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A parametric study of the effects of the noradrenaline neurotoxin DSP4 on avoidance acquisition and noradrenaline neurones in the CNS of the rat

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1 The effects of various doses of DSP4 on two-way active avoidance acquisition in rats and on central noradrenaline neurones were compared.

2 Doses of DSP4 from 3 mg kg^{-1} i.p. and upwards injected one week before the onset of the avoidance trials significantly impaired two-way avoidance learning.

3 The learning impairment caused by DSP4 (50 mg kg^{-1} i.p.) lasted for at least 10 weeks.

4 Desipramine (20 mg kg^{-1}) injected either 30 or 60 min before DSP4 (50 mg kg^{-1}) antagonized the active avoidance impairment.

5 A high dose of DSP4 (50 mg kg^{-1} i.p.) produced profound decreases in dopamine-β-hydroxylase activity in the frontal cortex and in the concentrations of noradrenaline in various brain regions indicating degeneration of the locus coeruleus noradrenaline system.

6 Low doses of DSP4 (3 and 6 mg kg^{-1} i.p.) produced small but significant decreases in the concentrations of noradrenaline (NA) in some regions, e.g. cerebral cortex, hippocampus, olfactory bulb and spinal cord.

7 The avoidance impairment caused by the low dose of DSP4 (3 mg kg^{-1}) was absent when rats were tested 10 weeks after treatment nor was NA depletion present when NA was analysed 3 months after treatment.

Introduction

Systemic administration of N-chlorethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP4) produces a longterm disappearance of biochemical and histochemical markers for noradrenaline (NA) neurones in brain regions innervated by the locus coeruleus NA system in the mouse and rat (Ross 1976; Ross & Renyi 1976; Jaim-Etcheverry & Zieher 1980; Jonsson *et al.*, 1981). These effects are most likely due to a DSP4-induced degeneration of NA nerve terminals. DSP4 also affects peripheral noradrenergic nerve terminals, e.g. in the heart and iris, but this effect is shortlasting with almost complete recovery within two weeks. DSP4 appears to be selective for the noradrenaline system, i.e. does not act on dopamine (DA) neurones and has very little effect upon 5-hydroxytryptamine (5-HT) neurones (Ross, 1976; Jonsson *et al.*, 1981). Since pretreatment of the animals with the NA uptake blocker desipramine (DMI) antagonizes the DSP4-evoked degeneration of the noradrenaline nerve terminals, the neurodegenerative effect seems to be dependent on the NA uptake system (Ross 1976; Jonsson *et al.*, 1981). The selectivity, the antagonism by desipramine, the relatively rapid recovery in peripheral systems but relative permanence in the CNS and the parenteral effectiveness make DSP4 a uniquely valuable tool for studies of the functional role of noradrenaline in the brain.

In a series of studies, we have used DSP4 as a tool to examine the role of noradrenaline in two-way avoidance learning. At a dose (50 mg kg^{-1}) producing pronounced degeneration of noradrenaline terminals in the locus coeruleus system, DSP4 caused marked impairments in the two-way avoidance and modified T-maze acquisition tasks (Ögren *et al.*, 1980; Archer *et al.*, 1981c; 1983; Archer, 1982a) Note however, that Spyraki *et al.* (1982) obtained no effects with DSP4 treatment on either positively-reinforced Y- or L-maze learning. The avoidance impairment is present up to 10 weeks after DSP4

treatment. Therefore, it seems likely that DSP4 acts by affecting central and not peripheral noradrenaline (Archer *et al.*, 1982b).

In the present study we have examined several pharmacological parameters of DSP4, including: (1) the effects of various doses of DSP4, (2) the effect of varying the recovery interval, (3) the dose of DMI required to block a 50 mg kg^{-1} dose of DSP4, and (4) the time at which DMI should be injected in order to block the effects of DSP4 on the two-way acquisition in rats. These parameters were compared with the biochemical effects on NA levels and turnover in various brain regions, the dopamine-β-hydroxylase activity in the frontal cortex region, and the post-decapitation reflex.

Methods

Male Sprague-Dawley rats weighing between 250 and 300 g, aged 55–65 days, were used throughout the experiments. They were randomly allocated to the different treatment conditions (in each group, $n = 8$) and were placed under laboratory conditions at least 10 days before two-way avoidance testing (carried out during the 'lights on' phase between 08 h 00 min and 15 h 00 min).

Treatments

All drugs were administered intraperitoneally (i.p.) in a volume of 5 ml kg^{-1} body weight. DSP4 (50 mg kg^{-1}, Astra Läkemedel, except in the dose study) was dissolved in distilled water. Desipramine (DMI, Ciba-Geigy AG) was dissolved in saline (0.9% w/v NaCl solution). All treatments were administered 7 days before the avoidance test.

Apparatus

The shuttleboxes with housing, shock generators and shock scramblers (Campden Instruments Ltd, London) have been described in detail previously (Archer *et al.*, 1982b).

Two-way active avoidance

A single 100 trial session was presented to each rat. At the start of the session each rat was placed in one compartment of the shuttlebox and allowed a 10 min period of free exploration before the onset of the first trial. The conditioned stimulus (CS signal) was a 10 s tone (1,000 Hz) which was immediately followed by the unconditioned stimulus (US), a 5 s scrambled shock (1.0mA) if no avoidance response to the signal had been made. Avoidance responses terminated the CS, while escape responses terminated the CS – US (signal – shock) compound. The intertrial interval was constant at 40 s. The number of conditioned avoidance responses (CARs) were recorded throughout by a PDP11/04 computer. Within a few hours of the avoidance session each rat was decapitated and the intensity of its post-decapitation reflex (PDR) was scored on a scale of 0 to +++. At the same time, the frontal cortex was removed from each of the animals and stored at −70°C until biochemical analysis.

Studies with a single 100 trial avoidance session: parametric studies

(a) Groups ($n = 8$) of rats were administered DSP4, i.p., at either the 50, 25, 12.5, 6.25, 3.0 or 1.5 mg kg^{1} dosages 7 days before the avoidance test. (b) Other groups were injected DSP4 (50 mg kg^{-1}) either one, two, four, ten or forty weeks before the 100 trial test session. (c) DMI 2.5, 5.0, 10.0 or 20.0 mg kg^{-1} was given 30 min before DSP4 (50 mg kg^{-1}). (d) DMI (20 mg kg^{-1}) was also administered at various time intervals (0, 30, 60, 120 min) before DSP4 (50 mg kg^{-1}).

Effect of a low (3 mg kg^{-1}) and a high (50 mg kg^{-1}) dose of DSP4 on avoidance acquisition: five sessions of 30 trials/session.

Four groups ($n = 8$) were injected with DSP4 (50 mg kg^{-1}), DSP4 (3 mg kg^{-1}), DMI(20 mg kg^{-1}) 30 min before DSP4 (3 mg kg^{-1}), or DMI (20 mg kg^{-1}), 30 min before DSP4 (50 mg kg^{-1}), 7 days before the first avoidance sessions. Five sessions, consisting of 30 signal-shock trials, were presented, one on each of 5 consecutive days. All other conditions of two-way avoidance were maintained as in the 100 trial procedure. The rats were killed 3 days after the last session and NA concentrations measured.

Biochemical determinations

Dopamine-β-hydroxylase (DBH) activity in homogenates of the frontal cortex was determined according to Coyle & Axelrod (1972) using a radioenzymatic assay with tyramine as substrate. The enzyme activity was expressed as nmol octopamine formed per g brain tissue during a 20 min incubation.

Assay

Endogenous catecholamine concentrations were determined using high pressure liquid chromatography with electrochemical detection (l.c.e.c.) according to

Keller *et al.* (1976): as modified by Jonsson *et al.* (1980). The rats were killed by decapitation and the regional CNS dissection performed mainly according to Jonsson & Sachs (1976) and Jonsson *et al.* (1982) The tissue was extracted with 320 µl 0.1 M perchloric acid containing 27–520 pmol of α-methyl-dopamine (internal standard) using a Branson B30 sonifier. After an Al_2O_3 prepurification step, the extracted catecholamines were determined by l.c.e.c. Endogenous 5-HT was assayed using l.c.e.c. according to Ponzio & Jonsson (1979). The catecholamine and 5-HT values were expressed as ng g^{-1} wet weight of the tissue, based on internal standard measurements.

Results

Single 100 trial acquisition sessions

Dose-response Various doses of DSP4 (1.5 to 50 mg kg^{-1} i.p.) were administered to groups of rats 7 days before the avoidance test, which was performed in single 100 trial sessions. The dose-response curve obtained appeared to be biphasic with a marked impairment of avoidance learning at 50 mg kg^{-1} i.p. and a significant avoidance impairment at 3 to 25 mg kg^{-1} i.p. (Figure 1a). The effects at the low doses (3.0 and 6.25 mg kg^{-1} i.p.) of DSP4

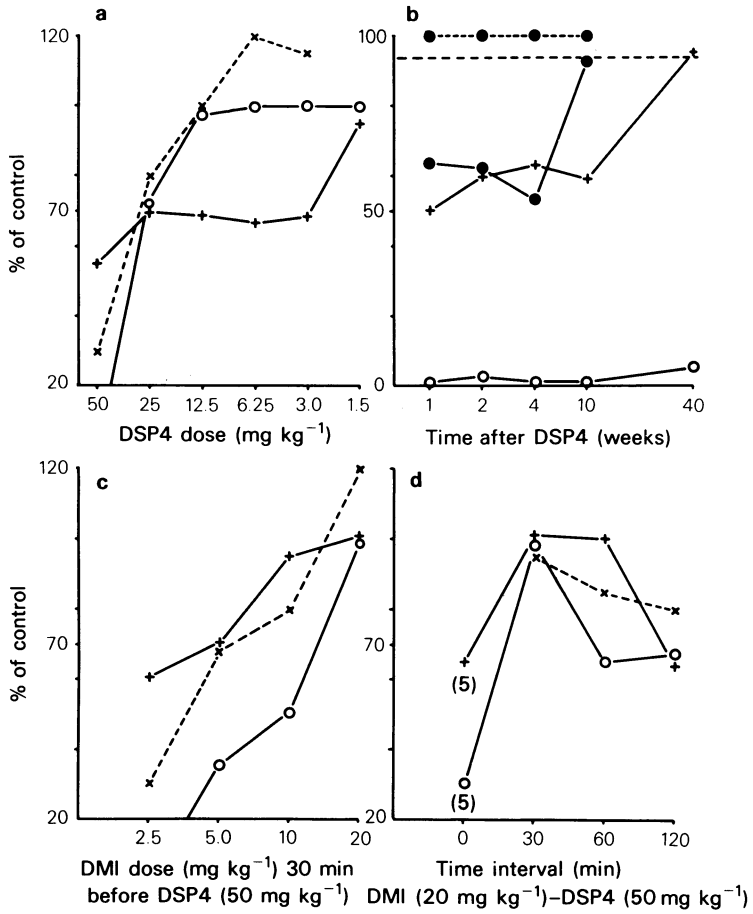

Figure 1 Impairment of two-way active avoidance learning (single 100 trials acquisition) in (a) rats by DSP4 (50 mg kg^{-1} i.p. (+−+)) in relation to decrease in dopamine β-hydroxylase activity in frontal cortex (×−−×) and loss of post decapitation seizures (O–O). Various doses of DSP4 were given one week before the test. (b) The duration of the effect of DSP4 (50 mg kg^{-1} i.p. (+−+), 3 mg kg^{-1} i.p. (●—●) conditioned avoidance response (CAR); (●− − −●) (post decapitation seizures)) in the avoidance test. (c) The antagonism of DSP4 (50 mg kg^{-1}) by various doses of desipramine (DMI) 30 min before the injection of DSP4. (d) The effect of varying the time interval between desipramine (DMI) (50 mg kg^{-1} i.p.) and DSP4 (50 mg kg^{-1} i.p.). *$P < 0.05$ versus control, Dunnett's t test.

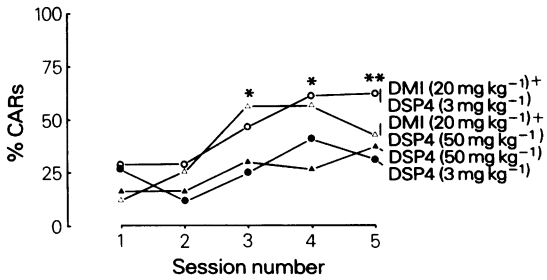

Figure 2 Two-way avoidance acquisition during 30 trial sessions on 5 consecutive days, commencing one week after the injection of DSP4 3 mg kg^{-1} i.p. (●) or 50 mg kg^{-1} i.p.(▲); desipramine (DMI) 20 mg kg^{-1} i.p. was injected 30 min before DSP4 3 mg kg^{-1} (○), 50 mg kg^{-1} (△). *$P<0.01$, Scheffes test; **$P<0.02$ (t test), cf without DMI.

were confirmed in additional experiments (data not shown).

Loss of decapitation seizures which is a measure of degeneration of noradrenergic nerves in the spinal cord (Roberts *et al.*, 1978; Mason & Fibiger, 1979a), was observed only at the two highest DSP4 doses.

Time course The acquisition performance was examined at various times after the DSP4 (50 and 3 mg kg^{-1} i.p.) injections. The effect of the high dose of DSP4 lasted 10 weeks but had disappeared 40 weeks after administration. The low dose caused an effect after 4 weeks but not after 10 weeks (Figure 1b).

Antagonism by desipramine (DMI)

As shown previously DMI pretreatment antagonizes the DSP4-induced degeneration of noradrenergic nerve terminals (Ross 1976; Jonsson *et al.*, 1982). The optimal dose of DMI antagonizing the effect of DSP4 (50 mg kg^{-1} i.p.) on DBH activity, decapitation seizures and acquisition appears to be 20 mg kg^{-1} i.p. (Figure 1c). The optimal time interval between the DMI and DSP4 dosage appears to be between 30 to 60 min (Figure 1d).

Five days repeated 30 trial sessions In order to establish the effect of the low dose (3 mg kg^{-1} i.p.) of DSP4 on the 2-way avoidance acquisition in the single 100 trial session the experiment was repeated with 30 trial sessions on 5 consecutive days. Two groups of 8 rats were given DSP4 3 and 50 mg kg^{-1} i.p. and two groups desipramine 20 mg kg^{-1} i.p. 30 min before each of these DSP4 doses. As shown in Figure 2 the two doses of DSP4 caused similar impairment of the learning. Desipramine significantly antagonized this impairment during sessions 3 to 5.

Biochemical analyses

DSP4 produced a dose-dependent reduction of the endogenous NA levels in all CNS regions analysed (Figure 3a). The most pronounced NA depletions were seen in locus coeruleus innervated areas (cerebral cortex, hippocampus, olfactory bulb, cerebellum and spinal cord, see Lindvall & Björklund, 1978), with an almost complete disappearance of NA after the highest dose of DSP4 (50 mg kg^{-1}). Regions innervated mainly by lateral tegmental NA cell-groups (hypothalamus, pons-medulla, see Lindvall & Björklund, 1978) were less affected. The dopamine levels were not significantly affected in most regions analysed, although a 30–40% reduction in dopamine was noted after the highest DSP4 dose in the hippocampus and the cerebellum (Figure 3b). The reason for the reduction in dopamine in these latter regions is

Table 1 Effects of low doses of DSP4 on the regional catecholamine levels

Dose DSP4 (mg kg^{-1})		*Cerebral cortex* *Front.*	*Cing.*	*Enth.*	*Occ.*	*Olf.bulb*	*Hipp.*	*Olf.Tub*	*Nuc.Acc.*	*Striatum*	*Sp.cord*
3[a]	NA	94±4.5	87±6.4	70±6.6**	80±6.5*	92±5.4	84±2.5*	107±12	91±7.4	117±9.1	94±6.6
(16 days)	DA	123±8.6	91±9.3	114±9.4	88±5.7	98±4.6	87±13	98±6.6	95±8.7	100±3.2	94±6.5
3[b]	NA	94±5.1	—	—	99±3.7	—	—	—	—	—	103±6.1
(3 months)	DA	108±7.0	—	—	92±8.3	—	—	—	—	—	105±9.2
6.25[c]	NA	87±6.7	75±4.5**	77±2.8**	—	87±7.4	89±4.3*	—	—	—	79±7.9*
(7 days)	DA	103±8.8	111±14	84±8.0	—	99±19	95±11	99±8.4	88±5.0	92±3.4	105±4.5

Mean ± s.e.mean of 4–6 determinations, expressed as % of respective control value.
[a]Control: desipramine (DMI) 20 mg kg^{-1} i.p. 30 min before DSP4. The rats were killed 16 days after drug administration
[b]Control: saline injection. The rats were killed 3 months after DSP4 or saline administration
[c]Control: saline injection. The rats were killed 7 days after DSP4 or saline administration
*$0.05 > P > 0.01$; **$0.01 > P > 0.001$ (Student's t-test).

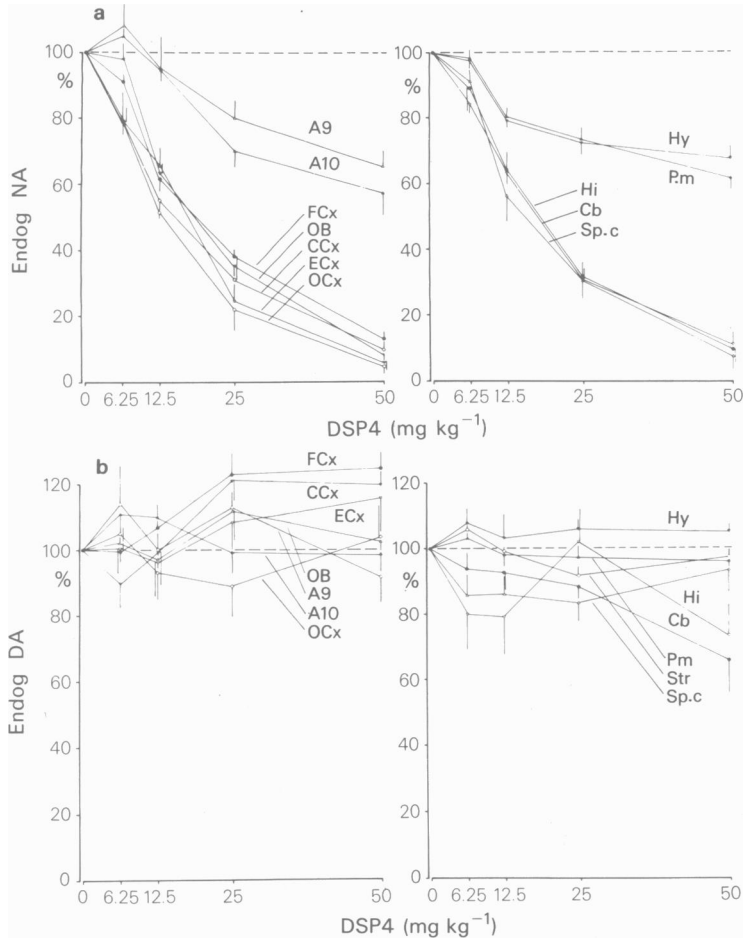

Figure 3 Effect of various doses of DSP4 (6.25–50 mg kg^{-1} i.p.) on the regional noradrenaline (NA) (a) and dopamine (DA) (b) concentrations in rat brain one week after the DSP4 injection. Each point represents the mean ± s.e.mean of 4 determinations, expressed as % of the respective control (saline injected). *A9* and *A10* = DA cell-body groups (according to Dahlström & Fuxe, 1964); *FCx* = frontal cortex; *CCx* = cingulate cortex; *OB* = olfacatory bulb; *ECx* = entorhinal cortex; *OCx* = occipital cortex; *P–m* = pons-medulla; *Hy* = hypothalamus; *Hi* = hippocampus; *Cb* = cerebellum; *Sp.c.* = spinal cord (lumbar); *Str* = striatum.
Control levels (ng g^{-1}) of NA and DA for the various regions were: *A9*: 287 ± 26 and 850 ± 41; *A10*: 588 ± 21 and 1353 ± 31; *FCx*: 205 ± 16 and 74 ± 15; *CCx*: 233 ± 13 and 55 ± 4.1; *OB*: 218 ± 12 and 7.2 ± 0.7; *ECx*: 284 ± 7.2 and 54 ± 6.9; *OCx*: 154 ± 2.2 and 7.3 ± 0.3; *P–m*: 705 ± 23 and 40 ± 1.8; *Hy*: 2715 ± 126 and 952 ± 39; *Hi*: 335 ± 12 and 13 ± 2.1; *Cb*: 144 ± 5.5 and 4.7 ± 0.3; *Sp.c.*: 358 ± 15 and 24 ± 1.4; *Str*: 9644 ± 81 (DA).

most likely related to the precursor of dopamine localized in NA nerve terminals constituting a substantial part of the total amount of dopamine present in these regions (cf. Jonsson *et al.*, 1981). Analysing the acute time-course of the NA depletion following DSP4 (50 mg kg^{-1}) showed that the neurotoxin produced a very rapid reduction in NA in both the frontal and occipital cortex where maximal effects were achieved between 8 and 16 h after DSP4 administration (Figure 4).

DBH is an enzyme localized in the storage granules of NA neurones. The activity of this enzyme in the frontal cortex was also markedly reduced (−70%) after 50 mg kg^{-1} of DSP4, while only slightly reduced after 25 mg kg^{-1} of DSP4 (Figure 1). The effect of DSP4 is thus somewhat less pronounced on the DBH activity compared to its effects on NA levels. The reason for this is not known, but could, at least to a certain extent, be related to part of the DBH being sequestered in glial cells after phagocytosis of degenerating NA nerve terminals.

Analysis of the effects of low doses of DSP4 (3 or 6.25 mg kg^{-1} i.p.) showed that significant reductions in NA were observed in some cerebral cortical reg-

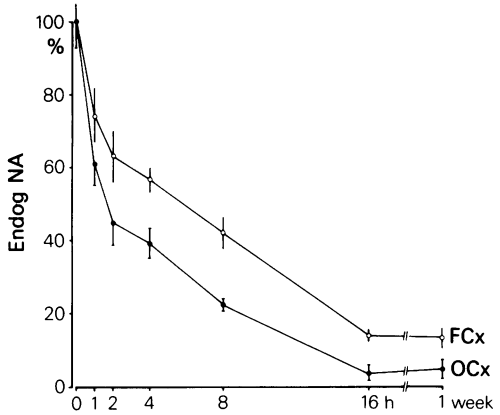

Figure 4 Time-course of the acute effect of DSP4 (50 mg kg^{-1} i.p.) on the noradrenaline (NA) levels in the frontal (FCx) and occipital (OCx) cortex. Each point represents the mean ± s.e.mean. of 5 determinations, expressed as % of control.

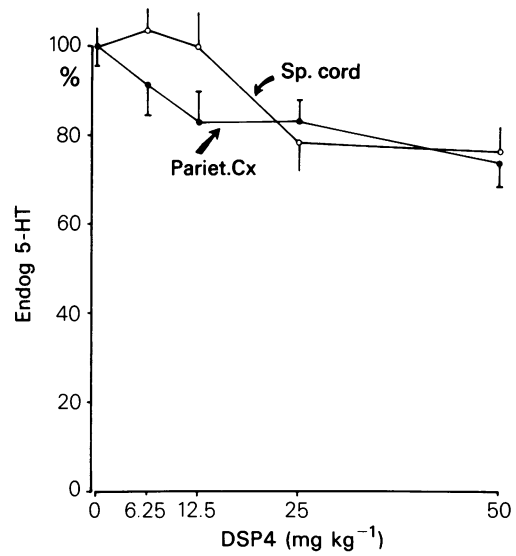

Figure 5 Effect of various doses of DSP4 (6.25–50 mg kg^{-1} i.p.) on the 5-hydroxytryptamine (5-HT) concentration in the parietal cortex and the spinal cord one week after the administration of DSP4. Each point represents the mean ± s.e.mean of 4 determinations, expressed as % of respective control. Control levels were: 186 ± 9.2 ng g^{-1} (parietal cortex) and 311 ± 13 ng g^{-1} (spinal cord).

ions, hippocampus and spinal cord (Table 1). It is of interest to note that 3 mg kg^{-1} DSP4 produced a significant depletion (−20%) of NA 16 days after drug administration while this effect was not present 3 months later. This long-term recovery is probably related to regeneration of NA nerve terminals.

Inhibition of NA biosynthesis at the rate-limiting step by administration of a tyrosine hydroxylase inhibitor (H44/68) showed that the relative NA reduction was very similar in animals treated with a low dose of DSP4 compared to controls (Table 2). If anything, there appeared to be a slight increase in the H64/68 induced NA reduction after DSP4 treatment in most regions studied. These results show that a small dose of DSP4 does not produce any reduction in NA turnover, and thus does not appear to produce any damage on the NA release properties of the terminals that remain after DSP4 pretreatment. It was also observed that low doses of DSP4 had very

Table 2 Effects of tyrosine hydroxylase inhibition with H44/68 on the regional catecholamine levels in DSP4-treated rats

	Cerebral cortex									
	Front.	*Cing.*	*Enth.*	*Occ.*	*Olf.bulb*	*Hipp.*	*Olf.Tub.*	*Nuc.Acc.*	*Striatum*	*Sp.cord*
Noradrenaline										
Control	69±0.7	68±3.0	65±5.8	66±7.4	60±5.5	78±2.5	104±2.6	109±233	129±7.5	63±5.6
DSP4 (3 mg kg^{-1}, 16 days)	49±2.6	50±1.2	57±5.3	41±2.9	46±3.2	61±3.3	91±12	82±12	74±11	61±4.0
Control	61±2.4	48±3.1	60±3.4	—	64±4.5	59±1.8	—	—	—	73±2.8
DSP4 (6.25 mg kg^{-1}, 7 days)	55±3.3	51±4.3	57±9.1	—	57±5.2	64±4.3	—	—	—	67±7.8
Dopamine										
Control	30±4.8	19±3.1	71±12	47±9.4	22±4.9	56±6.0	51±4.2	50±4.0	58±1.3	39±6.5
DSP4 (3 mg kg^{-1}, 16 days)	21±1.6	18±4.5	48±8.3	65±11	30±2.2	29±5.2	47±4.2	48±2.7	55±1.7	62±10.3
Control	24±5.0	34±13	31±1.3	—	10±5.0	5	49±4.1	44±6.1	48±3.4	29±2.5
DSP4 (6.25 mg kg^{-1}, 7 days)	17±6.1	34±7.6	38±8.3	—	16±5.8	26±8.1	51±1.5	42±1.5	43±2.3	28±2.4

[a]H44/68 (α-methyl-*p*-tyrosine methylester) 250 mg kg^{-1} i.p., 2 h.
Results are mean ± s.e.mean (n = 4–6), expressed as % of saline treated DSP4 or control rats (treated as in Table 1).

small effects on the H44/68 induced depletion of dopamine in all the regions analysed (Table 2).

Analysis of the effect of DSP4 on central 5-HT neurones showed that the neurotoxin produced very small decreases in 5-HT levels in the parietal cortex and spinal cord, the depletion of 5-HT being about 20–25% in both regions after the highest dose of DSP4 (50 mg kg^{-1} i.p.; see Figure 5).

Discussion

In accordance with results obtained in previous studies (Ögren *et al.*, 1980; Archer *et al.*, 1981b; 1982b) DSP4 50 mg kg^{-1} i.p. caused a marked impairment of two-way avoidance acquisition in rats; this lasted more than 10 weeks. At this dose the noradrenergic nerve terminals were profoundly degenerated as shown by the reduction in DBH in the frontal cortex and in NA levels in the various brain regions with NA terminals originating from the locus coeruleus system. The loss of decapitation seizures showed the same dose-response effect as the decrease in DBH activity and therefore provides a simple test for the central degeneration (spinal cord) of noradrenaline terminals evoked by DSP4.

The two-way avoidance impairment following NA depletion with systemic DSP4 is not easily reconciled with the lack of any two-way avoidance deficit following the dorsal bundle 6-hydroxydopamine (6-OHDA) lesion (e.g. Mason & Fibiger, 1979b) which produces similar NA depletions in the forebrain. There are several possible explanations for this discrepancy. Apart from the neurochemical depletion difference between the two techniques, Mason & Fibiger (1979b) used radically different behavioural parameters of two-way avoidance and the behavioural conditions have differed drastically from ours in other investigations (for review of NA and two-way avoidance, see Archer, 1982a,b). There exist instances of NA depletions, via techniques other than DSP4, causing two-way active avoidance impairment. Crow *et al.* (1977) have obtained a significant two-way avoidance impairment with rats that had received electrolytic lesions in the dorsal pontine tegmentum area which caused a drastic reduction in cortical NA concentration. At this stage we can only speculate that perhaps the functional effect of systemic DSP4 resembles the electrolytic dorsal pontine tegmentum area which caused a drastic reduction in cortical NA concentration.

However, low doses, down to 3 mg kg^{-1}, of DSP4 also produced significant impairments of two-way avoidance acquisition. These doses caused only marginal reductions (10 to 20%) in NA in the brain regions which are sensitive to DSP4. These reductions seem to be the result of partial degeneration of the noradrenaline terminals since the NA turnover was increased probably in order to compensate for the loss of some of the terminals. It is possible that this partial degeneration of NA terminals produces a functional deficit great enough to impair avoidance performance in the two-way task. Regions with NA terminal systems located most distant to the locus coerulus complex, e.g. the entorhinal and occipital cortices, are particularly sensitive to low doses of DSP4 (Jonsson *et al.*, 1981 and Table 1). The terminals in the olfactory bulbs belong also to the ones more sensitive to DSP4-treatment but some recent data indicate that this region does not appear to be involved in the learning defect caused by DSP4, since bulbectomy does not impair the two-way avoidance acquisition in rats (Archer *et al.*, 1984).

Since desipramine antagonized the impairment of the two-way avoidance acquisition by a low dose of DSP4, noradrenaline appears to be involved. As we have shown previously, however, DMI plus DSP4 treated rats have regional NA levels which are significantly reduced (by 20–35%) compared to saline treated rats (Archer *et al.*, 1982b). On the other hand, the reduction in DBH activity is completely blocked by the DMI pretreatment (Figure 1) as is the decrease in NA uptake (Archer *et al.*, 1982a). Obviously, the exact significance of the type of neurochemical analysis after DSP4 remains to be determined. It cannot, however, be excluded that DSP4 at low doses can affect the noradrenaline system without causing a degeneration of the terminals. DSP4 and particularly the aziridinum derivative formed upon cyclization are structually very similar to bretylium. Although it is very unlikely that free DSP4 or its aziridinum derivative is present in the brain one week after the injection, it is possible that, the reactive aziridinium derivative binds to a 'bretylium receptor' (if such a receptor exists), and thereby impairs the nerve function. However, the turnover of NA was increased and not decreased which is assumed to occur after neurone blockade. On the other hand, it has recently been shown that corticosterone and adrenal corticoids increased the NA receptor-coupled adenylate cyclase activity in the frontal cortex of rats (Mobley & Sulser, 1980a,b). Since the DSP4-induced effect upon avoidance acquisition does seem to be partially modulated by corticosterone (Archer *et al.*, 1981b) it is possible that even the lower doses of DSP4 may cause some alteration to the NA receptor-coupled adenylate cyclase system in the forebrain. DSP4 has also a transient α-receptor blocking effect similar to phenoxybenzamine which, however, dissipates within 4 days (Jonsson *et al.*, 1981). Although the idea that DSP4 may have a subtle, non-neurotoxic action that interferes with NA neurotransmission is interesting, the explanation is not easily reconciled to the existing

data since any non-neurotoxic mechanisms could apply also in the periphery. Our earlier findings (Archer *et al.*, 1982b) demonstrated that manipulations (systemic 6-OHDA) that caused notable disruptions of NA function peripherally caused no effect whatsoever upon the acquisitive performance of the two-way avoidance task. Obviously more experiments have to be performed in order to elucidate the effects of low doses of DSP4 on the brain noradrenaline systems.

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