IMMUNOHISTOCHEMICAL LOCALIZATION OF CATECHOLAMINE-SYNTHESIZING ENZYMES AND SEROTONIN IN THE BAT BRAIN

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Monoaminergic neuron systems have been studied in frontal, sagittal and horizontal sections of the bat brain by the indirect immunofluorescent method using antibodies to tyrosine hydroxylase (TH), dopamine- β -hydroxylase (DBH) and serotonin (5-HT).

In general, the topographical distribution of monoaminergic neurons was found to be similar to that of the rat by Dahlstrom & Fuxe (3). However, some differences were found in the bat brain; namely the A3 group in the nucleus olivaris accessorius dorsalis and the A4 in the lateral part of the roof of the fourth ventricle were not observed.

The 5-HT-immunofluorescent varicose fibers and terminals were widely spread throughout the brain, especially in the supra-ependymal parts of the ventricular walls, several cranial nerve nuclei such as V, VII, and Xth nerve nuclei, and the catecholaminergic cell groups (A1, A2, A6, A9, A10). In addition, both the dopaminergic neurons and 5-HT neurons were observed in the nucleus raphe dorsalis and the ventral tegmental area caudal to the interpeduncular nucleus.

The distribution of catecholaminergic and serotonergic neuron systems was first identified in the rat brain by a modified histofluorescence method (3, 4, 5, 11, 12, 20, 23). More recently, the monoaminergic pathways were clarified in the rodent brain with immunohistochemical techniques using antibodies to the monoamine-synthesizing enzymes or to monoamines themselves (6, 7, 8, 9, 10, 16, 21, 22). The purpose of this study has been to provide an immunohistochemical map of both cerebral catecholaminergic and serotonergic neurons in the bat in order to obtain the localization of these cells in a mammalian brain.

MATERIALS AND METHODS

Specific antisera to bovine adrenal TH, DBH and antiserum directed against 5-HT were used in the present experiments. Antisera to TH and DBH were produced in rabbits and the specificity of these antisera were tested as previously described (14, 17). Antiserum to 5-HT was purchased from RIA (UK).

Bats (*Pipistrellus abramus*, 7–12 g, body weight) were anesthetized with ether and perfused with Zamboni's solution (2% paraformaldehyde-0.2% picric acid in 0.1 M phosphate buffer, pH 7.2). The brains were removed and postfixed with Zamboni's solution for 5–17 hr. Subsequently they were washed and rinsed in phosphate buffer containing 10% sucrose and frozen on dry ice. Frozen sections of 10 μ m in thickness were cut in a cryostat and processed for the indirect immunofluorescent technique (14).

RESULTS

The general appearance of the bat brain differs somewhat from that of rodents such as the rat. As shown in Fig. 1, the most striking feature of the bat brain is the notable developed tectum (Colliculus inferior).

The catecholaminergic and serotonergic cell bodies are shown in the sagittal sections of the bat brain (Fig. 1).

FIG. 1. Sagittal sections of the bat brain in which the catecholaminergic neurons [TH-positive, DBH-negative dopamine neuron (○), TH- and DBH-positive noradrenaline neuron (●)] and serotonergic neuron (▲) are shown schematically.

FIG. 2.

FIG. 3.

FIG. 4.

Medulla oblongata

TH-positive neurons in the medulla oblongata were observed mainly in two areas, the ventrolateral part and the dorsomedial part which correspond to A1 (C1) and A2 (C2), respectively, according to Swedish investigators (3, 7). The ventrolateral group was identified from the level of the pyramidal decussation to the inferior olive and most of the cells in this group were present in the nucleus reticularis lateralis. These cells were large-sized, multipolar and had long processes (Fig. 2a).

The TH-positive neurons of the dorsomedial group were observed in the nucleus tractus solitarius (Fig. 2c), dorsal vagal nucleus, the nucleus commissuralis (Fig. 2d), and continued to the level of the caudal end of the nucleus facialis in the frontal section. These cells were medium-sized, oval-shaped and were surrounded by dense varicose fibers showing 5-HT-positive immunofluorescence.

In the area postrema, TH-positive small, oval cells were observed in close apposition to either the blood vessels or to the ventricular surface (Fig. 2b). However, the A3 (3) was not observed in the nucleus olivaris accessorius dorsalis. Almost all of the TH-positive cells in the medulla oblongata were DBH-positive too.

On the other hand, 5-HT-positive cells were seen in the ventromedial section throughout the medulla oblongata. This site coincides with the nucleus raphe pallidus (B1), nucleus raphe obscurus (B2) and the nucleus raphe magnus (B3) of Dahlström & Fuxe (3). Some 5-HT-positive cells were also found outside the raphe regions, the area dorsal to the inferior olive (Fig. 6a), the area lateral to the tractus corticospinalis (Fig. 6b) and the area medial to the nucleus facialis.

Pons and midbrain

TH-positive cells in the pons formed four separate groups: the ventrolateral pontine (A5), the medial border of the lemniscus lateralis (A7), the locus coeruleus

FIG. 2. Catecholamine neurons in the bat medulla oblongata. (a) Sagittal section of the ventro-lateral cell group (A1) incubated with TH-antiserum showing large multipolar cells with long processes. $\times 190$ (b) Horizontal section showing TH-positive cells in the area postrema. Small, oval cells were observed in close apposition to the blood vessels. These cells also showed DBH-positive immunofluorescence. $\times 220$ (c) Horizontal section through the rostral part of the dorso-medial cell group [A2 (C2)] in the nucleus tractus solitarius. $\times 70$ (d) Frontal section through the caudal brainstem. TH-positive neurons and dense varicose terminals were observed in the nucleus commissuralis (A2). $\times 135$

FIG. 3. Catecholamine neurons in the pons. (a) Frontal section through the pons showing TH-positive ventro-lateral pontine cells (A5). These cells were found in the area dorsal to the superior olive (os). $\times 200$ (b) TH-positive cells of A5 in the sagittal plane. These cells were also DBH-positive. $\times 130$ (c) Frontal section through the dorsal pontine tegmentum. TH-positive, medium-sized multipolar cells were observed in the locus coeruleus (A6). $\times 190$ (d) Frontal section through the lateral part in the rostral pons. TH-positive large cells with processes were found in the medial border of the lemniscus lateralis (LL). $\times 130$

FIG. 4. Catecholaminergic and serotonergic neurons in the midbrain and the locus coeruleus. (a) Mount of the dorsal pontine tegmentum and the lateral part of the ventral tegmentum in the sagittal plane. TH-positive immunofluorescent neurons were seen in the substantia nigra (A9) and locus coeruleus (lc). $\times 50$ (c, b) Mounts of the mesencephalon in the midline of sagittal consecutive sections. (b) TH-positive immunofluorescent neurons were observed in the ventral tegmental area (A10) and in the nucleus raphe dorsalis (rd). $\times 75$ (c) 5-HT-positive immunofluorescent neurons were found in the nucleus raphe dorsalis (B7) in the caudal area (B6). Note that 5-HT-positive neurons coexisted with TH-positive neurons in the rostral part of the rd and the decussation of the superior cerebellar peduncle (DPCS). $\times 75$

(A6) and the subcoeruleus. The multipolar and large ventrolateral cells were present among the area rostral to the nucleus facialis. In the frontal section, these cells were found in the site between the superior olive and the outgoing facial nerve fibers (Figs. 3a, 3b). In the lateral pontine tegmentum, medium-sized, multipolar cells were situated at the border of the griseum pontis. This cell group was identical with the locus coeruleus (A6) (3), and its site appeared to be more ventral than that of the rat (Fig. 3c). The TH-positive cells seemed to continue rostro-ventrally, and formed less densely packed large cells of the subcoeruleus group. More laterally and rostrally from the subcoeruleus, large-sized, multipolar TH-positive cells (A7) (3) were observed at the medial border of the lemniscus lateralis (Fig. 3d).

In the midline of the midbrain-sagittal section, TH-positive, DBH-negative dopaminergic neurons were found as the two main masses, the ventral tegmental cell group (A10) and the group in the rostral part of the nucleus raphe dorsalis in the central gray (Fig. 4b). The A10 cells continued dorsally to the decussation of the superior cerebellar peduncle crossing the fasciculus longitudinalis medialis and reached the nucleus raphe dorsalis. In the lateral part of the ventral tegmentum, dopamine neurons (A8) were present in a reticular formation and continued ventrolaterally to the substantia nigra. Large, multipolar cells (A9) were observed in the substantia nigra pars compacta (Fig. 4a). Varicose dendrites of these cells spread within the substantia nigra pars reticulata.

5-HT-positive cells were found in several discrete areas in the pons and midbrain. A small number of 5-HT-positive cells was seen in the areas dorsomedial to the nucleus tegmentalis dorsalis (B6) (Fig. 6d), near the genu of the facial nerve (B4) and in the corpus trapezoideum (Fig. 6c). In the midline area beneath the rostral part of the fourth ventricle and the caudal part of the aqueductus cerebri, the largest 5-HT-positive cell group (B7) was located within the nucleus raphe dorsalis mainly (Fig. 4c). As shown in Fig. 1, both the dopaminergic cells and the 5-HT cells were observed in the rostral part of the nucleus raphe dorsalis and in the decussation of the superior cerebellar peduncle (Figs. 4b, 4c). The cells of B7 group were large, multipolar and sent their axons rostro-ventrally toward the ventral tegmentum where they turned rostrally to enter the medial forebrain bundle.

Small numbers of TH-positive cells were observed along the aqueductus cerebri in the mesencephalic-hypothalamic junction and in the caudal hypothalamus. These cells (A11) (1, 3) were large with long processes and showed DBH-negative reaction.

Hypothalamus

Hypothalamic TH-positive neurons formed two distinct groups, the dorsal hypothalamic group (A13) in the zona incerta ventral to the fasciculus mammillo-

FIG. 5. Catecholamine neurons in the hypothalamus and in the preoptic area. (a) Ventral periventricular cells (A12) in the frontal section. TH-positive, small and oval-shaped cells were found around the third ventricle (V3). Some fibers from the cells of A12 extended to the ventricular surface. $\times 110$ (b) A13 group in the sagittal section revealing TH-positive, oval cells. These cells were sparse and few in number. $\times 190$ (c) TH-positive neurons in the medial preoptic area in the sagittal section. These cells were small and oval-shaped. $\times 190$ (d) Mount of the ventral periventricular cells (A12, A14) in the sagittal plane. TH-positive small neurons with processes were localized in the arcuate nucleus (A12) and some cells (A14) were also found in the area dorso-rostral to the arcuate nucleus. $\times 190$

FIG. 5.

FIG. 6.

thalamicus and the ventral hypothalamic group (A12, A14) in the periventricular area (Fig. 5d). The cells of the A13 were very small, oval-shaped and were very few in number (Fig. 5b). The cells localized mainly in the arcuate nucleus (A12) were also oval-shaped but with long processes (Figs. 5a, 5d). Some TH-positive fibers near A12 penetrated the ventricular ependyma into the third ventricle (Fig. 5a). These cells (A12) continued rostrally to A14 group in the anterior periventricular area. More rostrally, small numbers of TH-positive neurons were found in the medial and lateral preoptic areas (Fig. 5c). TH-positive neurons could be traced until the level of the anterior commissure in the frontal section. These hypothalamic TH-positive neurons were DBH-negative.

The 5-HT-immunofluorescent cells were not found in the bat hypothalamus or in regions of the forebrain except in the pineal body. However, there were dense plexuses of 5-HT-positive varicose fibers or terminals present throughout the forebrain, especially in the suprachiasmatic region (Fig. 6e), the hypothalamus, thalamus caudate nucleus, hippocampus and the cerebral cortex. 5-HT-positive nerve fibers and terminals were also observed on the supra-ependymal surface of all ventricles (Figs. 4c, 6d, 6f).

DISCUSSION

The cellular localization of catecholaminergic and serotonergic neurons in the bat brain was similarly arranged to that of the monoaminergic cell groups found in the rat brain by histofluorescence (3, 11, 20). However, the distribution of catecholamine cell groups was more extensive in the brainstem of the bat since these cells in the bat were observed below the nucleus facialis in contrast to those observed in the rat by histofluorescence. On the other hand, A3 and A4 cells reported in the rat brain (3) were not seen in the bat brain.

The catecholamine containing cells demonstrated in the medulla oblongata of the bat most likely represent adrenergic cell groups, since their localization is the same as for adrenaline containing cells demonstrated in the rat by immunohistofluorescence (7, 10).

Although the A6 TH- and probably DBH-positive cells in the locus coeruleus of the bat were similarly situated as those reported in the rat, the A4 cell group which extended their dendrites into the fourth ventricle as observed in the rat (6) was not found in the bat.

Furthermore, the noradrenergic and dopaminergic cells of A11 observed in the

FIG. 6. Immunofluorescent micrographs of 5-HT neurons and terminals in the bat brain. (a) Sagittal section through the ventro-medial part of the medulla oblongata. 5-HT-immunofluorescent neurons (B1) were found in the area dorsal to the inferior olive (oi). $\times 190$ (b) Horizontal plane through the area lateral to the tractus cortico-spinalis displaying 5-HT-positive neurons (B1) and varicose fibers. $\times 190$ (c) Sagittal section through the ventral part of the ponto-medullary junction. 5-HT-positive cells were found in the area ventro-medial to the nucleus facialis and in the corpus trapezoideum (CT). $\times 80$ (d) Sagittal section of the dorsal pontine tegmentum. 5-HT-positive neurons (B6) were found below the upper surface of the pontomesencephalic junction. 5-HT-positive varicose terminals were also found in the supra-ependymal part of the fourth ventricle (V4). $\times 75$ (e) Suprachiasmatic region in the sagittal plane. Note the very strong 5-HT-immunofluorescence of varicose structures. $\times 200$ (f) Frontal section through the lateral ventricle (Vla). 5-HT-immunofluorescent nerve terminals were found in the ventricular wall. $\times 250$

rat by microspectrofluorometry (1) were not observed in the bat, since they were found to be PNMT- and DBH-negative. Nevertheless, these findings are consistent with the results obtained by immunohistochemical investigation of rats (8, 16, 22).

The occurrence of dopamine and 5-HT containing neurons in the nucleus raphe dorsalis is in agreement with the reported findings of these cells in the rat (15, 16, 18).

The 5-HT-positive cells reported in the area postrema and the locus coeruleus of the rat (21) were scarcely seen in these areas of the bat.

As far as the serotonergic innervation of the ventricular wall is concerned, it was found to be same as in the rat (13, 21). In addition, the catecholaminergic varicose structure in the ventricular wall near the locus coeruleus and A12 group observed in rat by us (Nagatsu, I. *et al.*, unpublished data) was also seen in the present study of the bat. These observations raise the possibility of 5-HT and catecholamine involvement in the regulation of either formation of cerebro-spinal fluid (CSF) and/or barrier between CSF and blood.

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Abbreviations used in Figures.

ap	area postrema
ar	nucleus arcuatus
CA	commissura anterior
CC	canalis centralis
CI	colliculus inferior
co	nucleus commissuralis
CP	commissura posterior
cp	nucleus caudatus-putamen
CS	colliculus superior
CT	corpus trapezoideum
DPCS	decussatio pedunculi cerebellarium superiorum
FLM	fasciculus longitudinalis medialis
EM	fasciculus mamillothalamicus
FR	fasciculus retroflexus
hl	nucleus habenulae lateralis
hm	nucleus habenulae medialis
ip	nucleus interpeduncularis
lc	locus coeruleus
LL	lemniscus lateralis
ntm	nucleus tractus mesencephali

nts	nucleus tractus solitarii
nIII	nucleus motorius nervi oculomotorii
nVII	nucleus nervi facialis
nXII	nucleus nervi hypoglossi
oi	nucleus olivaris inferior
os	nucleus olivaris superior
OT	tractus opticus
POA	area preoptica
PCS	pedunculus cerebellaris superior
rd	nucleus raphe dorsalis
sl	nucleus lateralis septi
SM	stria medullaris thalami
vl	nucleus vestibularis lateralis
Vla	lateral ventricle
V3	third ventricle
V4	fourth ventricle
III	nervus oculomotorius
VII	nervus facialis