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Immunohistochemical Study of the Neuropeptides in the Stellate Ganglion of the Water Buffalo

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Abstract The localization of some neuropeptides including neuropeptide Y (NPY), substance P (SP), calcitonin gene related peptide (CGRP), vasoactive intestinal peptide (VIP), galanin (Gal), methionine enkephalin (M-ENK), tyrosine hydroxylase (TH) immunoreactivity was studied in the stellate ganglion (SG) of water buffalo. NPY, SP, Gal and TH immunoreactivities were present in almost all of the ganglion cells. NPY, SP, Gal, SP, CGRP, VIP and M-ENK immunoreactive nerve fibers were also seen in the SG. The localization and pattern of distribution of these peptides in the water buffalo stellate ganglion were compared with those in stellate ganglia of other mammalian species.

Key words : Neuropeptide, stellate ganglion, water buffalo

Introduction

The stellate ganglion (SG, cervicothoracic ganglion) is one of the paravertebral ganglia which form the sympathetic chains along the spinal cord and it receives short preganglionic axons from spinal cord and the ganglion send out their long postganglionic axons to the important organs in the thoracic cavity; lung and heart and to the arm via the brachial plexus.

Several studies have focussed on the neurotransmitters and neuropeptides of the mammalian stellate ganglia. However the studies about the SG are based mainly on the results of

immunohistochemical studies carried out on small laboratory animals⁷⁾¹⁰⁾¹⁴⁾¹⁸⁾²⁰⁾. There were few evidences available concerning the localization of neuropeptides in sympathetic paravertebral ganglia of farm animals⁴⁾, and the presence of neuropeptides in the water buffalo SG was not mentioned yet. Therefore, the present study was undertaken to clarify the distribution of neuropeptides in the water buffalo SG using antibodies against several neuropeptides.

Materials and methods

Adult water buffalo (carabao) were used in this study. These animals were killed at the slaughter house in Philippines. The SG of both sides were immediately removed and fixed by immersion into the 5% phosphate-buffered forma-

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lin. As a usual method, tissues were embedded in paraffin to cut at a thickness of $10\mu\text{m}$. Sections were processed for immunohistochemical detections of following neuropeptides and catecholamine-synthesizing enzyme neuropeptide Y (NPY), substance P (SP), calcitonin gene related peptide (CGRP), vasoactive intestinal peptide (VIP), galanin (Gal), methionine enkephalin (M-ENK), tyrosine hydroxylase (TH). Antisera for NPY, SP, CGRP, VIP, Gal and M-ENK were raised in rabbits (polyclonal antibodies: pa). Antiserum for TH was raised in mice (monoclonal antibody: ma). All of the antibodies were supplied from INCSTAR Corp. (Minnesota, USA). The sections were dewaxed and pretreated with a 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Immunohistochemical stain was achieved using an avidin-biotin-immunoperoxidase method (Histofine SAB-PO (R) for pa and SAB-PO (M) for ma, Nichirei co. Ltd., Tokyo). Incubation period for primary antisera is 12 hours in 37°C . The immunohistochemical reaction product was developed by incubation of the sections in diaminobenzidine and H_2O_2 in Tris-HCl buffer. After being washed, the sections dehydrated in a graded alcohol, and mounted under coverslips with Canada balsam. Controls included the bovine serum instead of the primary antibodies. None of the immunoreactivities were observed in the controls. The immunoreactive and non-immunoreactive principal cells of SG were counted. The total number of counted cells from four sections were 200, respectively.

Results

The stellate ganglion of the water buffalo lied on the ventral part of the costovertebral junction of the first rib and the first intercostal space. It was formed by the caudal cervical

ganglion and the first one or two thoracic sympathetic chain ganglia. The size of the SG was about 1.5 cm in length 1.0 cm in width.

Many nerve fibers arose from this ganglion. The ganglion was surrounded by dense connective tissue capsule and consisted of several bundles of nerve fibers and nerve cells. The nerve cells (principal neurons) were large, round shape and had spherical nucleus and display several processes (multipolar cell).

NPY immunoreactivity

A large number of NPY-immunoreactive (IR) principal neurons were found in the SG. Approximately 60–70% of principal neurons in the SG exhibited NPY-IR. In these positive neurons there were many granules. Some varicose NPY-IR nerve fibers were detected in the SG.

SP immunoreactivity (Fig. 1)

Almost all of principal neurons in the SG exhibited SP-IR. They scattered in the ganglion evenly. But these positive reactions were very weak in all sections. We could detect varicose SP-IR nerve fibers in some sections.

CGRP immunoreactivity

Approximately 80–90% of principal neurons exhibited CGRP immunoreactivity. These neurons exhibited weak CGRP immunoreactivity. Some varicose CGRP-IR nerve fibers were also found.

VIP immunoreactivity (Figs. 2, 3)

Some VIP-IR principal neurons (10–20%) were present in the SG. They were characterized that the some positive neurons gathered solitary. The IR principal neurons had granules which stained intensely in the cytoplasm. A few VIP-IR nerve fibers were found in the

SG.

Small number of varicose Gal-IR nerve fibers were observed in the SG.

Gal immunoreactivity (Fig. 4)

A large number of principal neurons of the SG exhibited galanin immunoreactivity. These neurons had positive granules in the cytoplasm.

M-ENK immunoreactivity

Numerous M-ENK-IR principal neurons were observed in the SG. Approximately 50–

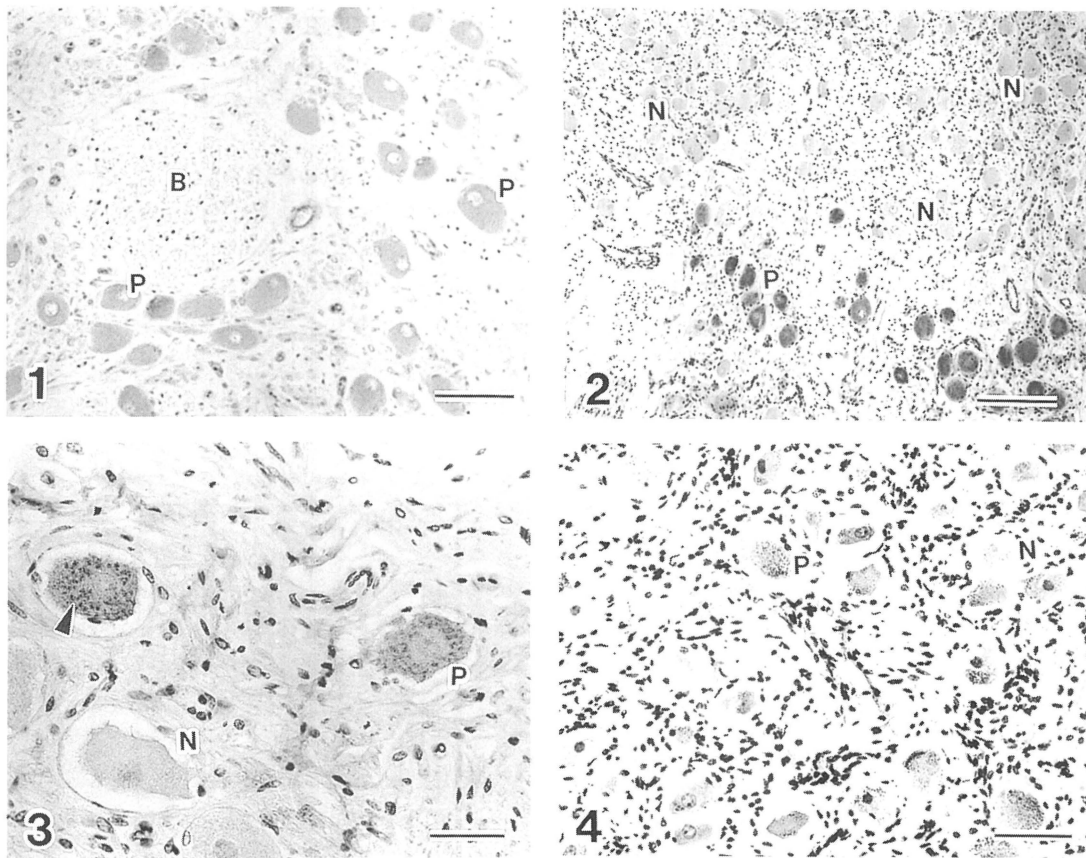


Fig. 1 SP-immunoreactive principal neurons in stellate ganglion of water buffalo. The immunoreactive neurons are scattered in the ganglion. The immunoreactivity of these cells is not intense.

B: bundle of nerve fiber, P: immunoreactive principal neuron. Bar = 70 μ m

Fig. 2 VIP-immunoreactive principal neurons in stellate ganglion. Some immunoreactive neurons gather solitary.

P: immunoreactive principal neuron, N: negative neuron. Bar = 150 μ m

Fig. 3 VIP-immunoreactive cell in the stellate ganglion. Many positive granules in the cytoplasm (arrow) are observed.

P: positive cell, N: negative cell Bar = 30 μ m

Fig. 4 Gal-immunoreactive neurons in stellate ganglion. Almost all of the neurons in the ganglion are Gal immunoreactive and they scattered evenly in the ganglion. Many immunoreactive granules in the cytoplasm are observed.

P: immunoreactive principal neuron, N: negative neuron. Bar = 70 μ m

60% of principal neurons in the SG showed M-ENK immunoreactivity. Small number of M-ENK-IR nerve fibers found were in the SG.

TH immunoreactivity

A large number of principal neurons of the SG (80–90%) showed intense TH immunoreactivity. But we could not find any TH-IR fibers surrounding the principal neurons of the SG.

Discussion

In this study we could detect seven types of neuropeptides in the stellate ganglion (SG) of the water buffalo.

Immunohistochemical investigations have revealed numerous NPY-positive principal neurons in sympathetic ganglia including SG of cat²⁰⁾, pig³⁾, rat¹⁰⁾ and man²¹⁾. In this study almost all of the principal neurons of the water buffalo SG exhibited NPY-immunoreactive reaction. This observation is similar to those of the guinea-pig²¹⁾, but SG of other animals³⁾²¹⁾²²⁾ showed fewer NPY-immunoreactive neurons than water buffalo one. Because of the thickness of the section, we could detect only a small number of NPY-IR nerve fibers in the water buffalo SG.

The principal neurons in mammalian SG¹⁾³⁾⁹⁾¹⁸⁾ and other paravertebral ganglion²⁾ appear to be negative for antibody to SP. In vitro study has shown the existence of SP-IR material in the rat superior cervical ganglion¹¹⁾. In the present study we could find many but weak SP-IR principal neurons in the SG. SP immunoreactive nerve fibers have been found in the SG¹⁾⁴⁾⁷⁾⁹⁾¹⁹⁾²¹⁾. The present result that a small number of SP-positive nerve fibers were found in the carabao SG is consistent with these views.

In the present study many CGRP-IR principal neurons were found in the SG, while solitary

principal neurons were found in the SG of the rat¹⁴⁾, cat¹⁷⁾, guinea pig¹³⁾, pig³⁾ and man²³⁾. We could also find CGRP-IR nerve fibers in the ganglion and this result is in agreement with recent investigations carried out on paravertebral ganglia of the guinea pig²⁴⁾, rat¹²⁾ and pig³⁾.

VIR-IR principal neurons have been shown to be present in various animals⁷⁾¹⁴⁾¹⁵⁾²⁰⁾²²⁾. In the present study solitary gathered VIP positive principal neurons were seen in some region of the SG. This is in accordance with pig data³⁾⁸⁾. Few VIR-IR nerve fibers were observed in the SG, but they did not surround the clustered VIR-IR principal neurons as reported in pig SG³⁾⁸⁾.

In the cat SG, there are many Gal immunoreactive principal neurons and these neurons also contained immunoreactivity to TH¹⁶⁾. We could observe the many Gal-immunoreactive principal neurons and degree of these cells is almost same as that of TH positive cells.

Numerous M-ENK immunoreactive principal neurons were observed in the water buffalo SG, but in the pig³⁾⁴⁾ fewer positive cells are found. No M-ENK immunoreactive principal neurons are found in man⁵⁾ and cat⁷⁾. M-ENK immunoreactive nerve fibers form the baskets around principal neurons⁵⁾⁷⁾, while in our observation the positive nerve fibers were sparse and did not form basket. It is in accordance with the result of pig and guinea-pig¹⁷⁾.

Many intense TH immunoreactive principal neurons were detected in the carabao SG. However, we could not find any fibers surrounding the principal neurons of the SG. This result is in accordance with the report of Heym et al.⁶⁾. TH is the enzyme of noradrenaline synthesis, so we could observe only immunoreactive principal neurons in the sympathetic ganglion.

In conclusion, this study provides new, detailed immunohistochemical evidence that the

water buffalo SG contains a number of different neuropeptides, located in neuronal cell bodies and in nerve fibers of unknown origin. The presence of NPY, SP, CGRP, VIP, M-ENK, Gal and TH in principal neurons and their processes suggests that they may play a functional role in the modulation of neurotransmission in target organs of the water buffalo SG.

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(和文抄録)

水牛星状神経節の神経ペプチドの分布

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**佐賀医科大学動物実験施設

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立山 晋・森本正敏**

神経終末の化学的伝達物質として、古典的神経伝達物質の他にペプチド性伝達物質が最近重要視され、各種の動物で様々な神経ペプチドが発見されている。星状神経節は心臓、肺などの胸腔臓器および前肢へ向かう交感神経節後線維を派出する重要な器官であり、各種の動物で、星状神経節の神経ペプチドが発見されているが、家畜では豚のみの報告しかない。

今回、我々はフィリピン大学の協力により、マニラ郊外の屠場で水牛の星状神経節を採取する機会を得た。

採取した神経節を5%ホルマリン液で固定後、常法に従いパラフィン包埋、10 μ の組織標本を作製し、市販されている抗体を用いて神経節内のペプチドの検索を免疫組織化学的に行った。各種の神経ペプチドについて、水牛星状神経節を免疫組織化学的に観察した結果、7種類(NPY, SP, CGRP, VIP, Gal, M-ENK, TH)の神経ペプチドが水牛星状神経節に存在することが分かった。