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Dose-dependent effects of noradrenergic denervation by DSP-4 treatment on forced swimming and β-adrenoceptor binding in the rat

J. Harro[1,2], **R. Pähkla**[3], **A.-R. Modiri**[2,4], **M. Harro**[2,5], **A. Kask**[3], and **L. Oreland**[2]

[1] Department of Psychology, [3] Department of Pharmacology, and [5] Department of Public Health, University of Tartu, Tartu, Estonia
[2] Department of Neuroscience, Pharmacology, University of Uppsala, Biomedical Centre, Uppsala, Sweden
[4] Pharmacia & Upjohn, Uppsala, Sweden

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Summary. DSP-4 is a neurotoxin highly selective for the noradrenergic nerve terminals originating from the locus coeruleus. Preliminary data suggested that its effect in a typical screening test for antidepressant drugs, the forced swimming test, is biphasic dependent on the dose. In the present study, DSP-4 was administered in four doses (5, 10, 30 and 50 mg/kg) to male Wistar rats. Administration of the neurotoxin had a dose-dependent biphasic effect on immobility time in the forced swimming test 8 and 9 days later. Thus, DSP-4 at the dose of 10 mg/kg increased immobility, but higher doses reduced this measure. The reduction of noradrenaline concentration in the frontal cortex and hippocampus was dose-dependent starting from the dose 10 mg/kg. Cortical β-adrenoceptor binding was increased by DSP-4 treatment at the doses 30 mg/kg and 50 mg/kg. These results suggest that the increase in immobility time in the forced swimming test is associated with presynaptic changes in noradrenaline availability, whereas the decrease in immobility observed after more complete denervation is associated with postsynaptic receptor supersensitivity.

Keywords: DSP-4, noradrenaline, depression, forced swimming, β-adrenoceptor, rat.

Introduction

Noradrenergic pathways from the locus coeruleus (LC) to the forebrain have been implicated in selective attention and general arousal (Foote et al., 1983). The neurones in the LC are very responsive even to mild non-noxious stimuli in the environment. External sensory events involving vigilance or orientation

towards stimuli increase LC cell firing rates (Aston-Jones et al., 1991). In stressful situations, the metabolism of noradrenaline (NA) is remarkably increased (Korf et al., 1973), which might mediate the response of the organism to challenging situations (Levine et al., 1990).

The neurotoxin DSP-4 [N(2-chloroethyl)-N-ethyl-2-bromobenzylamine] has, after its original description (Ross, 1976), been widely used for selective destruction of the nerve terminals originating from the LC (Jonsson et al., 1981; Fritschy and Grzanna, 1989). Peripheral administration of DSP-4 depletes NA in the brain regions which are innervated by the LC and has either zero or little effect on serotonin and dopamine levels. After DSP-4 treatment, upregulation of α_1-(Johnson et al., 1987; Nowak et al., 1991), α_2- and β-adrenoceptors (Dooley et al., 1983a) is observed, in parallel to biochemical supersensitivity to α_1-adrenoceptor agonists (Johnson et al., 1987), biochemical or behavioural supersensitivity to α_2-(Dooley et al., 1983b; Benkirane et al., 1995), and β-adrenoceptor agonists (Dooley et al., 1983b), and hyperresponsivity of hippocampal neurons to NA (Yasuda et al., 1986). Previous studies have shown that DSP-4-induced lesions cause no gross changes in spontaneous behaviour (Jonsson et al., 1982), but that the animals fail to engage in active behaviour necessary to cope with environmental changes (Archer, 1983; Archer et al., 1984) due to an increase in neophobia and possibly some reduction in their motivation to explore (Harro et al., 1995).

DSP-4 is used, by most investigators, in a standard dose of 50 mg/kg (or even higher) to reach maximal depletion of tissue NA levels. Despite of about 400 published papers in which DSP-4 has been used as a neurochemical tool, surprisingly little is known about the dose-dependency of its primary effect (degeneration of NA-ergic nerve terminals), and there are virtually no studies on the functional consequences of different degrees of LC denervation caused by DSP-4 treatment. An exception is the study by Archer et al. (1984) in which it was found that doses of DSP-4 from as low as 3 mg/kg (upwards) impaired two-way avoidance learning.

In a preliminary experiment, we have found that the effect of DSP-4 on the immobility scores in the forced swimming test, a common test for screening of antidepressants, can depend upon the dose of the neurotoxin administered (Harro and Oreland, 1996). From a theoretical point of view, if low levels of NA were responsible for depressed behaviour, DSP-4 treatment should increase the immobility time. However, we found that a standard dose of DSP-4 (50 mg/kg) which depletes LC projection areas of NA to 80% or more had a statistically significant antidepressant-like effect. Nevertheless, a low dose of DSP-4 (10 mg/kg) indeed produced a tendency towards increased immobility. This seemingly contradictory finding is even more surprising in the context that DSP-4 (50 mg/kg) has been found to reduce active coping behaviour, at least in tests which measure exploration and learning (Archer, 1983; Archer et al., 1984; Delini-Stula et al., 1984; Harro et al., 1995). Interestingly, there are other data suggesting that a standard dose (50 mg/kg) of DSP-4 has effects opposite to what is expected of a depressogenic compound. Thus, DSP-4 treatment prolongs the latency of paradoxical sleep, and reduces sleep rebound after deprivation (Gonzales et al., 1996).

The aim of this investigation was to compare forced swimming behaviour, NA tissue levels and β-adrenoceptor binding in the rat brain after administration of various doses of DSP-4.

Materials and methods

Animals

Male Wistar rats (200–250 g) from Grindex, Riga, Latvia, were housed 5 per cage after arrival and left to habituate to the housing conditions at the Laboratory Animal Department for at least seven days. The animal room had light schedule 12:12 (lights on at 8 a.m.). Food and water were available ad libitum. All rats living in a common cage received the same neurotoxin treatment.

Neurotoxin treatment and general procedure

DSP-4 [N(2-chloroethyl)-N-ethyl-2-bromobenzylamine] was injected in the volume of 2 ml/kg intraperitoneally. Each dose was weighed separately, dissolved in distilled water, and injected immediately. Control animals received an injection of distilled water. Doses are expressed as for hydrochloride. Seven days after the treatment the animals were submitted to the forced swimming test on two consecutive days. Twenty four hours after the last behavioural procedure the rats were decapitated. Some animals were left undisturbed until decapitation. Noradrenaline measurements were carried out on animals not subjected to forced swimming. Half of the animals (n = 4–5 depending upon group) included in the β-adrenoceptor analysis had been subjected to forced swimming.

Forced swimming test

The technique first characterized by Porsolt et al. (1978) was used after pharmacological validation for our laboratory conditions (Pähkla et al., 1996; Harro et al., 1997). Briefly, rats (9–10 per group) were forced to swim in a vertical glass box (floor 19 × 29 cm; height 39 cm) containing 25 cm of water maintained at 25°C. On the first day of experiments, the rats were forced to swim 15 min and were thereafter dried with laboratory tissues. On the following day, the rats were re-exposed to the forced swimming. The total duration of immobility was measured on both days, judging the rat to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its head just above the surface. The time of observation was the first 5 min of forced swimming on both days.

Noradrenaline concentration measurements

The sample preparation was performed as described by Felice et al. (1978) and the analytical procedure as by Sharp and Zetterström (1992). In brief, approximately 50 mg of brain tissue (frontal cortex, hippocampus) of each rat (n = 5 per group) was homogenized in 1.25 ml solution containing 1 ml 0.4 M $HClO_4$, 100 µl 10% EDTA and 50 µl 5% $Na_2S_2O_5$. Solution samples (100 µl) contained 100 ng of 3,4-dihydroxybenzylamine (DHBA) as internal standard. After centrifugation and decanting, catecholamines were extracted by alumina adsorption. The analytes were desorbed by addition of 0.125 M boric/citric acid and, after centrifugation, separated by reverse-phase HPLC and measured by electrochemical detection. All values were corrected for the recovery of the internal standard.

[^{3}H]-dihydroalprenolol binding

Brain tissue samples (frontal cortex) of each rat (n = 9 per group) were homogenized in ice-cold Tris-HCl (50 mM, pH 7.4) using a Potter-S glass-teflon homogenizer (1,000 rpm,

10 passes). The membranes were washed three times in the same buffer by centrifugation and resuspension. The final pellet was rehomogenized in Tris-HCl buffer (50 mM; pH 8.0). β-Adrenoceptor labelling was carried out in the presence of 0.05–3.2 nM tritiated ligand dihydroalprenolol ([^{3}H]-DHA, spec. act. 94 Ci/mmol, Amersham Radiochemicals) at room temperature with 2 mg w/w tissue per tube in a total incubation volume of 0.3 ml. Propranolol (5 μM) was added to determine nonspecific binding. The incubation medium contained 10 μM pargyline (a MAO inhibitor) and 10 μM 5-HT in order to prevent binding of the tracer to 5-HT receptors. Incubation was terminated after 40 min by rapid filtration over Whatman GF/B filters using Brandel Cell Harvester (M-24S). The filters were washed with 9 ml cold incubation buffer, dried and assayed for radioactivity by liquid scintillation spectrometry.

Data analysis

All binding data were analyzed by nonlinear least-squares regression analysis using a commercial program GraphPad PRISM™ 2.0 (GraphPad Software, San Diego, U.S.A.) and the statistical analysis was carried out with the StatView® 4.5 package (Abacus Concepts, Berkeley, U.S.A.) for Power Macintosh. Behavioural and biochemical data were treated with analysis of variance (ANOVA). Repeated measures analysis was performed for the behavioural data, collapsing the data collected on two different days. Group differences after significant ANOVAs were measured by post hoc Fisher's PLSD test.

Results

Repeated measures ANOVA demonstrated a significant effect of DSP-4 treatment on the immobility scores in the forced swimming test [$F(4,44) = 4.04$, $p < 0.01$]. There was no significant difference in the effect of the neurotoxin on the two days, as revealed by repeated measures ANOVA. ANOVA for simple main neurotoxin effect revealed a highly significant influence of DSP-4 on immobility [$F(4,93) = 6.72$, $p < 0.0001$]. This effect was dose-dependent and biphasic (Fig. 1): 10 mg/kg of DSP-4 prolonged the immobility time compared to the vehicle group but 30 mg/kg shortened it, and the shortening effect of 50 mg/kg just missed ($p = 0.083$) the conventional level of statistical significance in comparison with the vehicle group. The differences between the 10 mg/kg group with 30 mg/kg and 50 mg/kg groups were highly significant ($p < 0.0001$).

DSP-4 treatment caused a dose dependent decrease in NA levels both in the frontal cortex (Fig. 2A) and hippocampus (Fig. 2B) [$F(4,20) = 7.81$ or 8.53 for the frontal cortex and hippocampus, respectively, $p < 0.001$]. Even the lowest dose of DSP-4 (5 mg/kg) decreased the NA levels by 18 and 35% in the frontal cortex and hippocampus, respectively, and the lack of statistical significance in the latter region was probably only due to the spread of data in a small sample. In doses of 10 mg/kg and higher, DSP-4 reduced NA levels significantly and to a large extent in both brain regions. The maximal effect was obtained already with the dose 30 mg/kg.

DSP-4 treatment had also a significant effect on the maximal apparent number of [^{3}H]-DHA binding sites with the 5-HT sites masked [$F(4,40) = 2.63$, $p < 0.05$]. The treatment effect was statistically significant compared to the vehicle group with doses 30 and 50 mg/kg (Fig. 3). No effect on the binding affinity was found. Mean K_D values for all groups were within the range of

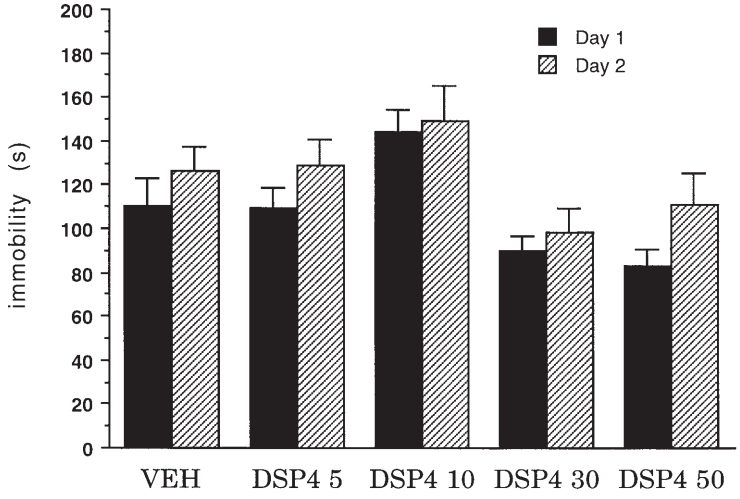

Fig. 1. Immobility time in the forced swimming test in rats after DSP-4 pretreatment. Animals were injected with either DSP-4 (5, 10, 30 or 50 mg/kg) or vehicle seven days prior to the experiment. Animals were forced to swim on two consecutive days and immobility was measured during first 5 min of swimming. Dark columns represent the immobility time on the first exposure to forced swimming and hatched columns show the duration of immobility on the subsequent day. The results are expressed as means ± SEM, n = 9–10 per group. For statistical evaluation of the data, see Results

0.48–0.58 nmol/l. Since half of the rats had been submitted to forced swimming, the possibility that this had influenced β-adrenoceptor binding was explored by using two-way ANOVA with Treatment and Swimming as independent factors. There was a tendency toward the reducing effect of forced swimming on the number of 5-HT-masked [^{3}H]-DHA binding sites ($p = 0.064$), but there was no interaction with the Treatment factor.

Discussion

These experiments show that the lesioning of the NA LC projections by the administration of DSP-4 can cause different changes in rat forced swimming dependent upon the extent of the denervation. After a low dose of DSP-4 (10 mg/kg), there was a significant reduction in tissue NA levels, no change in β-adrenoceptor density, and a behavioural effect opposite to that of antidepressant drugs. In contrast, after the administration of higher doses of DSP-4 which led to almost total depletion of NA levels, small but significant upregulation of β-adrenoceptors in the frontal cortex and a behavioural effect reminiscent of antidepressant action were observed.

Forced swimming behaviour after DSP-4 treatment has been studied in mice (Semba and Takahashi, 1988). DSP-4 (50 mg/kg) treatment caused a nonsignificant trend towards a decrease in immobility in that study. However, administration of L-threo-dihydroxyphenylserine, an artificial NA precursor, which did not affect forced swimming in control mice, potently reduced immobility in DSP-4-treated mice. This suggests that in animals with LC denerva-

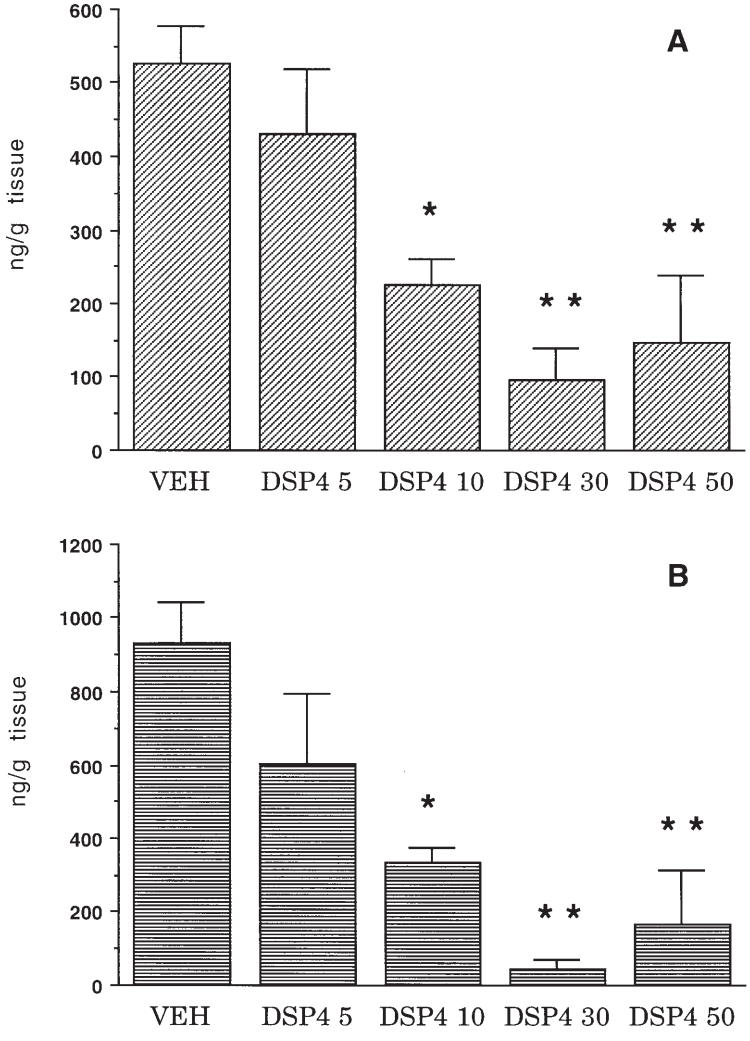

Fig. 2. Noradrenaline content in the frontal cortex (**A**) and hippocampus (**B**) of rats after DSP-4 pretreatment. Animals were injected with either DSP-4 (5, 10, 30 or 50 mg/kg) or vehicle nine days prior to decapitation. Results are expressed an ng/g tissue, means ± SEM, n = 5 per group. *$p < 0.01$, **$p < 0.001$, DSP-4 groups compared to the vehicle group

tion, NA has a more potent effect in the forced swimming test. This could be caused by denervation-induced receptor supersensitivity. As yet, it is not known, which subtype of adrenoceptors is involved and in which brain region.

Plaznik et al. (1988) administered 65 mg/kg of DSP-4 to rats and expressed their observations of forced swimming behaviour in terms of animal's behaviour directed to escape from the water tank. DSP-4 treatment had no significant effect of its own (despite of a tendency to increase the struggling time) but antagonized the anti-struggling effect of inescapable shock. On the other hand, the anti-exploration effect of inescapable shock in an open field

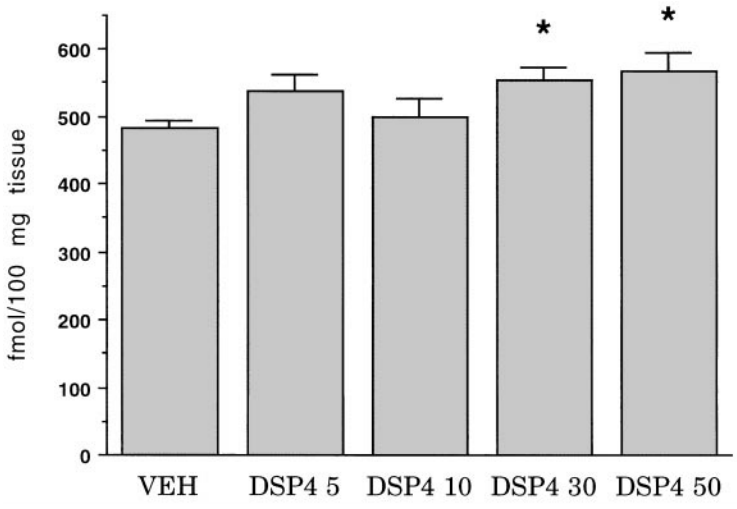

Fig. 3. Maximal apparent number (B_{max}) of [^{3}H]-dihydroalprenolol (DHA) binding sites with masking the 5-HT receptors in the frontal cortex of rats after DSP-4 pretreatment. Animals were injected with either DSP-4 (5, 10, 30 or 50 mg/kg) or vehicle nine days prior to decapitation. Results are expressed as fmol/100 mg tissue, means ± SEM, n = 9 per group. *$p < 0.05$, DSP-4 groups compared to the Vehicle group

test was potentiated by DSP-4 treatment which also reduced exploration by itself. One study has reported no effect of DSP-4 treatment (50 mg/kg) on forced swimming (Esposito et al., 1987). However, the immobility periods in this study were exceptionally long (about 85% of the time of observation), which may have been caused by the low level (17 cm) of water in the cylinders.

Previous studies (Dooley et al., 1983a; Eison et al., 1991; Theron et al., 1993; Wolfman et al., 1994) have found increased numbers of [^{3}H]-DHA binding sites in the neocortex and hippocampus after DSP-4 treatment. The increase in β-adrenoceptor binding is believed to be due to the reduction of synaptic NA. After DSP-4 (50 mg/kg) treatment, chronic treatment with various antidepressants does not elicit any reduction in β-adrenoceptor density, which is the characteristic response to these drugs in control animals (Hall et al., 1984; Eison et al., 1991).

This study does not provide direct evidence for associating pre- and postsynaptic changes in NA-ergic neurotransmission with the biphasic effect of DSP-4 on forced swimming in rats. Nevertheless, it is tempting to speculate that the increase in immobility after a low dose of DSP-4 is related to a decreased NA-ergic neurotransmission and the decrease in immobility after high doses of DSP-4 to a net increase during this highly stressful procedure because of receptor supersensitivity. It is plausible that the amount of releasable NA is lower after DSP-4 treatment in the LC projection areas, but this does not mean that there is no potential for substantial release. Recent in vivo microdialysis studies aimed at the measurement of extracellular NA after LC denervation by DSP-4 treatment (Kask et al., 1997; Hughes and Stanford,

1998) have provided evidence that despite profound reductions in NA tissue levels, there can still be a considerable NA release. In a study on the overflow of NA in the frontal cortex after DSP-4 (50 mg/kg) in anaesthetized rats (Kask et al., 1997), it was found that the basal extracellular level of NA was similar to control, even though tissue levels were very low. Stimulation of NA release by systemic administration of atipamezole, an α_2-adrenoceptor antagonist, resulted in a considerably lower but still significant increase in the extracellular levels of NA, which suggests that there was some release potential upon stimulation. These results are compatible with those of Abercrombie and Zigmond (1989), who demonstrated that the reduction of tissue NA content in the hippocampus after lesioning of the dorsal noradrenergic bundle by 6-OHDA treatment is greater than the reduction of extracellular NA in this brain region measured by microdialysis. In their study, the amounts of NA in the dialysates remained normal when the tissue NA content was not decreased more than 50%, and when the reduction of NA content in the tissue was 50–90%, it was of a lower magnitude in the dialysates. It was also found that both local perfusion with excess K^+ and administration of a stressful stimulus, a tail shock, increased NA content in the dialysates in control rats and in rats with up to 50% tissue depletion of NA to an equal level. In the first study in freely-moving rats, Hughes and Stanford (1998) demonstrated that a partial lesion of LC projections by DSP-4 treatment may lead even to increased basal levels of extracellular NA. After administration of a somewhat lower dose of DSP-4 (40 mg/kg), a 75% reduction of tissue NA levels was achieved. However, nearly two-fold higher basal extracellular NA levels were found, due to an increase in neuronal release in the DSP-4 treated rats. A depolarizing pulse of K^+ induced a proportionally larger NA release response after DSP-4 treatment in both anaesthetised (Kask et al., 1997) and awake animals (Hughes and Stanford, 1998). However, subsequent pulses of K^+ did not result in larger release of NA (Hughes and Stanford, 1998), probably reflecting the depletion of transmitter pools. As yet, it is unknown on which mechanisms such an overcompensation of NA-ergic neurotransmission is based, but it could be related to increased synthesis of NA in the remaining nerve terminals, together with the preferential release of newly synthesized NA (Carlsson, 1975).

LC denervation has been shown to reduce active behavioural strategies in moderately demanding tasks (Archer, 1983; Archer et al., 1984; Delini-Stula et al., 1984; Harro et al., 1995). On the other hand, extremely challenging situations such as immersion into water provoke more active behaviour in comparison with control animals. It has also been shown that DSP-4 (50 mg/kg) treated rats are more aggressive towards conspecifics when given electric footshocks (Mogilnicka et al., 1993), even though they are not more aggressive in home-cage conditions (Cornwell-Jones et al., 1990).

On the basis of the present and earlier studies, it seems likely that the behavioural response of the rat in a challenging situation depends upon the balance between the NA release and receptors responsiveness. This balance is likely to be different at different doses of DSP-4, which may explain some of the controversies in the literature on the topic. We have earlier presented a

hypothesis that modestly decreased tonic activity of the LC is of major importance for the development of depression (Harro and Oreland, 1996). The present results lend some support to this hypothesis and suggest that treatment of rats with low doses of DSP-4 might provide a relevant animal model of depression.

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Authors' address: Prof. J. Harro, Department of Psychology, University of Tartu, Tiigi 78, EE-2400 Tartu, Estonia.