DISTRIBUTION OF DOPAMINE TRANSPORTER IN THE RAT: AN IMMUNOHISTOCHEMICAL STUDY

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Objective. To investigate the organ distribution of dopamine transporter (DAT) in rats by immunohistochemical method.

Methods. Dopamine transporter (DAT) was identified immunohistochemically in the tissues using specific antipeptide antiserum raised in New Zealand white rabbits immunized with a conjugate of synthetic DAT peptide (29-45) with bovine serum albumin. Immunohistochemical analysis was performed by avidin-biotin complex method.

Results. DAT immunoreactivity was visualized in the neural perikarya, axons and dendrites of the central nervous system, retina, adrenal medulla, Auerbach's nervous branch and Meissner's nervous branch of the stomach, small intestine and colon, anterior pituitary, and lung. When using antiserum preincubated with synthetic DAT peptide (DAT, 29-45) or hypothalamus homogenate which contains DAT, no significant stain of neurons in the hypothalamus was detected.

Conclusion. These findings suggest that DAT is widely distributed and that the method used is valuable in studying the distribution of DAT in rats.

Key words: Dopamine transporter – Immunohistochemistry – Organ distribution – Rats

Recently, rat dopamine transporter (DAT) cDNA has been isolated (Giros et al. 1989; Kilty et al. 1991; Usdin et al. 1991; Shimada et al. 1992). DAT is 619 amino acid protein with 12 hydrophobic putative membrane domains which terminates dopaminergic transmission by sodium and chloride dependent re-accumulation of dopamine into presynaptic neurons (Horn 1990). Using *in situ* hybridization, DAT mRNA was found in the ventral midbrain neurons and hypothalamus neurons (Shimada et al. 1992; Meister et al. 1993). Repeated administration of cocaine induced down-regulation of DAT levels in the dopaminergic neurons of substantia nigra (Xia et al. 1992). Therefore, it is of interest to study the distribution of DAT in the rat body. However, since the precise distribution of DAT has not yet been reported, we investigated its distribution in rats using a specific antibody to DAT which was raised in rabbits with a conjugate of synthetic DAT protein fragment (DAT, 23-41) to bovine serum albumin.

Materials and Methods

Animals: Male Wistar rats weighing 250-280 g were housed in a temperature (22 °C) and humidity (60 %) controlled room under 12 h illumination cycle. They were fed with a laboratory chow and water ad libitum.

Preparation of anti-DAT antiserum: Peptides corresponding to the following sequences of the DAT were synthesized using a solid phase method and automated peptide synthesizer, followed by purifi-

Table 1

Distribution of dopamine transporter in the rat organs

Hypothalamus

n. paraventricularis, n. periventricularis, n. supraopticus, n. hypothalamicus anterior, n. preopticus suprachiasmatis, n. suprachiasmaticus (internal, external), area retrochiasmatica, n. arcuatus (I,II,III,IV,V), n. ventromedialis (anterior, pars med. ant., pars lat. ant., pars med. post., pars lat. post.), n. dorsomedialis (pars dorsalis, pars ventralis), n. perifornicalis, n. hypothalamicus posterior, medial forebrain bundle

Limbic system

limbic cortex, piriform cortex, entrohinal cortex, rostral limbic nuclei, olfactory tubercle, n. tractus diagonalis, n. interstitialis terminalis (dorsal, ventral), n. interstitialis striae medularis, area amygdaloidea anterior,

septal nuclei

n. septalis (medialis, dorsalis, lateralis, intermediate, triangularis)

Amygdaloid nuclei

n. amygdaloideus (medialis, lateralis, centralis, basalis), n. tractus olfactorii lateralis

Mammillary body

Other regions

n. paraventricularis, n. posterior thalami, n. nureins, n. lateralis thalami, n. reticularis thalami, substantia nigra, n. caudatus, caudate putamen, pallidum, n. ruber, n. raphe, locus coeruleus, cerebellum, spinal cord, retina

cation with HPLC: PREVLILVLKEQNGVQLT. The peptides were conjugated an equal weight basis to bovine serum albumin by the method previously described for anti-GHRH antibody (Mitsuma et al. 1986), using glutaraldehyde. New Zealand white rabbits were immunized with the emulsion of one mg of this conjugate in one ml water in complete Freund's adjuvant (1:2, v/v) which was injected into the foot pad at intervals of three weeks. Blood was drawn one week after each injection. The presence of anti-DAT was checked.

Preparation of tissue for DAT estimation: DAT contained fraction was obtained by the method of Grigoriadis et al. (1989). The hypothalami were removed from the rat and a pool of hypothalami weighing 200 mg was homogenized in 10 ml of cold buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM phenylmethylsulfonyl fluoride). The homogenate was centrifuged at 600 x g and the obtained supernatant was then centrifuged at 20,000 rpm for 10 min at 4 °C. The pellet was resuspended in 10 ml buffer and centrifuged again at 20,000 rpm for 10 min at 4 °C. The final pellets were resuspended in above buffer to working concentration of 20 mg/original wet weight and was used as DAT contained fraction.

Perfusion method and immunohistochemical method: The rats were anesthetized with sodium pentobarbital and transcardially perfused with 0.01 % glutaraldehyde and 4 % paraformaldehyde in Bouin's solution (pH 7.2). The brain, spinal cord, retina, pituitary gland, lung, heart, kidney, liver, thyroid, pancreas, testis and muscle were removed and post-fixed for additional hours at 4 °C, then cut at 4 μm using a vibratome.

Immunohistochemical treatment was performed by avidin-biotin complex (ABC) method, using Vecstatin kits (Vector Laboratories Inc., Burlingam, CA, USA). The primary antibody was used after dilution (1:50). To confirm the specificity of DAT antibody, the following methods were used: 1. omission of the primary antisera or the secondary antiserum in the peroxidase anti-peroxidase technique; 2. preabsorption of the antisera prior to the incubation of experimental tissues with hypothalamus homogenate containing DAT or DAT protein fragment (29-45) (1.0 mg/ml antisera). Specific immunohistochemical stain could not be seen in any of these control paradigms (Fig. 1); 3. serial dilution of primary antisera was used. Specific stain disappeared at 1:10000 dilution. The brain nuclei were determined using the map by Pellegrino et al. (1969).

Results

Specific DAT staining was found in the central nervous system, anterior pituitary, Auerbach's nervous system, Meissner's nervous system of the stomach, small intestine and colon, adrenal medulla and lung, but not in the liver, kidney, heart, testis, pancreas, thyroid gland, posterior pituitary and muscle (Tab. 1, Fig. 1-3). In the central nervous system, significant stain revealed neural perikarya, axons and dendrites.

Discussion

The distribution of DAT immunoreactivity in the rat body was estimated immunohistochemically. Anti-DAT antibody was raised in New Zealand white rabbits by repeated injection of synthetic peptide (DAT, 29-45) conjugated to bovine serum albumin

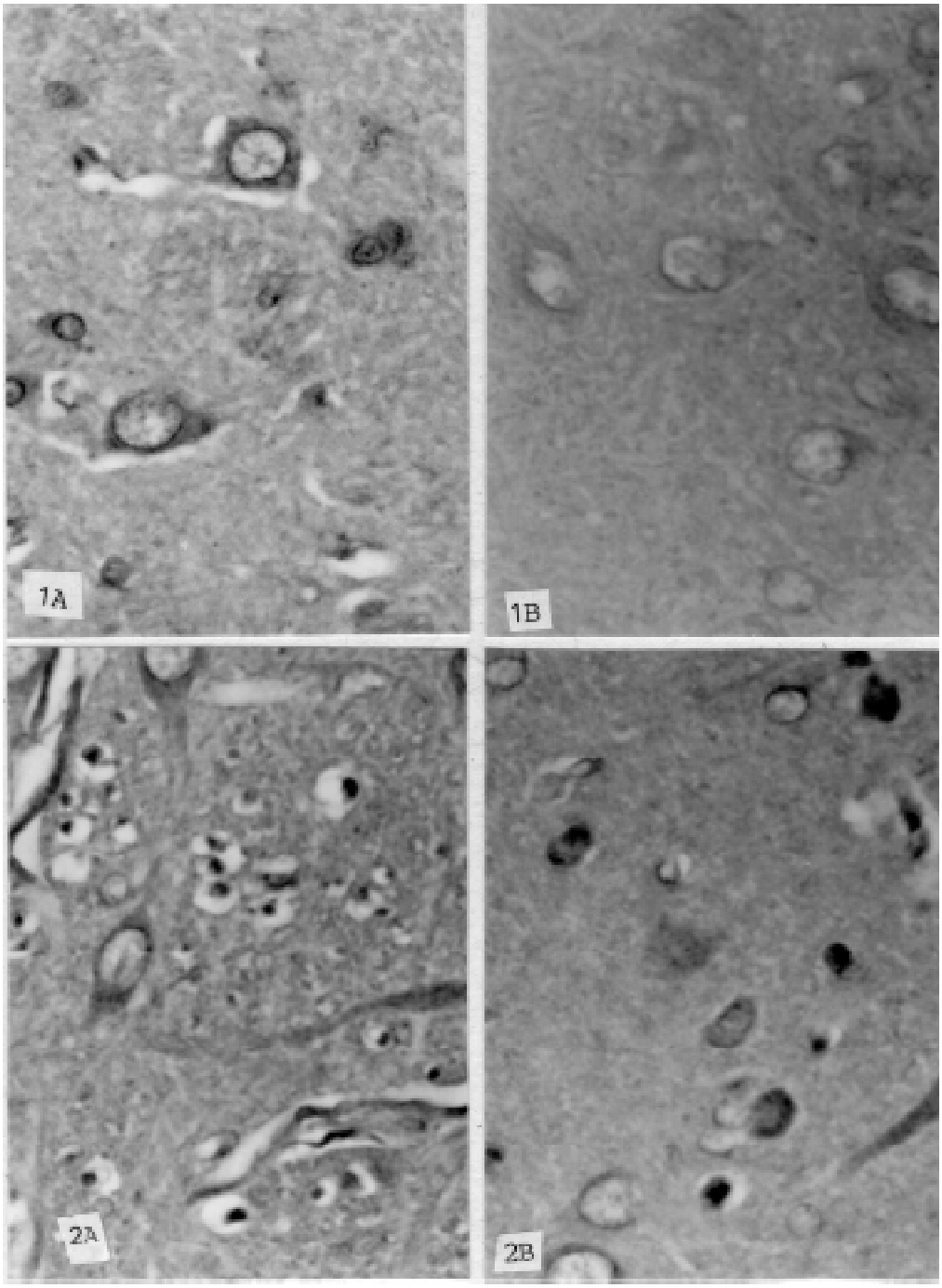

Fig. 1
Dopamine transporter (DAT) in the cerebrum with (A)and without anti-DAT antibody. Without anti-DAT antibody satin almost completely disappeared.

Fig. 2
Distribution of DAT immunoreactivity in the central nervous system. A: medulla oblongata, B: cerebrum

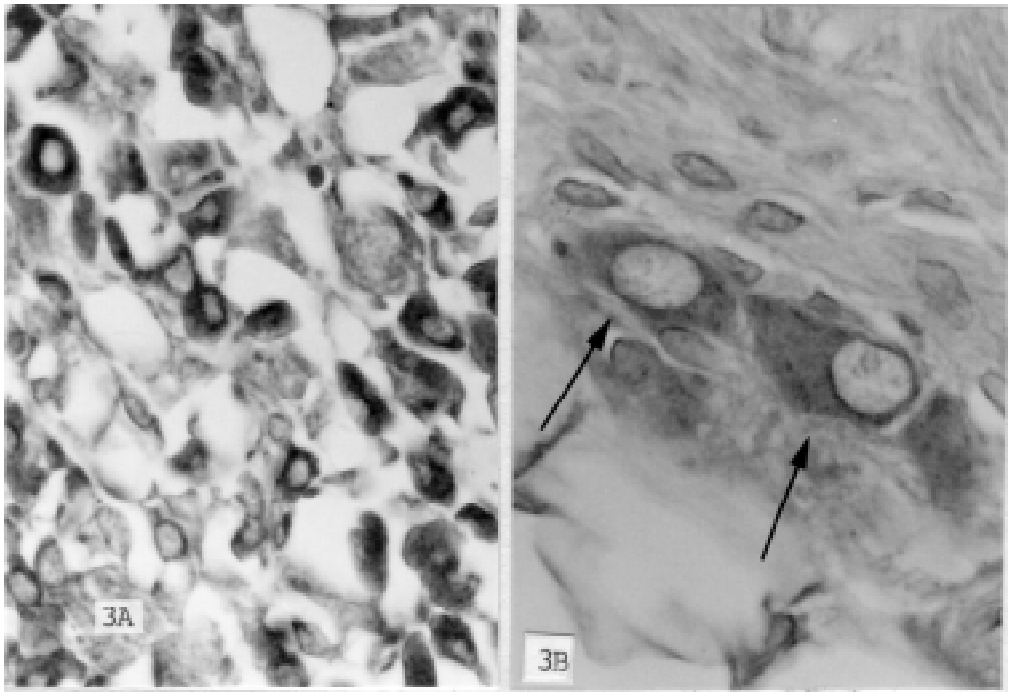

Fig. 3
Distribution of DAT immunoreactivity in the anterior pituitary and Auerbach's nervous branch of the stomach
A: anterior pituitary, B: Auerbach's nervous branch of the stomach. Arrow indicates positive satining cells

with complete Freund's adjuvant. This antibody was characterized by the immunohistochemical method, DAT immunoreactivity being specifically eliminated by the incubation of antiserum with an excessive amount of synthetic peptide (DAT, 29-45) or hypothalamus homogenates containing DAT fractions. The significant stain was not detected without this antiserum and, moreover, the dilution of antibody reduced the significant stain. These data indicate that the antiserum is specific and that this method can be used to detect the distribution of DAT in the rat body.

The present study clearly demonstrated that DAT is widely distributed in the rat body. In the central nervous system, it was found in the perikarya, dendrites and axons of the midbrain, pons, medulla oblongata, hypothalamus, cerebrum and cerebellum. These data are partly comparable with the previous reports in which DAT mRNA was found (Xia et al.; Shimada et al. 1992; Bannon et al. 1992; Meister et al. 1993). The present study confirmed these findings at the protein levels. These regions are also known to be found dopamine neurons (Brownstein et al. 1974; Palkovits et al. 1974). DAT is reported to play an important role in dopaminergic transmission (Horn 1990). It has been also reported that DAT mRNA in the substantia nigra was changed after cocaine treatment and deceased with age (Xia et al. 1992; Bannon et al. 1992). We demonstrated the presence of DAT in neural structures of gastrointestinal tract and lung, anterior pituitary in which DAT may play a certain physiological role. The data presented suggest that DAT plays an a important role in the dopaminergic system.

Moreover, it is widely distributed in the rat body as demonstrated by the histochemical method used which proved to be useful to study the organ distribution of DAT.

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