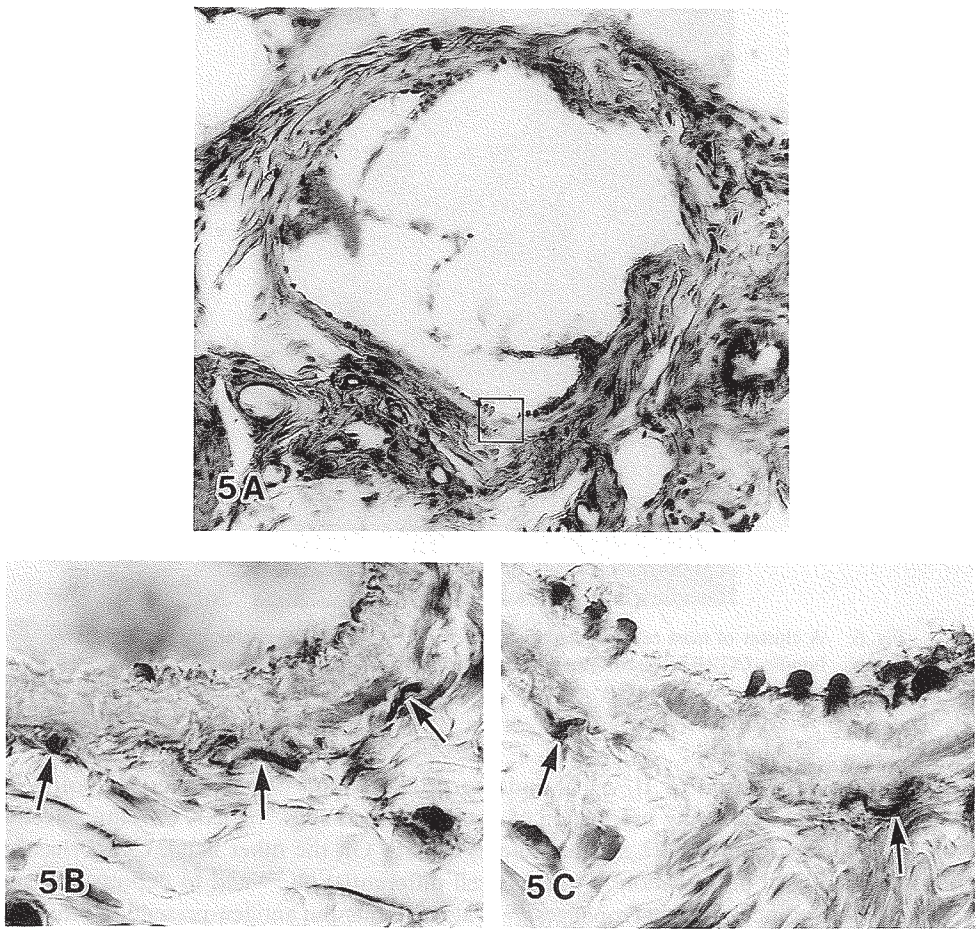

Fig. 5. A lymphatic vessel with a valve seen in the region just above the paw of the hind limb. 5A: This vessel is innervated by peptide-containing nerve fibers. 5B and 5C are serial sections. 5C is a higher magnification of the box in Figure 5A. Notice that these fibers reach the tunica media. (arrows: CGRP in 5B and SP in 5C) 5A: × 450 5B, 5C: × 1,100

a lymphatic vessel of the leg after a paw scalding injury in dogs. These studies indicate that CGRP and SP are released from the nerve fibers nonsynaptically by noxious stimulations to the skin, and are rapidly drained into lymphatic vessels. Both peptides, if released from the nerve fibers located close to the lymphatic vessels, may be drained directly into neighboring lymphatic vessels.

The effects of CGRP and SP on the blood vessels have been well documented. CGRP induces vasodilation to increase blood flow,[13,14] and SP causes an increase in the permeability of blood vessels.[15,16] Both peptides thus cooperate to produce edema. The close relationship of these peptide-containing nerve fibers to the blood vascular structures of the skin provides a morphological basis for these reactions. The edema increases at the lymph flow, and both peptides may also be drained into the lymphatic vessels after they stimulated blood vessels. Then they reach the lymph nodes.

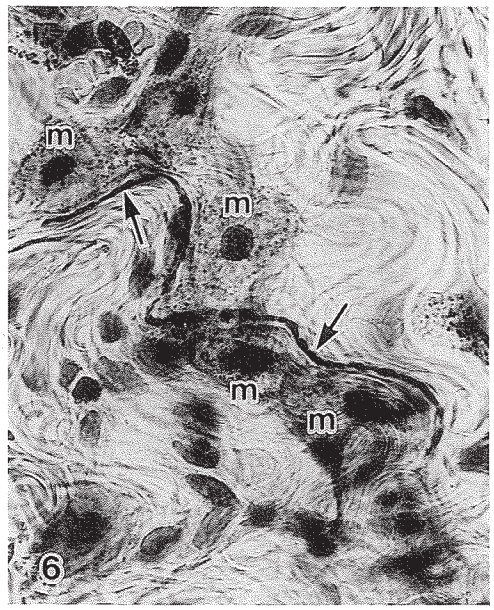

Fig. 6. A cluster of mast cells situated in the area close to a CGRP-containing nerve fiber bundle. CGRP released from nerves (arrows) may stimulate these mast cells (m). × 750

SP stimulates production of IL-2[27,29] and the proliferation[31,32] and differentiation[21,22] of lymphocytes, thereby increasing production of antibodies in B cells.[18,19] NK cells are selectively activated,[25] and macrophages are also activated[17] by SP. On the other hand, CGRP has been found to inhibit T cell proliferation[20,26] and B cell differentiation,[28] and to depress NK activity[24] as well as the function of macrophages.[23] It also inhibits the antigen-presenting function of Langerhans cells.[33] Thus CGRP and SP are antagonistic in their effects on immune system function.

All of the reports mentioned here suggest that CGRP and SP released from peptide containing fibers under noxious stimulation of the skin can modulate immunological function through their antagonistic effects on the immune system in the lymph nodes. It seems quite possible that noxious stimulation by acupuncture and moxibustion (therapeutic scalding) could serve to modulate the function of the immune system through a similar mechanism.

CGRP has been demonstrated to co-exist with SP in the sensory neurons of many species, while some neurons contain CGRP without SP.[2-4] Although double staining of both peptides was not carried out in the present study, the similar distribution pattern of both peptides and the more frequent occurrence of CGRP-containing fibers than SP-containing fibers observed in the present study seem to agree with the results of previous studies.

CGRP- and SP-containing nerve fibers have been demonstrated to associate closely with the lymphatic capillaries in the rat liver,[34] and in the central lacteal lymphatics of the small intestines of monkeys.[35] A baroreceptive function such as lymphatic luminal pressure has been suggested in these fibers.[34,35] We suggest that in addition to the proposed baroreceptor function the nerve fibers secrete their neuropeptides into lymphatic vessels under noxious stimulation and modulate immunological function in the visceral organs and the skin.

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