AROMATIC L-AMINO ACID DECARBOXYLASE (AADC)-IMMUNOREACTIVE CELLS IN THE TUBERAL REGION OF THE RAT HYPOTHALAMUS

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ABSTRACT

In the rat hypothalamic tuberal region, the distribution of aromatic L-amino acid decarboxylase (AADC)-immunoreactive cells was compared with that of tyrosine hydroxylase (TH)-immunoreactive cells. Two types of AADC-immunoreactive cells were found in the tuberal region; strongly stained cells with distinct processes and weakly stained cells visualized only after colchicine treatment. Most of the intensely AADC-immunoreactive cells were restricted to the dorsomedial arcuate nucleus and neighbouring periventricular nucleus. The second type of AADC-immunoreactive cells was found in the ventromedial and lateral parts of the nucleus. In the ventrolateral part of the arcuate nucleus and its neighbouring periarculate region, we identified a dense aggregate of TH-immunoreactive cells, but there were no AADC-immunoreactive cells in the homologous region.

Aromatic L-amino acid decarboxylase (AADC) is involved in the decarboxylation step in many amino acids. In the catecholamine synthetic pathway, AADC is the second step enzyme which converts DOPA to dopamine. According to the immunocytochemical studies by Jaeger *et al.* (9-13), AADC is contained not only in well-known monoaminergic neurons in the central nervous system, but also in many other non-monoaminergic cells such as in the spinal cord, dorsolateral parabrachial nucleus and suprachiasmatic nucleus of the hypothalamus, and this finding has recently been confirmed in the cat hypothala-

mus (14).

On the other hand, neurons in the hypothalamic tuberal region including the arcuate nucleus of the hypothalamus have been reported to show catecholamine histofluorescence and tyrosine hydroxylase (TH)-immunoreactivity in the rat and cat (1, 2, 4-6, 8, 15, 19) and are believed to be dopaminergic. In the ventrolateral arcuate nucleus, however, TH-immunoreactive cells are reported (5, 8) without any evidence for existence of catecholamine-fluorescent cells. This discrepancy has been ascribed to the low sensitivity of classical histofluorescence method. Therefore, demonstration of AADC, enzyme responsible for dopamine synthesis, in the tuberal cells has been awaited to solve this problem.

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However, detailed description of tuberal AADC-containing neurons has not yet been available, partly because of the difficulty in staining them immunocytochemically. In the present study, we used a specific and potent anti-AADC serum suitable for immunohistochemistry and revealed the distribution and cell types of AADC-immunoreactive neurons in this region. Furthermore, we compared them with those of TH-immunoreactive cells to examine the possible differences in the respective distributions of AADC- and TH-immunoreactive cells.

MATERIALS AND METHODS

Fifteen male Sprague-Dawley rats were used in this study. In some animals, colchicine (60 μ g/animal) was given into the lateral ventricle 48 h before sacrifice. Under deep pentobarbital anesthesia, animals were perfused with saline, followed by perfusion with a fixative consisting of 4% paraformaldehyde, 0.35% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, brains were removed and postfixed with 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer at 4°C for 48 h, and then immersed in 0.1 M phosphate buffer containing 20% sucrose at 4°C for 48 h. Frontal sections, 25 μ m thick, were made in a cryostat.

The sections were then sequentially immersed in 1) normal bovine serum, 2) antiserum raised against AADC (dilution 1:5,000) or TH (dilution 1:10,000) for 4 days at 4°C, 3) anti-rabbit IgG (DAKO, Denmark) (dilution 1:400) overnight at 4°C, and 4) PAP complex solution (DAKO) (dilution 1:400) for 2 h at room temperature. All sera were diluted in 0.1 M phosphate-buffered saline containing 0.3% Triton X-100 and the sections were washed for 15 min in the same solution between each step. Sections were treated with 0.02% diaminobenzidine tetrahydrochloride solution containing 0.003% hydrogen peroxide and 0.6% nickel ammonium sulfate in 0.05 M Tris buffer (pH 7.6). After the final rinse, specimens were mounted on chromogelatin-coated glass slides and examined by light microscopy and traced by a camera lucida. Nomenclatures we cited were from Paxinos and Watson (17).

The antibody to AADC was raised against enzyme purified from pig kidney (3) and characterized (14, 18). The characteristics and specificity of anti-TH serum has been described elsewhere (16).

RESULTS

Colchicine treatment not only enhanced the staining of AADC-immunoreactive cell bodies and the proximal processes, but also made possible to visualize small, weakly immunoreactive cells in the ventral part of the arcuate nucleus. Although the description below is mainly based on the data from colchicine-treated rats, it is also true for normal animals unless otherwise specified.

At the level of the retrochiasmatic area (Bregma: -1.9 mm; reference 17), numerous strongly AADC-immunoreactive cells of a medium size (long diameter: 10–16 μ m) were distributed in the periventricular and anterior hypothalamic areas (Fig. 3a), although there existed only a few labeled cells in the most rostral part of the arcuate nucleus. At the level of the anterior portion of the arcuate nucleus (Fig. 3b, Bregma: -2.3 mm; 17), intensely AADC-immunoreactive perikarya were clustered in the periventricular dorsomedial arcuate nucleus and the neighbouring periventricular nucleus (DMAR/PV) (Fig. 1, a and c). These cells were generally small (long diameter: 10–13 μ m) and ovoid or fusiform. At this level, in the other areas of the arcuate nucleus, AADC-immunoreactive cells were rarely detected even in colchicine-treated rats.

Fig. 1 Photomicrographs of AADC-immunoreactive cells (a) and TH-immunoreactive cells (b) in the anterior portion of the arcuate nucleus (Bregma: -2.3 mm, according to reference 17). (c) and (d): High-power magnifications of a part of (a) and (b), respectively. Arrowheads, example of long processes stained by anti-AADC antiserum. Bars, 100 μ m. Note the absence of AADC-immunoreactive cells in the ventrolateral part of the nucleus where many TH-immunoreactive cells are present. DMAR, dorsomedial part of the arcuate nucleus; ME, median eminence; PA, periaruate region; PV, periventricular nucleus; RCh, retrochiasmatic area; VLAR/PA, ventrolateral part of the arcuate nucleus and its neighbouring lateral periaruate region; VHM, ventromedial hypothalamic nucleus; V3, the third ventricle

Fig. 1

Fig. 2 Photomicrographs of AADC-immunoreactive cells (a) and TH-immunoreactive cells (b) in the middle portion of the arcuate nucleus (Bregma: -3.3 mm; 17). (c) and (d): High-power magnifications of a part of (a) and (b) respectively. There are no AADC-immunoreactive cells equivalent to medium-sized TH-immunoreactive cells in VLAR/PA, but small and weakly AADC-immunoreactive cells are visualized in the ventromedial and lateral parts of the nucleus after colchicine treatment. Bars, $100\text{ }\mu\text{m}$. For abbreviations, see Fig. 1 legend.

Fig. 3 Distribution of AADC-immunoreactive cells in the arcuate nucleus and its adjacent areas in the colchicine-treated rat. Displayed in rostrocaudal order from bregma -1.9 , -2.3 to -3.3 mm (17). Large black dots show the medium-sized and intensely AADC-immunoreactive cells, and small dots the small weakly stained cells. For abbreviations, see Fig. 1 legend.

Fine varicose fibers and terminals were abundantly and diffusely labeled throughout the arcuate nucleus. Dense immunoreactive terminals were seen in the external layer of ME. TH-Immunoreactive cells were detectable as clusters not only in DMAR/PV, but also in the ventrolateral arcuate nucleus and its neighbouring periarculate region, located just dorsal to the basal surface of the brain (VLAR/PA) (Fig. 1, b and d). The TH-immunoreactive cells in VLAR/PA were larger than those in DMAR/PV (long diameter; VLAR=15–18 μ m, DMAR=10–13 μ m). Although TH-immunoreactive fibers and terminals were found throughout the arcuate nucleus, their number was smaller than AADC-immunoreactive fibers and terminals.

At the level of the middle portion of the arcuate nucleus (Fig. 3c, Bregma: -3.3 mm; 17), strongly AADC-immunoreactive cells were detected in DMAR/PV (Fig. 2, a and c), as at the level of the anterior part of the arcuate nucleus mentioned above. Additionally, in the colchicine-treated rat, small and weakly AADC-immunoreactive cells (long diameter; 8–10 μ m) were found in the ventromedial and lateral parts of the arcuate nucleus. These small labeled cells were not detected in the normal rat. We observed a complete difference between the distribution of AADC-immunoreactive cells and that of TH-immu-

noreactive cells in the ventral part of the arcuate nucleus in the adjacent sections (Fig. 2, c and d). Moreover, in this region, small, weakly AADC-immunoreactive cells were round or ovoid with very short processes, but TH-immunoreactive cells were medium-sized, bipolar to multipolar with distinct immunoreactive processes.

DISCUSSION

Findings in this study are summarized as follows: 1) intensely AADC-immunoreactive cells are clustered in DMAR/PV, and 2) weakly AADC-immunoreactive cells are present in the ventromedial and lateral parts of the arcuate nucleus although this distribution and cell type are completely different from those of TH-immunoreactive cells. Jaeger *et al.* (9–13) reported that monoaminergic cells show stronger AADC-immunoreactivity as compared to non-monoaminergic cells. The intensely AADC-immunoreactive cells in DMAR/PV may be monoaminergic as TH-positive or catecholamine-fluorescent cells are also reported in this area. Since AADC decarboxylates many aromatic L-amino acid including DOPA, 5-hydroxytryptophan, tyrosine, tryptophan and phenylalanine, it is possible that AADC-immunoreactive cells are distributed more widely than catecholamine cells. Indeed, in the present study, we found small and weakly AADC-immunoreactive cells in the ventromedial and lateral parts of the arcuate nucleus as well as medium-sized strongly AADC-immunoreactive cells in the anterior hypothalamic area, in which AADC-immunoreactive cells show completely different distribution compared to TH-immunoreactive cells.

The most striking finding in our study is that AADC-immunoreactive cells are not detected in VLAR/PA where a dense aggregate of TH-immunoreactive cells is present. The result is not due to the difference in the potency of antisera used because 1) although our antiserum is raised against the purified AADC from pig kidney, the clear immunocytochemical image obtained in this study indicates that the antiserum can recognize rat AADC in cells and fibers, and 2) the distribution of AADC-immunoreactive cells stained by our antiserum in other brain areas (as mentioned above) is homologous to the other

previous reports using other AADC-antisera (9-13). It is possible that TH-positive but AADC-negative cells in VLAR/PA do not synthesize dopamine, because dopaminergic neurons should contain both TH and AADC. To our knowledge, catecholamine-fluorescent cells have not been reported in the VLAR/PA. Wide distribution of TH-positive/AADC-negative cell bodies in hypothalamic tuberal region raises the question about the validity of TH-immunocytochemistry as a definite criterion for dopaminergic cells. Application of the recently introduced immunocytochemical method in conjunction with anti-dopamine antisera (7) may solve the problem.

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Note added in proof: After submitting this article, Meister *et al.* (*J. Chem. Neuroanat.* 1, 59-64, 1988) reported the results similar to ours. However, they did not describe the small AADC-immunoreactive cells in the ventral part of the arcuate nucleus which manifest themselves in the colchicine-treated rat. This may be attributable to differences in the characteristics of antisera used and in the immunocytochemical procedures employed.

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