Br. J. Pharmac. (1976), **58**, 521–527

LONG-TERM EFFECTS OF *N*-2-CHLOROETHYL-*N*-ETHYL-2-BROMOBENZYLAMINE HYDROCHLORIDE ON NORADRENERGIC NEURONES IN THE RAT BRAIN AND HEART

S.B. ROSS

Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden

1 *N*-2-Chloroethyl-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP 4) 50 mg/kg intraperitoneally, produced a long-term decrease in the capacity of brain homogenates to accumulate noradrenaline with significant effect 8 months after the injection. It had no effect on the noradrenaline uptake in homogenates from the striatum (dopamine neurones) and on the uptake of 5-hydroxytryptamine (5-HT) in various brain regions.

2 *In vitro* DSP 4 inhibited the noradrenaline uptake in a cortical homogenate with an IC_{50} value of 2 μM but was more than ten times less active on the dopamine uptake in a striatal homogenate and the 5-HT uptake in a cortical homogenate.

3 DSP 4 (50 mg/kg i.p.) inhibited the uptake of noradrenaline in the rat heart atrium *in vitro* but this action was terminated within 2 weeks.

4 DSP 4 (50 mg/kg i.p.) caused a decrease in the dopamine-β-hydroxylase (DBH) activity in the rat brain and heart. The onset of this effect was slow; in heart a lag period of 2–4 days was noted. In brain the DBH-activity in cerebral cortex was much more decreased than that in hypothalamus which was only slightly affected. A significant effect was still found 8 months after the injection. The noradrenaline concentration in the brain was greatly decreased for at least two weeks, whereas noradrenaline in heart was only temporarily reduced.

5 The long-term effects of DSP 4 on the noradrenaline accumulation, the DBH activity and noradrenaline concentration in the rat brain were antagonized by desipramine (10 mg/kg i.p.).

6 It is suggested that DSP 4 primarily attacks the membranal noradrenaline uptake sites forming a covalent bond and that the nerve terminals, as a result of this binding, degenerate.

Introduction

In previous papers (Ross, Johansson, Lindborg & Dahlbom, 1973; Ross & Renyi, 1976) it was reported that the alkylating compound *N*-2-chloroethyl-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP 4) has a very sustained inhibitory action on the uptake of noradrenaline in mouse brain slices. The action was specific to the noradrenergic neurones, since no effect was obtained on the uptake of dopamine or 5-hydroxytryptamine (5-HT). DSP 4 was also found to be an active inhibitor of the noradrenaline uptake *in vitro*. One possible explanation of the effect is that the compound is covalently bound to the uptake sites *via* the reactive aziridinium derivative. However, the long-term effect can also be due to damage of the noradrenergic neurones resulting in their degeneration. In the present study the action of DSP 4 on the noradrenergic neurones was examined by determining the long-term effects on the concentration of noradrenaline, the enzyme dopamine-β-hydroxylase (DBH) activity and the noradrenaline accumulation in rat brain and heart. If the long-term effect is due to degeneration of the neurones all activities located in the noradrenaline neurones should decrease simultaneously.

Methods

Male Sprague-Dawley rats weighing 150–180 g at the time of the injection of DSP 4 were used. The injections were given intraperitoneally and DSP 4 was dissolved in water immediately before use.

Uptake of [^{3}H]-(–)-noradrenaline, [^{3}H]-dopamine and [^{14}C]-5-HT in homogenates of various regions of the brain was determined according to Snyder & Coyle (1969) modified to the double labelling technique (Ross & Renyi, 1975). The brains were homogenized in 0.25 M sucrose (1:20 w/v) with

an all-glass Potter-Elvehjem homogenizer. The homogenates were not centrifuged before use when the *in vivo* activity of DSP 4 was determined. The incubation was performed at 37°C in PVC centrifuge tubes for 5 min if not stated otherwise. The incubation mixture consisted of 100 µl of the homogenate, 0.2 nmol [^{3}H]-noradrenaline or [^{3}H]-dopamine, 0.2 nmol [^{14}C]-5-HT, 11 µmol glucose, 0.48 µmol pargyline, 2.2 µmol ascorbic acid and 0.26 µmol ethylenediaminetetra-acetic acid disodium salt (EDTA) in 1.8 ml of Krebs-Henseleit buffer, pH 7.4 (modified according to Snyder & Coyle, 1969). Immediately after incubation the tubes were chilled on an ice bath and centrifuged at 20,000 *g* for 20 min at 0°C. The pellets and the tubes were washed thrice with 5 ml of cold 0.9% w/v NaCl solution (saline). The pellet was dissolved in 1.0 ml of Soluene-350 (Packard) at room temperature; 10 ml of the scintillation liquid (Permablend III, Packard) was added and the radioactivity was measured in two separate channels in a liquid scintillation spectrometer as described previously (Ross, Renyi & Ögren, 1972).

Dopamine-β-hydroxylase activity in brain homogenates was determined according to Coyle & Axelrod (1972) and that in heart homogenates according to Molinoff, Weinshilboum & Axelrod (1971) with the modification that the pH of the DBH assay was 5.5. Tyramine, 1 mM, was used as the substrate. The optimal $CuSO_4$ concentration was determined for each tissue and brain region.

Noradrenaline and dopamine in the rat brain were analyzed according to Chang (1964). 5-HT was determined according to Bogdanski, Pletscher, Brodie & Udenfriend (1956).

Drugs

DSP 4 was synthesized by Dr Richard Dahlbom and collaborators, University of Uppsala, Sweden. Desipramine was a gift of Geigy AG and pargyline of Abbott Lab. Inc. (−)-Noradrenaline-[7-^{3}H-(N)] (3.8 Ci/mmol), dopamine-[^{3}H(G) (2.0 Ci/mmol) and *S*-adenosyl-L-methionine-[^{14}C-CH_3] (52 mCi/mmol) were purchased from NEN Chemicals GmbH, Frankfurt/Main, Germany and 5-hydroxytryptamine [2-side chain-^{14}C] creatinine sulphate (54 mCi/mmol) from The Radiochemical Centre, Amersham.

Results

Inhibition of noradrenaline uptake

The accumulation of [^{3}H]-(−)-noradrenaline in the synaptosomes of the homogenates of various regions of the brain was greatly decreased for a long time by a single intraperitoneal injection of DSP 4 50 mg/kg (Table 1); an exception was the uptake of noradrenaline by homogenates of the striatum which was not inhibited by DSP 4. This region is rich in dopaminergic neurones and the conclusion is that DSP 4 does not inhibit the uptake neurones in these neurones. The uptake of 5-HT was also unaffected by DSP 4 (Table 1). The small inhibition of the 5-HT uptake in the hypothalamus may be due to uptake of 5-HT into noradrenergic neurones. The selectivity of DSP 4 on noradrenaline uptake is in accordance with its *in vitro* activity. The IC_{50} value for the inhibition of the noradrenaline uptake in homogenates of cerebral cortex was 2 µM whereas the corresponding value for the inhibition of the 5-HT uptake in the same homogenate was >30 µM (20% inhibition at this concentration). The dopamine uptake in a striatal homogenate was also only partially inhibited (IC_{50} > 30 µM, actually 40% inhibition at this concentration). Thus the affinity of DSP 4 for the noradrenaline uptake sites was more than ten times higher than for the 5-HT and dopamine uptake sites.

Table 1 Effect of DSP 4 (50 mg/kg i.p.) on the accumulation of [^{3}H]-(−)-noradrenaline ([^{3}H]-NA) and [^{14}C]-5-hydroxytryptamine ([^{14}C]-5-HT) in homogenates of various regions of the rat brain one week after the injection

	Amine uptake as % in controls [a]	
Brain region	*[^{3}H]-(−)NA*	*[^{14}C]-5-HT*
Frontal cerebral cortex [b]	40 ± 5*	102 ± 3
Hypothalamus [c]	50 ± 6*	87 ± 3
Striatum [d]	100 ± 2	98 ± 6

Each value is the mean ± s.e. mean for 5 rats.
[a] Brain tissue was homogenized in 20 vol of 0.25 M sucrose. The incubation mixture consisted of 100 µl of the homogenate, 0.2 nmol [^{3}H]-(−)NA, 0.2 nmol [^{14}C]-5-HT in a final volume of 2.0 ml of Krebs buffer, pH 7.4. The incubation time was 10 min at 37°C. [b] Control uptake (nmol/g tissue (wet weight) in 10 min): [^{3}H]-NA: 0.34 ± 0.01; [^{14}C]-5-HT: 0.68 ± 0.05. [c] Control uptake: [^{3}H]-NA: 0.48 ± 0.04; [^{14}C]-5-HT 0.68 ± 0.05. [d] Control uptake: [^{3}H]-NA: 4.80 ± 0.22; [^{14}C]-5-HT: 1.98 ± 0.04.
*$P < 0.01$ (Student's *t*-test).

The dose-response of the inhibition of the noradrenaline in homogenates of frontal cerebral cortex one week after the intraperitoneal injection of DSP 4 gave an ED_{50} value of approximately 20 mg/kg (Table 2).

In order to examine whether DSP 4 acutely inhibited the membranal uptake of noradrenaline, rats were treated with reserpine (5 mg/kg i.p.) one day before DSP 4, 50 mg/kg i.p., and the animals were killed 2 h after the injection of DSP 4. Although the uptake of the noradrenaline in the homogenates from the reserpine-treated rats was significantly lower than that in the control animals, DSP 4 caused an additional decrease, demonstrating that it did not act on the same sites as reserpine (Table 3). Further support for the hypothesis that DSP 4 acts primarily on the membranal transfer sites was obtained in experiments in which rats were pre-treated with desipramine (10 mg/kg i.p.) 15 min before the injection of DSP 4. As shown in Table 4 this pre-treatment antagonized the long-term effect of DSP 4 (50 mg/kg i.p.).

The noradrenaline accumulation in homogenates of frontal cerebral cortex and hypothalamus was compared at various times after the injection of DSP 4 (Figure 1). There was no significant difference in the inhibition in these two regions during the first weeks after the injection. However, the uptake of noradrenaline in the cerebral cortex was severely impaired at 14 weeks (59% inhibition, $P < 0.01$) and at 8 months (31%, $P < 0.05$) after a single injection of DSP 4 (50 mg/kg i.p.) but the noradrenaline uptake in hypothalamus was not reduced 14 weeks after the injection although there was still a significant inhibition (29%, $P < 0.05$) after 8 weeks.

The very long inhibitory effect of DSP 4 contrasted with its considerably shorter action on the uptake in the rat heart (Figure 1). The inhibition of the uptake of

Table 2 Dose-response of the long-term effect of DSP 4 on the uptake of [^{3}H]-noradrenaline and dopamine-β-hydroxylase (DBH) activity in homogenate of the frontal cerebral cortex and the noradrenaline concentration in the whole rat brain

Dose	*[^{3}H]-Noradrenaline uptake*[a] *(% of control)*	*DBH activity*[b] *(% of control)*	*Noradrenaline concentration*[c] *(% of control)*
12.5	76 ± 8*	96 ± 11	95 ± 5
25	40 ± 10**	88 ± 15	58 ± 3**
50	29 ± 10**	8 ± 1**	30 ± 3**

The rats were killed one week after the injection of DSP 4 (50 mg/kg i.p.). Each value is the mean ± s.e. mean from 5 rats.
*$P < 0.05$; **$P < 0.001$.
[a] Control uptake: 0.24 ± 0.01 nmol/g in 5 min; [b] Control activity: 171 ± 10 nmol g^{-1} g/h^{-1}; [c] Control concentration: 0.476 ± 0.024 µg/g.

Table 3 Effect of reserpine on the inhibitory effect of DSP 4 on the noradrenaline uptake by cerebral cortical homogenate of the rat brain

	[^{3}H]-Noradrenaline uptake (nmol/g in 5 min)	*% Inhibition*	P*
1. Control	0.29 ± 0.02		
2. DSP 4	0.11 ± 0.04	62	<0.01 (1–2)
3. Reserpine	0.17 ± 0.01		<0.001 (1–3)
4. Reserpine + DSP 4	0.09 ± 0.01	47	<0.01 (3–4)

Reserpine (5 mg/kg i.p.) was injected 16 h before DSP 4 (50 mg/kg i.p.). The rats were killed 2 h after DSP 4. Frontal cortex was homogenized in 20 vol of 0.25 M sucrose and 100 µl of the homogenate was taken for the assay. The incubation time was 5 minutes. The concentration of [^{3}H]-(−)-noradrenaline was 0.1 µM. Each value is the mean ± s.e. mean for 5 rats.
* Student's *t*-test.

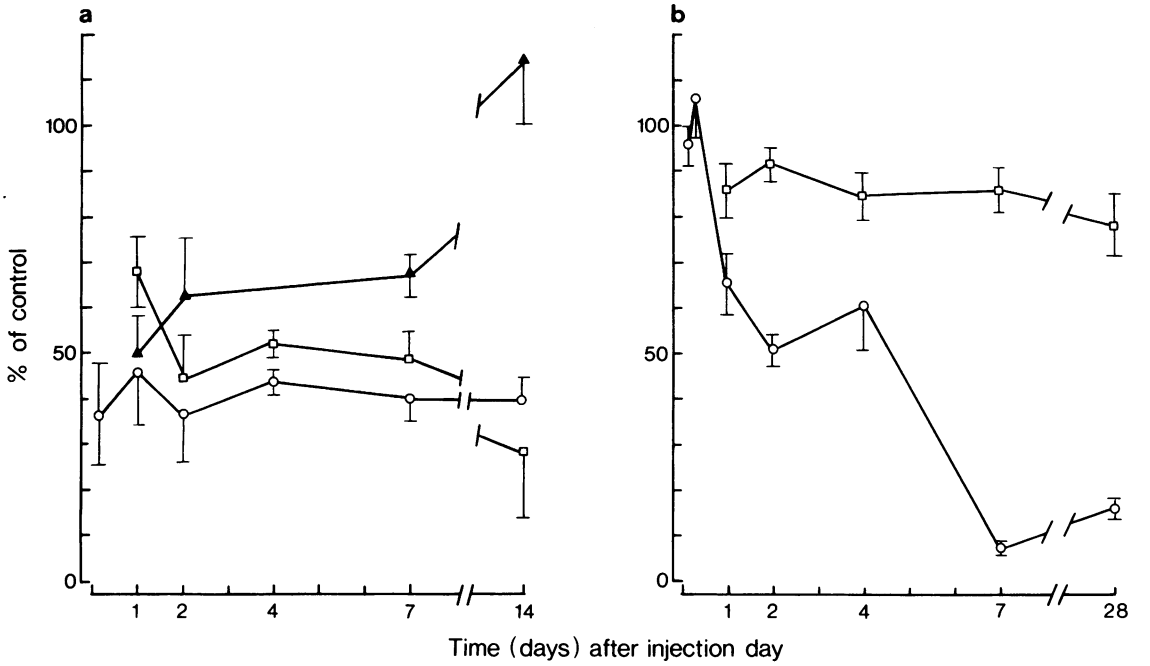

Figure 1 Effects of DSP 4 (50 mg/kg i.p.) on (a) accumulation of [^{3}H]-noradrenaline (NA) in homogenates of frontal cerebral cortex (O), hypothalamus (□) and in heart atrium (▲) and (b) dopamine-β-hydroxylase (DBH) activity in homogenates of frontal cerebral cortex (O) and hypothalamus (□) of the rat at various time after the injection. Each value is the mean ± s.e. mean (vertical lines) for at least 5 rats and is expressed as a percentage of the control values, which were: [^{3}H]-NA uptake in cerebral cortex 0.28 ± 0.02 (n=28) nmol/g in 5 min; in hypothalamus: 0.38 ± 0.02 (n=24) nmol/g in 5 min; in atrium (cocaine-sensitive part): 0.181 ± 0.010 (n=16) nmol/g in 10 minutes. DBH activity in cerebral cortex (0.14 mM $CuSO_4$): 128 ± 4 (n=6) nmol octopamine g^{-1} h^{-1} and in hypothalamus (0.11 mM $CuSO_4$): 340 ± 15 (n=6) nmol g^{-1} h^{-1}.

Table 4 Antagonistic effect of desipramine (10 mg/kg i.p.) on the long-term action of DSP 4 (50 mg/kg i.p.) on the accumulation of [^{3}H]-noradrenaline (NA) in homogenates of frontal cerebral cortex, on the dopamine-β-hydroxylase (DBH) activity in the rat brain and heart and the noradrenaline concentration in the whole rat brain

Treatment	*[^{3}H]-NA uptake* [a] *(% of control)*	*DBH activity* (% of control) *Brain* [b]	Heart [c]	*Noradrenaline in brain* [d] *(% of control)*
DSP 4	29 ± 10 (n=6)	42 ± 5 (n=7)	74 ± 5 (n=5)	49 ± 7 (n=5)
Desipramine + DSP 4	68 ± 11* (n=5)	102 ± 7** (n=6)	95 ± 6* (n=5)	77 ± 7* (n=5)

Desipramine was injected 15 min before DSP 4. The rats were killed one week later. Each value is the mean ± s.e. mean expressed as a percentage of the control values. The number of animals are given in brackets.
* Control uptake: 0.44 ± 0.03 nmol/g in 10 min; [b] Control activity: 150 ± 7 nmol octopamine formed g^{-1} h^{-1};
[c] Control activity: 202 ± 10 nmol octopamine formed g^{-1} h^{-1}; [d] Control level: 0.426 ± 0.025 µg/g.
*P < 0.05; **P < 0.001 (Student's t-test).

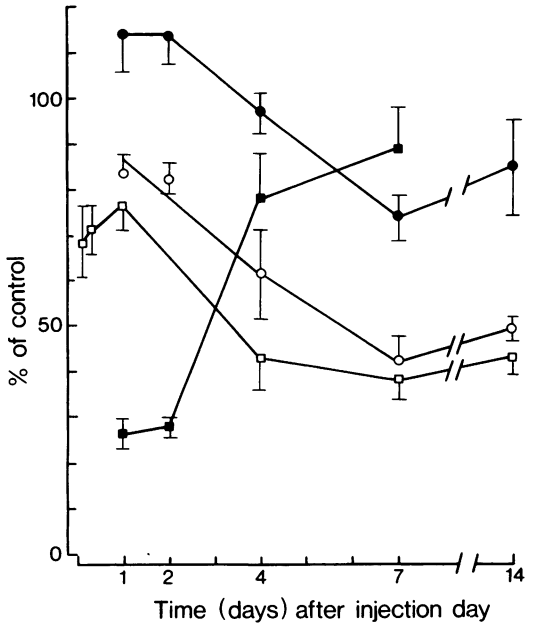

Figure 2 Effects of DSP 4 (50 mg/kg i.p.) on the noradrenaline concentrations (squares) and the dopamine-β-hydroxylase (DBH) activity (circles) in whole brain (open symbols) and heart (solid symbols) of the rat at various time after injection. Each value is the mean ± s.e. mean (vertical lines) for 6 rats and is expressed as a percentage of the control values, which were: noradrenaline concentration in brain: 0.392 ± 0.031 μg/g; in heart: 0.716 ± 0.045 μg/g. DBH activity in brain: 145 ± 4 nmol g^{-1} h^{-1} and in heart 185 ± 11 nmol g^{-1} h^{-1}.

noradrenaline in the rat atrium *in vitro* lasted only between one and two weeks after the injection of DSP 4.

Dopamine-β-hydroxylase

The DBH activity of brain homogenates was decreased by DSP 4 but in contrast to the inhibition of the noradrenaline uptake there was a slow onset of the decrease in DBH (Figure 2). In the heart there was a lag period of a few days before the DBH activity started to decrease, which then followed at the same rate as that in the brain. The DBH activity in heart did not decrease as much as in brain and recovered much earlier. DSP 4 added to brain homogenate did not inhibit the DBH activity at the concentration 3 μM.

The DBH activities in frontal cerebral cortex and hypothalamus were compared at various times after the injection of DSP 4 (Figure 1). The enzyme activity in the cortex was almost completely abolished after one week and stayed low for a long time. Thus, it was significantly ($P < 0.05$) lowered 8 months after the intraperitoneal injection of 50 mg/kg DSP 4 (control: 228 ± 16 nmol g^{-1} h^{-1}; $n = 5$; DSP 4: 131 ± 28 nmol g^{-1} h^{-1}; $n = 5$). The DBH activity in the hypothalamus was, on the other hand, much less affected. The lack of effect of DSP 4, 1 and 4 h after the injection, on the DBH activity indicates that DSP 4 in itself did not inhibit the enzyme. The dose-response curve (Table 2) showed that doses of DSP 4 lower than 50 mg/kg did not produce any long-term decrease in the DBH activity in cerebral cortex.

Since it has been shown that the DBH activity is regulated by a feed-back mechanism *via* the postsynaptic receptors (Molinoff, Brimijoin, Weinshilboum & Axelrod, 1970) it was of interest to examine whether inhibition of the membranal re-uptake of noradrenaline for a period of one week produced any effect on the brain DBH activity. Rats were therefore daily injected with desipramine (20 mg/kg i.p.) for one week and the DBH activity in the whole brain was determined on the eighth day. No difference between the treated and the control animals was obtained (DBH activity 134 ± 2 and 138 ± 3 nmol g^{-1} h^{-1}, respectively).

Although the inhibition of the adrenaline uptake did not *per se* cause the fall in DBH activity it is possible that the binding of DSP 4 to the neuronal membrane produced such damage that a degenerative process was started. This hypothesis is supported by the observation that desipramine (10 mg/kg i.p.), which antagonized the inhibition of the noradrenaline uptake, completely prevented the decrease in brain and heart DBH activity (Table 4).

Noradrenaline in brain and heart

The noradrenaline concentration in the whole rat brain was decreased by DSP 4 (50 mg/kg i.p.) in a biphasic way (Figure 2). After an initial fall of approximately 30% a second fall in the noradrenaline concentration occurred one to 4 days after the injection whereafter it stayed approximately constant at 40% of the control level for at least two weeks. Desipramine (10 mg/kg i.p.) significantly antagonized the long-term decrease in noradrenaline produced by DSP 4 (Table 4).

A dose-response study performed one week after the injection of DSP 4 showed that 12.5 mg/kg was without any effect whereas 25 mg/kg had about half of the effect of 50 mg/kg on the brain noradrenaline (Table 2). Determination of noradrenaline in the cerebral cortex and hypothalamus one week after 50 mg/kg of DSP 4 showed that a marked decrease had occurred in both regions (Table 5).

The noradrenaline in the heart was differently affected compared with that in the brain (Figure 2) in that it rapidly decreased during the first two days but had recovered almost to normal after 4 days.

Table 5 Effect of DSP 4 (50 mg/kg i.p.) on the noradrenaline concentration in cerebral cortex and hypothalamus one week after the injection

	Noradrenaline (μg/g)		
	Control	*DSP 4*	*% of control*
Cerebral cortex	0.133 ± 0.004 ($n=6$)	0.046 ± 0.006** ($n=5$)	35
Hypothalamus	0.608 ± 0.071 ($n=5$)	0.272 ± 0.049* ($n=5$)	45

Each value is the mean ± s.e. mean of the number rats given in the brackets.
*$P<0.01$; **$P<0.001$ (Student's t-test).

Dopamine and 5-hydroxytryptamine in brain

Dopamine in whole rat brain was not changed by DSP 4 (50 mg/kg i.p.) when examined at various times during the first week after the injection (Table 6). On the other hand the 5-HT concentration was significantly reduced by about 25% when determined one week after the injection (Table 6).

Discussion

The long-term effect of DSP 4 on the accumulation of noradrenaline in synaptosomes of homogenates of the rat brain is in accordance with the previous observation of a sustained decrease in the uptake of noradrenaline in mouse brain slices (Ross *et al.*, 1973; Ross & Renyi, 1976). Since this effect was selective, rapid in onset, antagonized by desipramine and was independent of a functional intraneuronal storage mechanism, i.e. not sensitive to reserpine, it is likely that the primary action of DSP 4 is a selective inhibition of the membranal noradrenaline carrier sites. DSP 4 is an alkylating compound and may accordingly be covalently bound to these sites. The considerably shorter effect of DSP 4 on the noradrenaline uptake in heart atrium indicates a difference in the stability of this binding. The reaction sequence of the hypothelial binding may be as follows: DSP 4 having selective affinity for the noradrenaline transfer sites is bound by electrostatic attraction to the uptake sites and is there transformed to the aziridinium derivative, which reacts covalently with an anionic component of the site, the latter probably being identical with that group attracting the cationic amino group of noradrenaline. The stability of this covalent bond is dependent on the nature of this group, which can be a carboxylate, phosphate or sulphhydryl group. The difference in the sensitivity for DSP 4 between central and peripheral noradrenergic nerve terminals may be due to different stabilities of this bond. DSP 4 is rather rapidly cyclized to the aziridinium derivative under *in vitro* conditions ($T_{\frac{1}{2}}=7$ min at pH 7.4 (Ross *et al.*, 1973)), which means that the cyclization terminates within a rather short time after the injection.

Table 6 Effect of DSP 4 (50 mg/kg i.p.) on the dopamine and 5-hydroxytryptamine (5-HT) concentrations in the whole rat brain.

Time after injection (days)	*Dopamine* [a] *(% of control)*	*5-HT* [b]
1	106 ± 4	88 ± 4*
2	—	84 ± 5*
4	97 ± 3	78 ± 5*
7	115 ± 11	77 ± 6*

Each value is the mean ± s.e. mean from at least 5 rats.
*$P<0.05$ (Student's t-test).
[a] Control value: 0.543 ± 0.038 μg/g($n=5$); [b] control value: 0.734 ± 0.021 μg/g ($n=10$).

The decrease in DBH activity in the rat brain and heart indicates another effect of DSP 4. Since this effect had a much slower onset than the decrease in uptake and was antagonized by desipramine it seems to be secondary to the uptake inhibition. The observation that higher doses of DSP 4 had to be given in order to affect DBH activity than are needed to affect noradrenaline uptake also supports this hypothesis. The finding that DBH in cerebral cortex was much more sensitive to DSP 4 than was that in the hypothalamus is interesting, since it indicates that the noradrenergic nerve terminals in the cortex are much more sensitive to this effect of DSP 4 than are the noradrenergic axons and cell bodies in the hypothalamus. This regional difference was also observed for the long-term inhibitory effect of DSP 4 on the noradrenaline uptake but because of the sustained inhibition of the uptake mechanism this difference could not be demonstrated until after two

months. These results suggest that a degenerative process is taking place in the nerve terminals. Such degeneration will of course also affect other constituents of the noradrenergic nerves, e.g. noradrenaline itself.

The finding of a long-term decrease in the noradrenaline content of the rat brain after DSP 4 gives further support to the suggestion that a degeneration of noradrenergic nerve terminals takes place. However, the effect on noradrenaline differed from that on DBH in that the hypothalamus was affected almost as much as the cerebral cortex. One possible explanation of this discrepancy may be that whole cerebral cortex was used for the determination of noradrenaline whereas about 40 mg of the frontal cerebral cortex was used for the assay of DBH activity. The significant antagonism by desipramine of the reduction in the noradrenaline concentration is in accordance with the hypothesis that the long-term decrease in noradrenaline is a secondary phenomenon and that the primary attack is in alkylation of the membranal uptake sites.

Since the 25% fall in the 5-HT concentration induced by DSP 4 in the whole rat brain did not correspond to a similar decrease in the 5-HT uptake this effect of DSP 4 does not appear to be due to a degeneration of 5-HT neurones. The origin of this effect has to be examined in further experiments.

Because of its long-term selective degeneration of the noradrenergic nerve terminals DSP 4 may become a valuable tool in studies of the functional actions of noradrenline in the brain. Compared with 6-hydroxydopamine, the compound now used for this purpose, DSP 4 has the advantage of readily passing the blood-brain barrier and can accordingly be injected parenterally. Furthermore, it seems to be more selective than 6-hydroxydopamine, which also affects dopamine neurones (Ungerstedt, 1968). However, before DSP 4 can be used as a pharmacological tool its action on central and peripheral monoaminergic receptor mechanisms has to be evaluated. Such studies are now being performed.

I thank Mrs Öie Frödén and Miss Diana Kelder for skilful technical assistance and Professor Richard Dahlbom and Dr Björn Lindborg for the synthesis of DSP 4.

References

BOGDANSKI, D.F., PLETSCHER, A., BRODIE, B.B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharm. exp. Ther.*, **117**, 82–88.

CHANG, C.C. (1964). A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmac.*, **3**, 643–649.

COYLE, J.T. & AXELROD, J. (1972). Dopamine-β-hydroxylase in the rat brain: developmental characteristics. *J. Neurochem.*, **19**, 449–459.

MOLINOFF, P.B., BRIMIJOIN, S., WEINSHILBOUM, R. & AXELROD, J. (1970). Neurally mediated increase in dopamine-β-hydroxylase activity. *Proc. Nat. Acad. Sci., USA*, **66**, 453–458.

MOLINOFF, P.B., WEINSHILBOUM, R. & AXELROD, J. (1971). A sensitive enzymatic assay for dopamine-β-hydroxylase. *J. Pharmac. exp. Ther.*, **178**, 425–431.

ROSS, S.B., JOHANSSON, J.G., LINDBORG, B. & DAHLBOM, R. (1973). Cyclizing compounds. I. Tertiary *N*-(2-bromobenzyl)-*N*-haloalkylamine with adrenergic blocking action. *Acta pharmaceutica Suecica*, **10**, 29–42.

ROSS, S.B. & RENYI, A.L. (1975). Tricyclic antidepressant agents. I. Comparison of the inhibition of the uptake of ^{3}H-noradrenaline and ^{14}C-5-hydroxytryptamine in slices and crude synaptosome brain. *Acta pharmac. tox.*, **36**, 382–394.

ROSS, S.B. & RENYI, A.L. (1976). On the long-lasting inhibitory effect on *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP 4) on the active uptake of adrenaline. *J. Pharm. Pharmac.*, **28**, 458–459.

ROSS, S.B., RENYI, A.L. & ÖGREN, S.-O. (1972). Inhibition of the uptake of noradrenaline and 5-hydroxytryptamine by chlorphentermine and chlorimipramine. *Eur. J. Pharmac.*, **17**, 107–112.

SNYDER, S.H. & COYLE, J.T. (1969). Regional difference in ^{3}H-norepinephrine and ^{3}H-dopamine uptake into rat brain homogenates. *J. Pharmac. exp. Ther.*, **165**, 78–86.

UNGERSTEDT, U. (1968). 6-Hydroxydopamine induced degeneration of central monoamine neurones. *Eur. J. Pharmac.*, **5**, 107–110.

(Received March 15, 1976.)