IMMUNOHISTOCYTOCHEMICAL LOCALIZATION OF CATECHOLAMINE-SYNTHESIZING ENZYMES IN SUPRARENAL, SUPERIOR CERVICAL AND NODOSE GANGLIA OF DOGS

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The localization of three catecholamine-synthesizing enzymes, tyrosine hydroxylase (TH), dopamine- β -hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) in some ganglia of dogs was studied by immunohistochemical methods.

The suprarenal and superior cervical ganglion cells showed TH-, DBH-positive and PNMT-negative or TH-positive, DBH- and PNMT-negative reactions by immunofluorescent staining. In the superior cervical ganglia, small numbers of TH-positive, small intensely fluorescent (SIF) cells formed clusters. By immunoelectron microscopy, TH-positive reaction products were observed in the cell matrix and granules of the superior cervical ganglion. In the nodose ganglion, small numbers of TH-positive, DBH-negative dopamine cells with varicose fibers were scattered among many TH-negative principal ganglion cells.

Several investigators have studied in detail the distribution of peripheral catecholaminergic neurons by the formaldehyde-induced fluorescence method (5, 17) and by the immunohistochemical technique (1, 3, 13). Thus, the majority of principal ganglion cells in sympathetic ganglia of mammals contain mostly noradrenaline, however, in the inferior mesenteric ganglia of guinea pigs, some principal ganglion cells have been reported to be lacking in DBH (1).

It has also been known that the classical transmitter of the vagus nerve is acetylcholine. However, it has been recognized that other components are present in this nerve, particularly a large proportion of sensory fibers and a small amount of catecholaminergic fibers (9). In the inferior vagal (nodose) ganglion, the catecholamine-containing SIF cells have been reported to exist in some mammals such as cats (2, 4), rats (2) and rabbits (7) but little work has been done to study what kinds of amines are contained in the nodose ganglia.

In the present experiment, distribution of TH, DBH and PNMT in the suprarenal, superior cervical and nodose ganglia of dogs has been examined by immunofluorescent staining and by immunoelectron microscopy. Preliminary reports have appeared (14, 16).

MATERIALS AND METHODS

Preparation of antisera:

The enzymes, TH, DBH and PNMT from bovine adrenal medulla were purified and the antibodies to these enzymes were raised in rabbits and tested for specificity as described previously (10, 11, 15).

Preparation of tissue:

Animals (4 days and 14 weeks old dogs) were anesthetized with ether and perfused through the right atrium with Zamboni's solution (2% paraformaldehyde-0.2% picric acid in 0.1 M phosphate buffer, pH 7.2). Suprarenal, superior cervical and nodose ganglia were removed and prepared by the following techniques.

1) Frozen section for immunofluorescence: Tissues were immersed in the same fixative overnight, washed and rinsed in phosphate buffer containing 10% sucrose, and frozen on dry ice. Frozen sections of 10 μm in thickness were cut on a cryostat and processed for the indirect immunofluorescence technique.

2) Polyethylene glycol section for PAP method: Superior cervical ganglion was postfixed for 6–17 hr in Zamboni's solution containing 0.02% glutaraldehyde at 4°C, washed overnight in the same way as the tissue for immunofluorescence and embedded in polyethylene glycol. The tissues were cut on a rotary microtome at 5–10 μm in thickness at 4°C. The sections were processed according to the peroxidase-antiperoxidase (PAP) method as described earlier (12). Ultrathin sections were observed with a JEM-100CX electron microscope without counter-staining.

RESULTS

The suprarenal ganglia:

After incubation with TH, DBH or PNMT antiserum, specific immunofluorescence was observed in this ganglion. The principal ganglion cells were moderately (after anti-DBH, Fig. 1-b) or strongly (after anti-TH, Fig. 1-a) fluorescent with a dense plexus of fluorescent fibers. However, no specific PNMT-positive reaction was found in this ganglion (Fig. 1-c).

The superior cervical ganglia:

According to Grimelius silver impregnation, larger or smaller cells of argyrophilic reaction were observed (Fig. 2-a). By immunofluorescent staining, the principal ganglion cells showed a very strong TH-positive reaction (Fig. 2-b). Some ganglion cells showed moderate and others almost no DBH-positive, but PNMT-negative reaction. There were also very small numbers of principal ganglion cells showing TH-negative reaction (arrow, Fig. 2-b). In addition, small numbers of TH-positive SIF cells formed clusters among the ganglion cells.

By electron microscopy, TH-positive reaction products were distributed throughout the cytoplasm and associated with granules of the principal ganglion cells, but not in the nucleus, nuclear membrane, mitochondria or endoplasmic reticulum (Fig. 2-c). Around the ganglion cells, TH-positive reactions were also found in some of the unmyelinated nerve fibers or terminals (Fig. 2-d).

The nodose ganglia:

The majority of principal ganglion cells showed TH-negative reaction, however,

FIG. 1. Immunofluorescence micrographs of the suprarenal ganglia of the dog after incubation with antiserum to TH (a), antiserum to DBH (b) or antiserum to PNMT (c). The principal ganglion cells show TH- and DBH-positive but PNMT-negative reaction. Adrenal cortex (C). $\times 94$.

FIG. 2. Photomicrographs of the superior cervical ganglia (SCG). a: Grimelius silver impregnation. Larger (arrow) or smaller (arrow head) types of argyrophilic cells are observed. $\times 440$ b: An immunofluorescence micrograph of a dog SCG after incubation with antiserum to TH. The majority of principal ganglion cells are TH-positive, and there exists a dense plexus of TH-positive fibers, whereas small numbers of principal neurons lack TH-positive immunofluorescence (arrow). $\times 192$ c, d: Electron micrographs of a SCG cell (c) and nerve fibers (d) of a dog SCG after incubation with TH-antiserum. TH-positive reaction products are observed in the ground substance, granules (arrow) in the cell (c) and in their processes (d) but neither in the nucleus (N), nuclear membrane, mitochondria or endoplasmic reticulum (c) nor in some of the unmyelinated axons or terminals (d). c: $\times 7,075$ d: $\times 12,000$

small numbers of TH-positive and DBH-negative dopamine cells were scattered in this ganglion and in the vagus nerve trunk (arrow, Fig. 3-a). These dopamine cells were mostly not clustered, round shaped and resembled to the principal ganglion cells. In the marginal portion of the ganglia, varicose fibers with TH-positive, DBH-negative immunofluorescence ran in close proximity to and surrounded the ganglion cells which showed no immunofluorescence (Fig. 3-b).

DISCUSSION

Cellular localization of three catecholamine-synthesizing enzymes, TH, DBH and PNMT in suprarenal, superior cervical and nodose ganglia of dogs were revealed in the present immunofluorescent study using specific antisera to these enzymes from bovine adrenal medulla.

In the suprarenal and superior cervical ganglia, the majority of principal neurons appeared to contain both TH and DBH but not PNMT and thus seemed to store noradrenaline. This finding supports previous reports on the superior cervical ganglia of other mammals (1).

Ultrastructurally, it was identified that the subcellular localization of TH in dog superior cervical ganglion cells was mainly in the cytoplasm and granules. This is also in agreement with some reports on TH in the adrenal medulla (12) or the noradrenaline-containing neurons in the brain (18) of rats. In addition, except TH-positive, DBH-negative ganglion cells (14), a minor cell population which contained no catecholamine-synthesizing enzymes existed in dog superior cervical ganglia. Thus, at least a part of the principal ganglion cells may be dopaminergic or non-catecholaminergic.

In the superior cervical ganglion of the rat, Verhofstad *et al.* (19) found 3 different types of SIF cells such as a dopamine-, noradrenaline-, and serotonin-storing SIF cell type. In our dog experiment, SIF cells showed a positive reaction towards anti-TH and some to anti-DBH, but not towards anti-PNMT (14). Our work is already under way as to whether there are serotonin positive cells as mentioned by Verhofstad *et al.* (19).

Previous researchers have noted the presence of SIF cells or monoamine-containing cells in the nodose ganglia of the mammals. Jacob and Comroe (4) found that these cells were clustered and much smaller than the principal neurons in the cat. Kojima *et al.* (7) mentioned that SIF cells of the rabbit nodose ganglion contained the monoamine by microspectrofluorimetric analysis, but the principal neuron was lacking in monoamine fluorescence.

In contrast, dopamine-containing cells recognized by the present immunofluorescent staining in the dog nodose ganglia were not SIF cells but seemed to be

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FIG. 3. Immunofluorescence micrographs of the dog nodose ganglion (NG), vagus nerve (V) and superior cervical ganglion (SCG). a: After incubation with TH-antiserum. The majority of principal ganglion cells in the dog nodose ganglion show TH-negative, but a small numbers of TH-positive cells exist. In the vagus nerve trunk, a few TH-positive cells (arrow) are observed. $\times 75$. b: A higher magnification of the marginal portion of the NG. TH-positive cells similar to principal ganglion cells in size or shape are observed. TH-positive nerve fibers with varicosity run among the TH-negative principal ganglion cells. $\times 192$.

the principal ganglion cells. In the present study, we chose young dogs (4 days and 14 weeks after birth) as the materials. In contrast to the adult dogs, the nodose ganglia of young dogs may contain catecholaminergic neuron, due to the immaturity or undifferentiation of the young dogs.

If these dopaminergic cells were the principal ganglion cells, our finding may be the first indication that the visceral primary afferent neurons contain dopamine as a chemical transmitter. In the nodose ganglia of rabbits and pigeons, Katz and Karten (6) found substance P-like immunoreactive cells and their processes. Substance P-positive processes encircled individual substance P-negative cells in the nodose ganglia.

Further studies are necessary on these dopamine or substance P-containing cells.

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