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Effects of DSP-4 on the Demonstration of Dopaminergic Terminals in Rat Frontal Neocortex by Immunohistochemistry for Tyrosine Hydroxylase and Dopamine-β-Hydroxylase

Kikuko IMAMOTO

Department of Anatomy, Shiga University of Medical Science

The effects of DSP-4 on the demonstration of dopaminergic (DA) terminals were examined in the rat frontal neocortex by means of immunohistochemistry for tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH), and by electronmicroscopy.

The specific neurotoxic effect of DSP-4 on noradrenaline (NA) terminals was clearly noted on day 2, 3 and 4 after the 2nd i. p. injection of the compound (50mg/kg). Using a computer image analysis system, the number of DBH immunopositive varicosities was calculated in a given area of 14000μm^2 : 40 in layer I and 20 in the other layers in the control neocortex, while apparently decreasing to zero in the DSP-4 injected rat. A few thick non-terminal axons were left as a sign of the abnormality. Concomitantly, TH immunopositive terminals also decreased in number in the fields innervated by NA fibers, while a half of them in the prelimbic, anterior cingulate and agranular insular neocortices remained. They were regarded as DA fibers showing the regional and laminar distribution of the mesocortical DA system. The density of DA varicosities in this system was highest in layers V/VI of the prelimbic area (76/14000μm^2) and high in layers II/III of the ventral and dorsal parts of the anterior cingulate cortex (70/14000μm^2). Although DSP-4 induced a pronounced depletion of DBH immunoreactivity, degeneration of NA fibers was hardly noted at the level of the ultrastructure.

Key words: DSP-4, DA terminal, immunohistochemistry, tyrosine hydroxylase, dopamine-β-hydroxylase, frontal neocortex

Introduction

It is well known that the cerebral cortex receives a number of aminergic fibers, such as noradrenaline (NA), serotonin (5-HT) and dopamine (DA) ones arising from the nuclei in the brainstem. NA and 5-HT fibers innervate

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今本喜久子

Mailing address : Shiga University of Medical Science, Otsu, Shiga 520-21, JAPAN

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nearly the entire cortex forming a network. They show a uniform distribution over the different cortical subareas with only minor regional difference in comparison with DA terminals (Ader et al., '80 ; Lidov, et al., '80 ; Lindvall et al., '78). Concerning DA terminals in the cerebral cortex, a diffuse distribution organizing a reticular system similar to the other aminergic ones was reported in early experiments. Up to date, however, the pattern of the origin and projection of DA terminals was pursued in detail. Then, the previous investigators came to an agreement about the regional and laminar specificity of DA distribution in the frontal neocortex, being summarized as the mesocortical DA system (Berger et al., '76 ; '85 ; Björklund and Lindvall, '84 ; Descarries et al., '87; Kalsbeek et al., '87). Namely, DA terminals innervating the layers of the anteromedial frontal (pregenual), anterior cingulate (supragenual) and dorsal agranular insular (suprarhinal) areas in the neocortex are components of the so-called mesolimbocortical DA terminal system (Björklund and Lindvall, '84 ; Palkovits et al., '79).

From a neuroanatomical point of view, it is very interesting to examine the synaptology in the areas included in the mesocortical DA system. Not only DA terminals from the ventral tegmental area (VTA) and substantia nigra (SN), but also other non-DA inputs from the thalamus, striatum, posterior cingulate, amygdala and hypothalamus may be connected with each other in the mesocortex for functions related to the limbic system (Berger et al., '85, Björklund and Lindvall, '84 ; Divac et al., '78 ; Kalsbeek et al., '87; Krettek and Price, '77 ; Lindvall et al., '78 ; Swanson '82 ; Van Eden and Uylings, '85 ; Vogt and Miller, '83). In order to establish the functional property of DA terminals and their fiber connections, it may be necessary to improve methods allowing for precise identification of DA terminals or selective destruction of the other aminergic ones.

Recently, Jonsson et al. ('81, '82) have introduced that DSP-4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride) is a useful chemical denervation tool specific for NA terminals in the cerebral cortex. They mentioned that this compound is capable of crossing the blood-brain barrier (BBB) and the blood-placenta barrier (BPB) and induced a marked reduction of NA content in the cerebral cortex of the adult and developing brains. The functional mechanism of DSP-4 producing neurotoxic effects on NA terminals is still obscure. Although Fritschy and Grzanna ('89) stressed different sensitivity of noradrenergic axons to DSP-4 due to distinct differences in their morphology and their topographic projections, several investigators have already used it as a denervation tool and obtained good results at least in the works on DA terminals of the cerebral cortex (Berger et al., '85 ; Lindvall et al., '84 ; Jonnson et al., '81, '82). Thus, the neurotoxic effect of DSP-4 on NA terminals seems now to be beyond any doubt, but the ultrastructural changes in NA terminals have not yet been reported. Before using DSP-4 for synaptology of aminergic terminals, it might be necessary to confirm the degenerating profiles induced by this compound at the level of electronmicroscopy. The present study was undertaken in order to establish the effects of DSP-4 on monoaminergic terminals in the cerebral cortex using immunohistochemical and electronmicroscopical techniques.

Materials and Methods

Sixteen male Sprague-Dawley rats weighing 200-250g were used in this experiment. DSP-4 powder (Res. Biochem. Incorp., Mass, U. S. A.) was dissolved in the physiological saline and injected intraperitoneally into rats. Three different dosages were tried : 1) one injection of 60mg/kg b. w., 2) two injections of 60mg/kg with a 24h interval, 3) two injections of 50mg/kg with a 24h

interval. Under anesthesia, the rats were perfused with 4% paraformaldehyde in 0.12 M phosphate buffer 8 hours, 1, 2, 3 and 4 days after the last injection. The brains were kept in the same fresh fixative for 2h at 4°C and soaked in 10% sucrose phosphate buffer overnight. Cryostat sections of 10μm in thickness were cut serially and mounted on gelatine coated slide glasses. Immunostaining for TH and DBH was done by the complete streptavidin-biotin method. First incubation : anti-TH antibodies diluted to 1/4000 in PBS and anti-DBH antibodies diluted to 1/2000, overnight in a humid box at room temperature. Second incubation : donkey biotinylated anti-rabbit Ig diluted to 1/200 in PBS, for 2h at room temperature. Third incubation : streptavidin peroxidase complex diluted to 1/400, for 2h at room temperature. Reaction products were visualized with a mixture of 0.05% of 3, 3'-diaminobenzidine in 0.12M PB, to which 0.025% of cobalt chloride, 0.02% of nickel ammonium sulfate and 0.05% hydrogen peroxide were added. Immunopositive varicosities were counted in a given area measuring 14000μm^2 of the various cortical regions with the help of a computerized image analyzer (Nexus 6400). Some of the 100μm-vibratome sections were postfixed with 1% osmium tetroxide for 1 h and prepared for epon-embedded materials. Ultrathin sections were examined under an electron microscope after double metallic staining.

Results

1) *The numbers of TH and DBH positive varicosities in the control rat*

In the present experiment, the neocortex was examined at three levels of the frontal plane, namely, the pregenual, just genual and rostral supragenual parts of the corpus callosum. The number of immunopositive varicosities was calculated in the 5 cortical subareas of each frontal plane shown in Fig. 1 (PL, ACv, ACd, Fm, FPss, AId) using a computer image analysis system (Nexus 6400). The topography of the different cortical subareas was derived from several sources (Berger et al., '85 ; Krettek and Price, '77; Palkovits et al., '79 ; Van Eden and Uylings, '85).

In the control rat, TH immunopositive varicose fibers were most numerous in layers V/VI of the PL and layer I of the ACv and ACd (110/14000μm^2). The high density observed in layers V/VI of the PL extended to the deep layers of the supragenual part of the ACv and ACd along the corpus callosum with a gradient decreasing from the rostral to the caudal and from the medial to the lateral. Then, in the vicinity of the rhinal fissure, the suprarhinal area (AId), a moderate number of TH immunopositive fibers were again observed. The number of TH positive fibers in layer I also decreased gradually from the ventral (Fig. 2A, 3A : PL, ACv) to the dorsal parts (Fig. 4A : Fm, FPss). In layers II/III of the ACv and ACd, well-ramified arborous fine varicose fibers were densely distributed (Fig. 3A). Such a characteristic innervation of TH positive fibers was not seen in layers II/III of the PL and in the posterior cingulate cortex.

On the other hand, the number of DBH immunopositive fibers was moderate in layer I of most parts of the cerebral cortex (40-50/14000 μm^2) and sparse in other layers (20-30/14000μm^2). The regional and laminar distribution of DBH positive terminals was not so distinct as in the case of the TH positive ones, although the density of DBH varicosities was slightly higher in the inner half of layer I. As DBH is a synthetic enzyme for NA, DBH immunopositive terminals may correspond to NA terminals. In double labeling experiments using our antibodies (TH and DBH), all the DBH positive fibers also became TH positive (Berger et al. : unpublished observations). Thus, TH immunohistochemistry suggests that not only DA fibers but also NA ones contain DA as the precursor for NA. Since the density of the TH varicosities was almost equal to that of

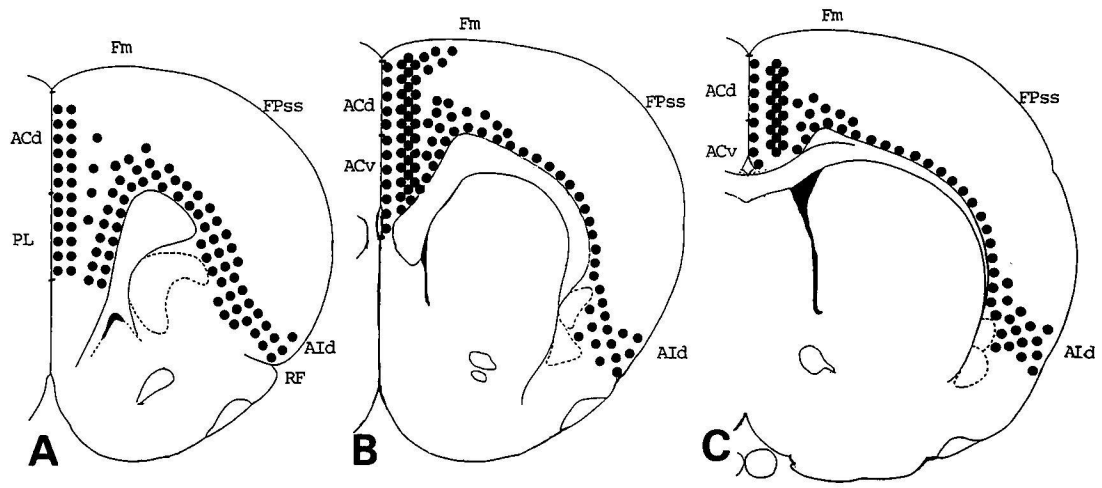

Fig. 1. Schematic representation of the distribution of DA varicosities in the cerebral cortex of a rat after DSP-4 injection, depicted on three frontal planes : pregenual (A), just genual (B) and supragenual (C) parts. The density of DA varicosities is high in the PL, ACv, ACd and AId, corresponding well to three DA terminal systems of the pregenual, supragenual and suprarhinal parts.

Abbreviations
ACd : anterior cingulate cortex pars dorsalis
ACv : anterior cingulate cortex pars ventralis
AId : agranular insular cortex pars dorsalis
Fm : frontal cortex motor area
FPss : frontoparietal cortex somatosensory area
PL : prelimbic area
RF : rhinal fissure

the DBH ones in the superficial layers of the Fm and FPss (Fig. 4), DA terminals are probably rare in these areas. In the other parts of the subareas, the density of DA terminals seemed to predominate over that of NA ones. The results of varicosity counting are summarized in Table 1.

2) *Alteration in DBH immunopositive terminals*

In order to induce a complete reduction of DBH immunoreactivity, a dosage of 60 mg/kg was insufficient, and that of 60 mg/kg x2 was too much for rats to survive for a few days. An optimum

Table 1. Numbers of TH and DBH immunopositive varicosities in a given area measuring 14000μm^2 of various cortical fields of a control rat, obtained by a computer image analysis system (Nexus 6400)

field \ layer	I	II/III	V/VI
A. PL	98 (49)	66 (28)	109 (28)
ACd	90 (42)	68 (26)	91 (23)
Fm	48 (47)	29 (30)	65 (15)
FPss	39 (35)	50 (30)	35 (12)
AId	67 (33)	50 (25)	61 (23)
B. ACv	105 (50)	84 (21)	97 (27)
ACd	96 (49)	79 (25)	89 (21)
Fm	43 (41)	23 (22)	62 (16)
FPss	38 (37)	31 (21)	29 (15)
AId	73 (44)	57 (19)	68 (19)
C. ACv	105 (43)	98 (19)	80 (17)
ACd	101 (47)	93 (27)	86 (23)
Fm	55 (42)	40 (24)	52 (27)
FPss	48 (39)	33 (26)	35 (19)
AId	67 (35)	55 (21)	64 (21)

TH (DBH)

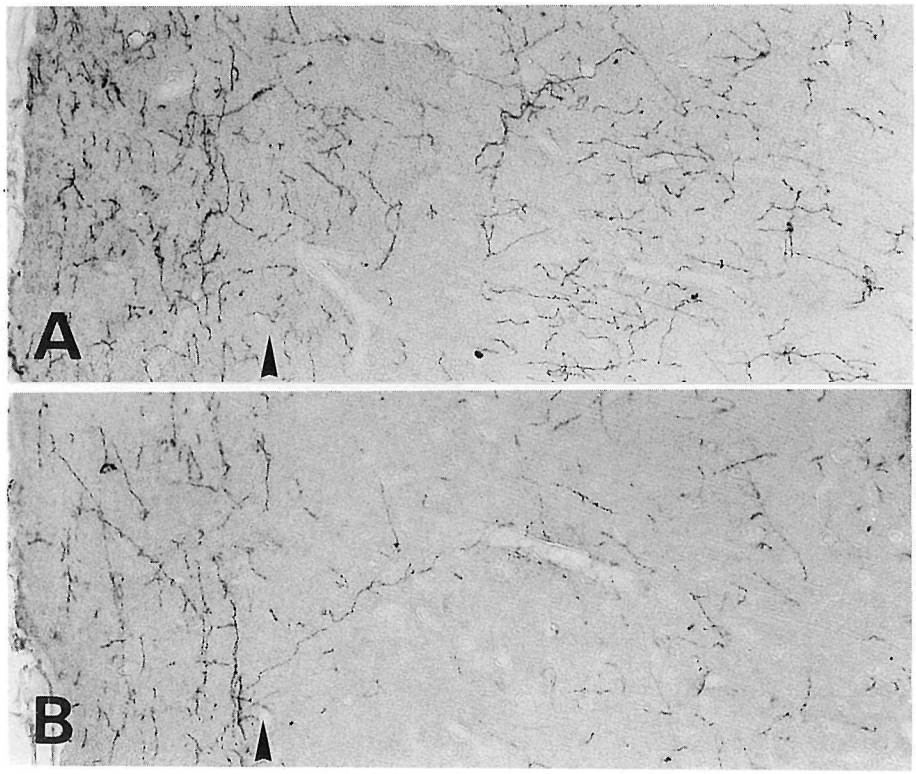

Fig. 2. Photomicrographs of TH (A) and DBH (B) immunoreactivity in the superficial layers (I-III) of the PL of the control rat. The arrowhead indicates the border between layers I and II. ×220

result showing a marked depletion of DBH immunoreactivity and sufficient TH immunoreactivity in DA terminals was obtained in rats receiving two injections of 50 mg/kg and sacrificed after 2, 3, and 4 days (Fig. 5, 6).

DBH immunopositive fibers decreased in number throughout the cerebral cortex after DSP-4 injection. Only a few DBH positive thick nonterminal axons were occasionally observed as a sign of abnormality in the deeper area of layer I and layers II/III (Fig. 6B). In the fronto-parietal cortex, all of the DBH and TH immunopositive fibers disappeared concomitantly except for layer VI. The TH positive fibers in these areas were considered to be NA fibers containing DA as the precursor of NA.

3) *Alterations in TH positive terminals in the cerebral cortex*

TH immunopositive fibers corresponding to NA fibers appeared to reduce TH immunoreactivity after DSP-4 injection. However, the activity in DA fibers was almost unaffected. Thus, the distribution of DA fibers in the neocortex was made clearly evident. The density of DA terminals was moderate in layer I of the PL, ACv and ACd (40-50/14000μm^2), almost equal to that of the NA fibers in layer I of the control, but rather higher in layers V/VI (60/14000μm^2). The

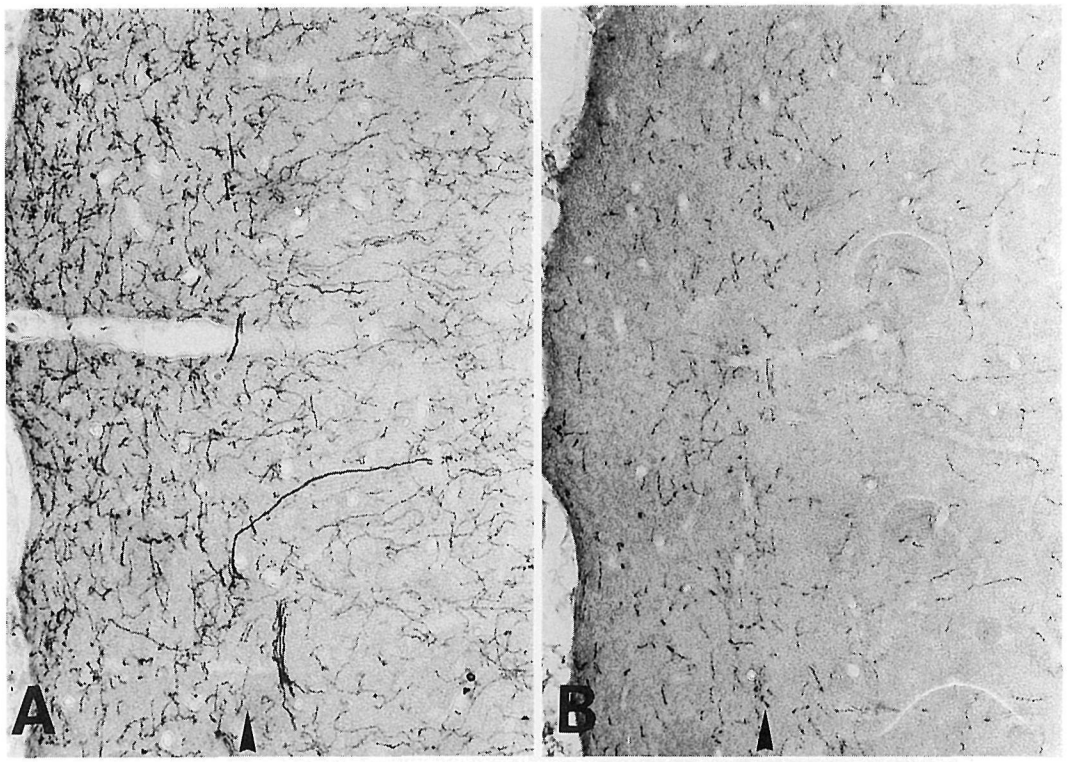

Fig. 3. Photomicrographs of TH (A) and DBH (B) immunoreactivity in the superficial layers (I-III) of the medial frontal from ACv to AVd of the control rat. The TH immunopositive fibers densely innervating layers II/III mainly consist of DA ones because there are only a few DBH positive fibers in this area. ×220

Table 2. Numbers of TH and DBH immunopositive varicosities in a given area measuring 14000μm^2 of various cortical fields of the rats 2 and 3 days after i. p. injection of DSP-4, obtained by a computer image analysis system

field \ layer	I	II/III	V/VI
A. PL	39 (8)	31 (0)	76 (5)
ACd	48 (4)	47 (0)	61 (2)
Fm	5 (1)	2 (0)	65 (0)
FPss	2 (0)	0 (0)	30 (3)
AId	32 (2)	26 (0)	31 (1)
B. ACv	45 (5)	64 (0)	55 (4)
ACd	47 (3)	68 (2)	50 (3)
Fm	2 (3)	68 (2)	50 (2)
FPss	1 (4)	0 (0)	33 (3)
AId	24 (2)	25 (0)	35 (0)
C. ACv	52 (12)	75 (1)	54 (2)
ACd	45 (1)	70 (2)	61 (0)
Fm	3 (0)	0 (0)	22 (3)
FPss	2 (0)	0 (0)	28 (2)
AId	38 (1)	31 (0)	36 (6)

TH (DBH)

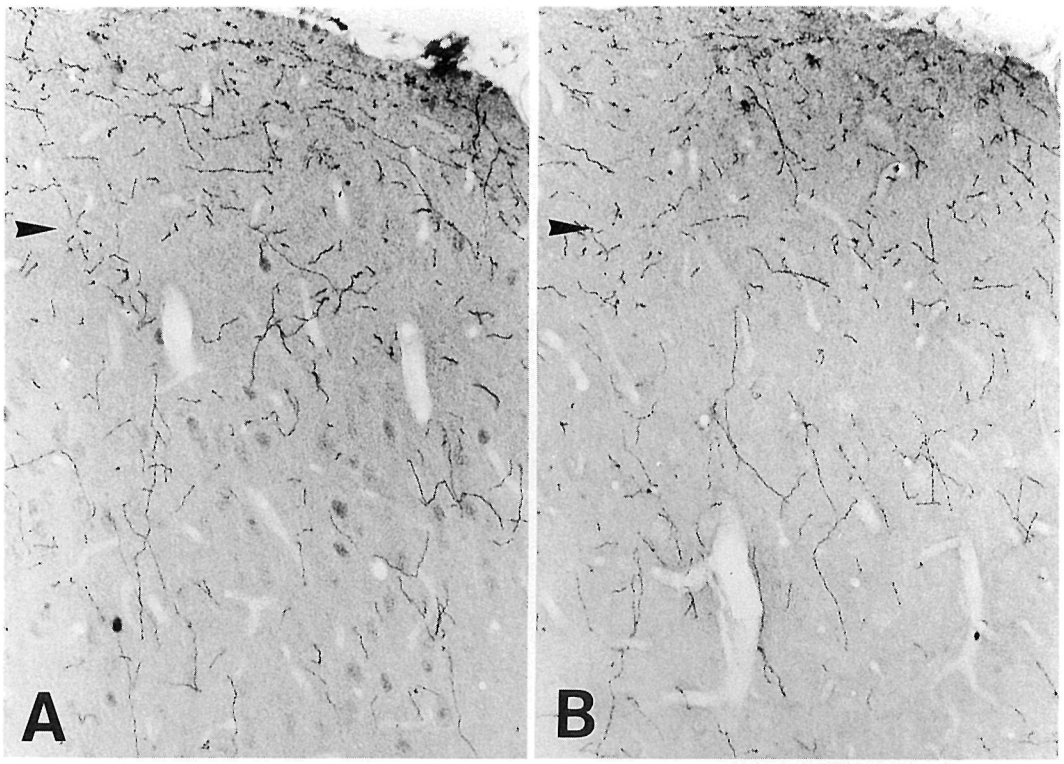

Fig. 4. Photomicrographs of TH (A) and DBH (B) immunoreactivity in the somatosensory area of the fronto-parietal cortex of the control rat. The pattern of distribution of TH immunopositive fibers is quite similar to that of DBH ones, suggesting that all of them are NA fibers. ×220

highest density of DA terminals was seen in layers V/VI of the PL and layers II/III of the ACv and ACd (70/14000μm^2). Arborization of DA fibers in layers II/III of the anterior cingulate cortex was clearly visible after DSP-4 treatment. Ramification of DA fibers extended laterally from these areas to layers II/III of the motor area(Fm : Table 2, Fig. 1). However, this dense innervation of DA fibers seemed to be restricted to these areas. The results of varicosity counting in DSP-4 injected rats are summarized in Table 2 and the DA terminal distribution is depicted schematically in the form of maps (Fig. 1).

4) *Ultrastructural changes of NA terminals in the cerebral cortex*

Since a severe reduction of DBH immunoreactivity was confirmed in the cortex of the DSP-4 injected rat, ensuring terminal degeneration was expected. In fact, a few abnormal terminals containing dense inclusion bodies or swollen vesicles were observed in layer I of the ACd and Fm and FPss (Fig. 7). However, the frequency of such abnormal terminals in this layer was absolutely low in comparison with the number of NA varicosities counted in the control. Therefore, the features of degenerating NA terminals were left undetermined.

Discussion

The distinct regional and laminar distribu-

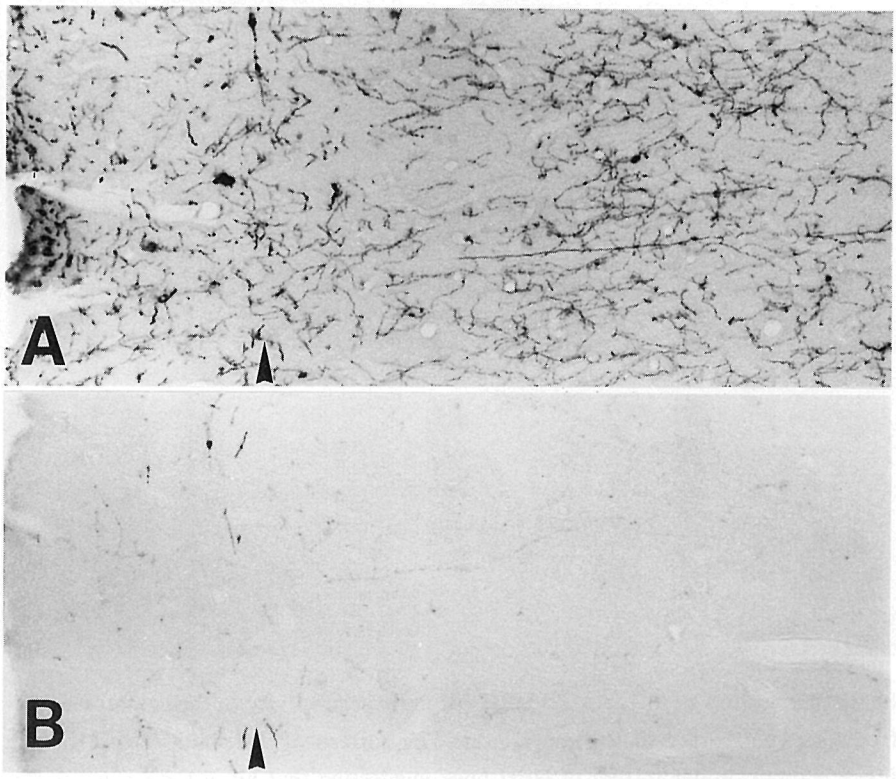

Fig. 5. Photomicrographs of TH (A) and DBH (B) immunoreactivity in the ACv of a DSP-4 injected rat, 2 days after the second injection. DBH immunoreactivity markedly reduced in all cortical layers (B), while TH immunoreactivity in DA terminals remained unchanged in layers II/III (A). ×220

tion of DA terminals in the neocortex has been the subject of much speculation concerning the functions of DA including not only the transmitter or modulator property but also others implicated with stereotyped behavior, motor function, feeding, clinical syndromes such as schizophrenia and Parkinsonism, and the neurotrophic role in the development of the cortex (Berger et al, '85 ; Björklund and Lindvall, '84 ; Kalsbeek et al., '87). In order to provide evidence to support such speculation, it is necessary to improve the reliability of methods for identification of DA, NA and 5-HT terminal profiles in the CNS. For this purpose, several neurotoxic compounds have already been tried. Neurotoxic compounds such as 6-hydroxydopamine, 5, 6- and 5, 7-dihydroxytryptamine have frequently been used as experimental tools to induce denervation of both central and peripheral catecholamine and serotonin neurons (Baumgarten et al., '71, '73 ; Malmfors & Thoenen, '71). Although these compounds are of great value in experimental work on monoamine neurons, their use seems to be limited because of their non-specific effects on aminergic neurons and inability to cross the BBB. In the search for new monoamine neurotoxins, Ross ('76) and

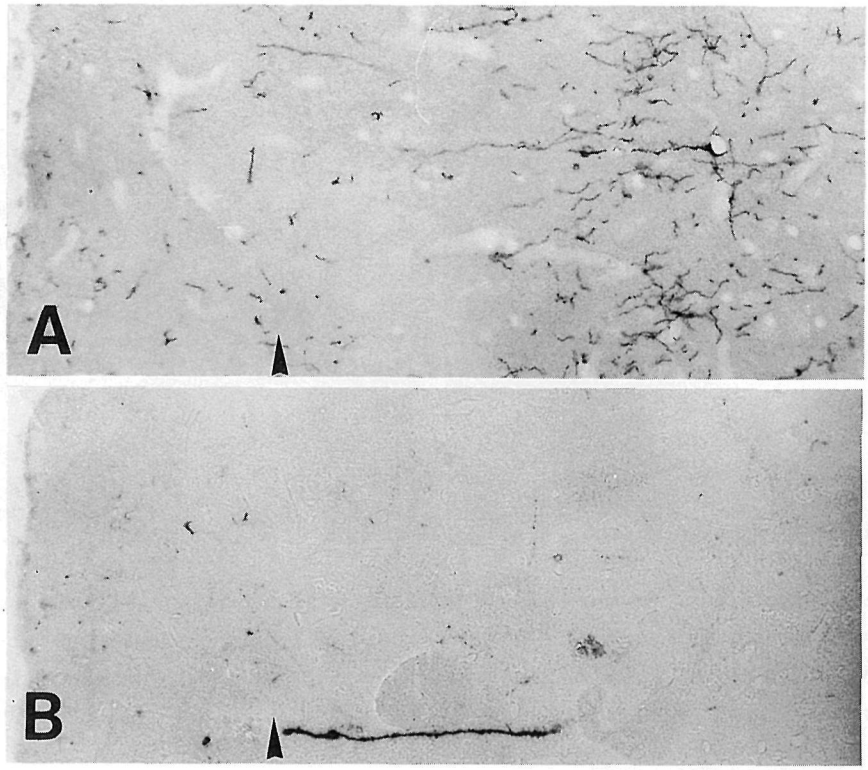

Fig. 6. Photomicrographs of TH (A) and DBH (B) immunoreactivity in the ACd of a DSP-4 injected rat, 3 days after the second injection. Note a marked reduction of DBH immunoreactivity leaving a thick non-terminal axon as a sign of the affection (B). TH immunopositive fibers are well preserved in layers II/III (A). ×220

Jonsson et al. ('81) have found that DSP-4 causes a inhibition of NA uptake in brain slices and a long-lasting reduction in DBH activity. Furthermore, they have emphasized that DSP-4 serves as a useful denervation tool with the advantage of readily passing the BBB and the BPB.

In the present experiment, we confirmed the usefulness of DSP-4 in causing a selective depletion in the number of cortical NA terminals and in DBH immunoreactivity. However, the effect of DSP-4 on the ultrastructure of NA terminals remains to be solved. Although DBH immunoreactivity has been affected within the first few days after DSP-4 injection, morphological damage probably occurs somewhat later. Since it has been reported that DSP-4 induces a long-lasting effect on the NA terminals, it is necessary to examine rats which have survived for longer periods.

In a combination with DSP-4 injection, TH immunohistochemistry clearly showed up the mesocortical DA system, i. e. components of the so-called mesolimbocortical DA system. There was no indication of DSP-4 affecting DA fibers.

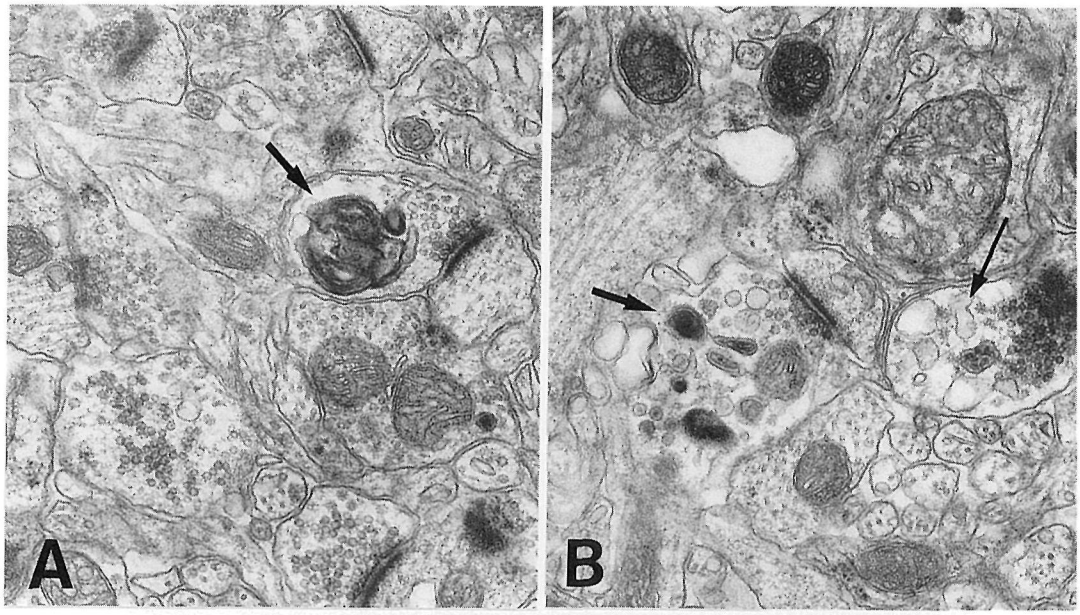

Fig. 7. Electronmicrographs of some abnormal terminals in the medial frontal (A) and the fronto-parietal (B) cortices. Only a small number of abnormal terminals containing dense granular bodies (thick arrow) or swollen vesicles (thin arrow) are seen. It is not clear whether these terminals indicate degenerating features of NA terminals induced by DSP-4. ×30,000

The pattern of DA distribution in the neocortex of the DSP-4 injected rat corresponded closely with previous findings obtained by histofluorescent, biochemical, radioautographic and immunohistochemical techniques (Björklund and Lindvall, '84; Lindvall et al., '78 ; Palkovits et al., '79 ; Yoshida et al., '88). In the present experiment the density of DA fibers in the mesocortex seems to predominate over that of the NA fibers although some investigators have mentioned a higher content of NA than DA in the cortex (Audet et al., '88 ; Palkovits et al., '79). Tentatively, the quantitative data of TH positive terminals after DSP-4 injection were compared with these obtained in radioautographic demonstration by Descarries et al. ('87). The number of DA vasicosities in the prelimbic area (76/14000μm^2 corresponding to 5400/mm^2) is approximately 4 times as many as those reported by radioautography (1490/mm^2). This may be attributed to the difference of the thickness of the sections examined: 4μm in radioautography and 10μm in immunohistochemistry.

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References

Ader, J. -P., Room, P., Postema, F. and Korf, J. (1980) Bilaterally diverging axon collaterals and contralateral projections from rat locus neurons, demonstrated by fluorescent retrograde double labeling and norepinephrine metabolism. J. Neural Transm. 49, 209-218.

Audet, M. A., Doucet, G., Oleskevich, S. and Descarries, L. (1988) Quantified regional and laminar distribution of the noradrenaline innervation in the anterior half of the adult rat cerebral cortex. J. Comp. Neurol. 274, 307-318.

Baumgarten, H. G., Björklund, A., Lachenmayer, L. and Nobin, A. (1973) Evaluation of the effects of 5, 7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. Acta Physiol. Scand. Suppl. 391, 1-20.

Baumgarten, H. G., Björklund, A., Lachenmayer, L., Nobin, A. and Stenevi, U. (1971) Long-lasting selective depletion of brain serotonin by 5, 6-dihydroxytryptamine. Acta Physiol. Scand. Suppl. 373, 1-15.

Berger, B., Verney, C., Febvret, A., Vigny, A. and Helle, K. B. (1985) Postnatal ontogenesis of the dopaminergic innervation in the rat anterior cingulate cortex (area24). Immunocytochemical and catecholamine fluorescence histochemical analysis. Develop. Brain Res. 21, 31-47.

Berger, B. Thierry, A. M., Tassin, J. P. and Moyne, M. A. (1976) Dopaminergic innervation of the rat prefrontal cortex : A fluorescence histochemical study. Brain Res. 106, 133-145.

Björklund, A. and Lindvall, O. (1984) Dopamine-containing systems in the CNS. In : Handbook of chemical neuroanatomy (Eds) Björklund, A. and Hökfelt, T. vol 2. Classical transmitters in the CNS. Part 1. Elsevier, Amsterdam pp. 55-122.

Descarries, L., Lemay, B., Doucet, G. and Berger, B. (1987) Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. Neurosci. 21, 807-824.

Divac, I., Björklund, A., Lindvall, O. and Passingham, R. E. (1978) Converging projections from the mediodorsal thalamic nucleus and mesencephalic dopaminergic neurons to the neocortex in three species. J. Comp. Neurol. 180, 59-72.

Fritschy, J. -M. and Grzanna, R. (1989) Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. Neurosci. 30, 181-197.

Jonsson, G., Hallman, H., Ponzio, F. and Ross, S. (1981) DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)—a useful denervation tool for central and peripheral noradrenaline neurons. Europ. J. Pharmacol. 72, 173-188.

Jonsson, G., Hallman, H. and Sundstrom, E. (1982) Effects of the noradrenaline neurotoxin DSP-4 on the postnatal development of central noradrenaline neurons in the rats, Neurosci. 7, 2895-2907.

Kalsbeek, A., Buijs, R. M.., Hofman, M. A., Matthijssen, M. A. H., Pool, C. W. and Uylings, H. B. M. (1987) Effects of neonatal thermal lesioning of the mesocortical dopaminergic projection on the development of the rat prefrontal cortex. Develp. Brain Res. 32, 123-132.

Krettek, J. E. and Price, J. L. (1977) The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J. Comp. Neurol. 171, 157-192.

Lidov, H. G. W., Grzanna, R. and Molliver, M. E. (1980) The serotonin innervation of the cerebral cortex in the rat. An immunohistochemical analysis. Neurosci. 5, 207-227.

Lindvall, O., Björklund, A. and Divac, I. (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. Brain

Res. 142, 1-24.

Lindvall, O., Björklund, A. and Skagerberg, G. (1984) Selective histochemical demonstration of dopamine terminal systems in rat di- and telencephalon : New evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. Brain Res. 306, 19-30.

Malmfors, T. and Thoenen, H. (eds) (1971) 6-hydroxydopamine and catecholamine neurons. North-Holland publ. Comp., Amsterdam.

Palkovits, M., Záborszky, L., Brownstein, M. J., Fekete, M. I. K., Herman, J. P. and Kanyicska, B. (1979) Distribution of norepinephrine and dopamine in cerebral cortical areas of the rat. Brain Res. Bull. 4, 593-601.

Ross, S. B. (1976) Long-term effects of N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurons in the rat brain and heart. Br. J. Pharmacol. 58, 521-527.

Swanson, L. W. (1982) The projections of the ventral tegmental area and adjacent regions : A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res. Bull. 9, 321-353.

Van Eden, C. G. and Uylings, H. B. M. (1985) Cytoarchitectonic Development of the prefrontal cortex of the rat. J. Comp. Neurol. 241, 253-267.

Vogt, B. A. and Miller, M. W. (1983) Cortical connections between rat cingulate cortex and visual, motor and postsubicular cortices. J. Comp. Neurol. 216, 192-210.

Yoshida, M., Sakai, M., Kani, K., Nagatsu, I. and Tanaka, M. (1988) The dopaminergic innervation as observed by immunohistochemistry using antidopaminergic serum in the rat cerebral cortex. Experientia 44, 700-702.

ラット前頭皮質のドーパミン線維終末を，検索する上でのDSP-4の効果

今本喜久子

滋賀医科大学解剖学第一講座

成熟雄ラットに，生理食塩水に溶かしたDSP-4（60mg/kg；60mg/kg x2；50mg/kg x2）を腹腔投与し，8h，1-4日生存させた．麻酔下で4％パラホルムアルデヒドによる灌流固定を行い，10μmの凍結脳切片を作製し，complete streptavidin-biotin法でTH及びDBHの免疫染色を施した．DSP-4の50mg/kgを二回投与後の2-4日例でNA線維終末の阻害効果は最も顕著であった．前頭皮質全域の14000μm^2に40個の割合で認められていたDBH陽性バリコーシティは，ほぼ完全に消失し，太く短い陽性線維が異常像としてまれに見られた．対応してNA神経線維のTH免疫活性は減少したが，従来よりmesocortical DA systemと呼ばれているDA分布部位では，TH陽性線維の減少はなく，DA線維終末へDSP-4の影響は認められなかった．辺縁前部のV/VI層と前内側の前頭皮質のII/III層ではDAバリコーシティが最も多く14000μm^2に70-80個であった．DSP-4は，DA終末に影響を与えないでNA終末のみを特異的に抑制する効果があることを明確に示したが，電顕的にはDSP-4によるNA線維終末の変性を確認するのは困難であった．