Psychopharmacology (1998) 136 : 299–303

ORIGINAL INVESTIGATION

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A partial noradrenergic lesion induced by DSP-4 increases extracellular noradrenaline concentration in rat frontal cortex: a microdialysis study in vivo

Received: 4 September 1997/Final version: 7 September 1997

Abstract The effect of systemic administration of the selective neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) on noradrenaline efflux in the frontal cortex was studied in freely-moving rats using microdialysis in vivo. Five days after treatment with DSP-4 (40 mg/kg IP), the noradrenaline content of the frontal cortex was reduced by 75%. Yet, noradrenaline efflux in the frontal cortex was nearly two-fold greater in DSP-4 treated rats than in saline-injected controls. Local infusion of the noradrenaline-selective uptake blocker, desipramine (5 μM), via the microdialysis probe, increased noradrenaline efflux in rats from both groups. Perfusion of Ringer's solution, containing 80 mM K^+, also increased noradrenaline efflux in both groups, but the increase after DSP-4 pretreatment was greater than in the controls. In contrast, removal of Ca^{2+} from the infusion medium reduced noradrenaline efflux in both treatment groups. These results indicate that, at this dose, DSP-4 increases the extracellular concentration of noradrenaline in rat frontal cortex despite causing a partial lesion of noradrenergic neurones. This is due to an increase in the release of noradrenaline, although reduced clearance is also likely. These data challenge the assumption that depletion of noradrenaline content after treatment with DSP-4 invariably translates into diminished noradrenergic transmission.

Key words Desipramine · DSP-4 · In vivo microdialysis · Noradrenaline

Introduction

Systemic injection of the neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) causes marked depletion of noradrenaline, particularly in brain regions innervated by the locus coeruleus (Jonsson et al. 1981; Fritschy and Grzanna 1989). After an initial surge of transmitter release, immediately after administration of DSP-4 (Smith et al. 1996), depletion of central noradrenaline stores is evident several hours later (Ross 1976; Fritschy et al. 1990). Within 3 days of DSP-4 treatment, a number of additional features of noradrenergic function are altered. For instance, there is a substantial reduction in the number of noradrenaline uptake sites in the cortex labelled by the radioligands, [^{3}H]desipramine (Lee et al. 1982) or [^{3}H]nisoxetine (Cheetham et al. 1996). In fact, a decrease in the uptake of [^{3}H]noradrenaline into synaptosomes prepared from the cortex of DSP-4-treated rats is evident as early as 1 day after treatment (Ross 1976). Heal and colleagues (1993) have also reported a reduction in the number of cortical presynaptic α_2-adrenoceptors 3 days after DSP-4 administration, while β-adrenoceptor numbers are increased in the cortex and hippocampus after only 1 day (Theron et al. 1993).

In addition to these early changes, an abrupt and profound loss of the noradrenaline synthesising enzyme, dopamine-β-hydroxylase, from cortical tissue is evident between 4 and 5 days after DSP-4 injection (Ross 1976; Fritschy et al. 1990). All these findings support the view that, within 5 days of administration, DSP-4 causes marked functional changes which could affect noradrenergic transmission in the brain.

Previous experiments from this laboratory have indicated that, despite a marked depletion of noradrenaline in the tissues, uptake of [^{3}H]noradrenaline into rat cortical synaptosomes ex vivo was unaffected by pretreatment with DSP-4 (Hughes and Stanford 1996). However, the inhibition of [^{3}H]noradrenaline uptake by low concentrations (0.5 or 5 μM) of the selective noradrenaline uptake inhibitor, desipramine, was reduced by DSP-4 pretreatment (Hughes and Stanford 1996). These findings raise the question of how

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DSP-4 treatment affects the extracellular concentration of noradrenaline in the brain? This is an important point, because noradrenergic transmission will be determined by the concentration of extraneuronal transmitter. For this reason, microdialysis in freely moving rats was used to evaluate the effects of DSP-4 treatment on the concentration of extracellular noradrenaline in the frontal cortex of freely moving rats. In the course of this work, it became evident that, 5 days after injection of DSP-4, when there is an extensive depletion of noradrenaline, efflux of noradrenaline was increased rather than reduced. Consequently, further experiments went on to investigate whether diminished reuptake of noradrenaline and/or an increase in its rate of release could contribute to this increase.

Materials and methods

Subjects

Outbred male Sprague-Dawley rats (280–320 g), derived from a colony at University College London, were used throughout. They were housed in groups of four and maintained on a 12-h light/dark cycle (lights on at 0800 hours) with unlimited access to food and water. Drug-naïve animals were used for every experiment and all procedures complied with the UK Scientific Procedures (Animals) Act, 1986.

DSP-4 treatment

Rats were injected systemically with DSP-4 (40 mg/kg IP). Control animals were given an equivalent injection of saline vehicle (2 ml/kg). Five days later, microdialysis was carried out on animals from both treatment groups. At the end of every experiment, rats were killed and a sample of the frontal cortex dissected from the side which was not used for microdialysis. These tissue samples were stored at −20 °C for measurement of noradrenaline, 5-hydroxytryptamine (5-HT) and dopamine content by high pressure liquid chromatography coupled to an electrochemical detector (HPLC-ECD). The mobile phase comprised (mM): sodium dihydrogen orthophosphate 100, sodium octanesulfonic acid 2.8, EDTA 0.7, 20% methanol, adjusted to pH 3.2 with orthophosphoric acid. Monoamines were detected using a glassy carbon electrode at an oxidising potential of 600 mV.

Intracerebral microdialysis

Microdialysis probes were constructed of Filtral 12 membrane (Hospal Industrie, France) with a 5 mm conducting zone; outer diameter 300 μm, inner diameter 200 μm with a 20 kD relative molecular mass cut-off. Four days after injection of DSP-4 or vehicle, Ringer-primed dialysis probes were implanted stereotaxically into the right or left frontal cortex, (A 3.5, L ± 1.5, V 5.0 mm from bregma; Paxinos and Watson 1986) of halothane anaesthetised rats. On the following day (i.e. 5 days after DSP-4 or vehicle injection), after recovery from the anaesthesia, the probe was perfused at 1.0μl/min with modified Ringer's solution comprising (mM): NaCl 145, KCl 4, $CaCl_2$ 1.3, (pH 6.1). Rats were left for 90 min after starting perfusion, after which dialysates were collected at 20-min intervals into 5 μl 0.01 M perchloric acid. The noradrenaline content of these samples was measured using HPLC-ECD as described in Dalley and Stanford (1995).

Changes in noradrenaline efflux induced by desipramine

Four basal samples of dialysate were collected in order to define spontaneous ("basal") noradrenaline efflux. The perfusion medium was then changed to a modified Ringer's containing desipramine (5 μM). Thereafter, dialysis samples were collected every 20 min for 3 h and their noradrenaline content measured by HPLC-ECD. Changes in noradrenaline efflux were compared in control and DSP-4-pretreated rats.

Ca^{2+}-dependence of noradrenaline efflux

After collecting four basal dialysates, the normal perfusion medium was replaced with modified Ringer's, from which $CaCl_2$ had been omitted (KCl 4, NaCl 145 mM). This Ca^{2+}-free Ringer's was infused for 80 min, after which the normal perfusion medium was reinstated. Subsequent recovery of noradrenaline efflux was monitored for a further 120 min.

$[K^+]$-sensitivity of noradrenaline efflux

Once stable basal noradrenaline efflux was established, the perfusion medium was changed to one containing an increased concentration of K^+: this modified Ringer's solution comprised (mM): KCl 80, NaCl 71, $CaCl_2$ 1.3. After 40 min, this was replaced with normal Ringer's. This cycle was repeated twice more with intervening recovery periods of 60 min.

Statistical analysis

Drug-induced changes in the concentration of noradrenaline in cortical microdialysates were analysed by split-plot analysis of variance (ANOVA) as described in Dalley and Stanford (1995). Data were split into bins of four consecutive samples, with "time" or "bin" as the "within-subjects" factor and "treatment" as the "between-subjects" factor. Significance of changes in monoamine content was analysed using the Mann-Whitney *U*-test.

Drugs

Desipramine HCl was purchased from Sigma Chemical Co., UK and DSP-4 HCl from Research Biochemicals International. DSP-4 was dissolved in sterile saline and administered in a volume of 2 ml/kg.

Results

Cortical monoamine content

DSP-4 caused a 75% reduction in the noradrenaline content when compared with that of saline-injected rats ($P < 0.001$, Table 1). Neither the 5-HT ($P = 0.78$) nor the dopamine ($P = 0.39$) content of the cortex was affected by treatment with DSP-4.

Table 1 Concentrations of noradrenaline, dopamine and 5-HT in the frontal cortex of saline- or DSP-4-pretreated rats. Data are pooled from all experiments and show mean ± SEM ng/g wet tissue weight with sample size in parentheses. * $P < 0.001$ with respect to saline control group (Mann-Whitney *U*-test)

	Concentration (ng/g)	
	Saline	DSP-4
Noradrenaline	854.7 ± 118.6 (9)	189.7 ± 22.4 (20)*
Dopamine	24.4 ± 7.0 (9)	65.4 ± 17.5 (20)
5-HT	187.1 ± 39.5 (9)	176.0 ± 25.5 (20)

Effects of desipramine

Basal noradrenaline efflux was significantly greater in DSP-4-pretreated rats than in saline-injected controls (DSP-4: 50.2 ± 1.8 fmol/20 min; saline: 27.2 ± 0.5 fmol/20 min, $F_{1,13} = 14.97$; $P = 0.02$) (Fig. 1a).

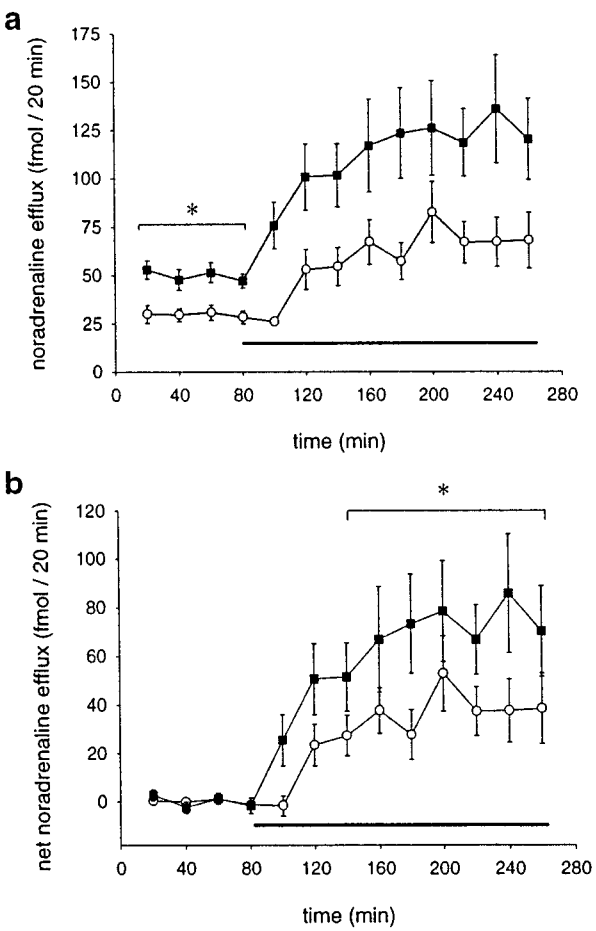

Fig. 1 a,b Effects of local infusion of desipramine (5 μM) on noradrenaline efflux in the frontal cortex of saline- (○) ($n = 5$) or DSP-4 (■) treated ($n = 9$) rats. *Solid bar* indicates 3 h duration of drug infusion. Data expressed as mean ± SEM noradrenaline efflux (fmol/20 min). *$P < 0.05$ cf. saline controls (ANOVA). **a** Results show absolute levels of noradrenaline in dialysates. **b** The change in noradrenaline concentration of dialysates expressed as "net" noradrenaline efflux

Infusion of 5 μM desipramine caused a statistically significant increase in noradrenaline efflux in the frontal cortex of both DSP-4 ($F_{3,66} = 3.68$; $P = 0.02$) and saline-pretreated rats ($F_{2,27} = 5.57$; $P < 0.01$). Because of the difference in basal efflux in the two treatment groups, "net efflux" was calculated. This involved subtracting the mean of the four basal samples, from each subsequent point, for each subject (Fig. 1b). This showed that net noradrenaline efflux, during hours 2 and 3 of desipramine infusion, was greater in the DSP-4-pretreated group when compared with saline controls ($F_{1,12} = 5.92$; $P = 0.03$).

Ca^{2+}-dependence

Once again, noradrenaline efflux was significantly greater in basal samples collected from DSP-4-treated rats than saline controls (saline: 26.3 ± 2.5 fmol/20 min; DSP-4: 42.8 ± 7.3 fmol/20 min; $F_{1,8} = 5.41$; $P < 0.05$) (Fig. 2). In both groups, noradrenaline efflux was reduced to approximately 10 fmol/20 min when Ca^{2+} was excluded from the perfusate. This decrease was statistically significant compared to basal samples in both groups (saline: $F_{1,6} = 12.8$; $P = 0.01$; DSP-4: $F_{1,8} = 10.7$; $P = 0.01$).

Depolarising pulses of K^+

Basal noradrenaline efflux in DSP-4-treated rats was again greater than that in saline controls (saline: 32.2 ± 4.4 fmol/20 min; DSP-4: 52.9 ± 4.9 fmol/20 min; $F_{1,27} = 29.8$; $P < 0.01$) (Fig. 3a). Because of this difference, net efflux of noradrenaline during perfusion of Ringer's containing 80 mM K^+ was

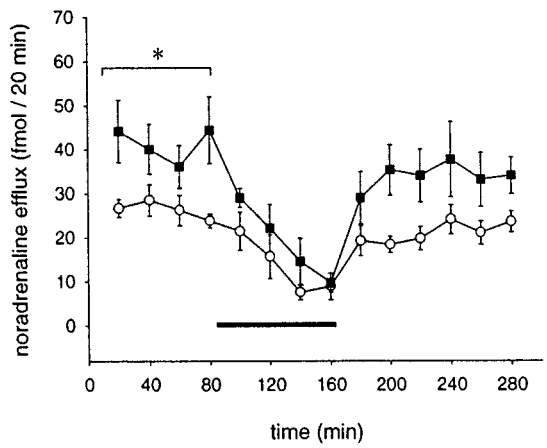

Fig. 2 The dependence of noradrenaline efflux in the frontal cortex of either saline- (○) ($n = 4$) or DSP-4-treated rats (■) ($n = 5$) on extracellular Ca^{2+}. *Solid bar* indicates 80 min infusion of Ca^{2+}-free Ringer's. Data expressed as mean ± SEM noradrenaline efflux (fmol/20 min). *$P < 0.05$ cf. saline controls (ANOVA). Results show absolute levels of noradrenaline in dialysates

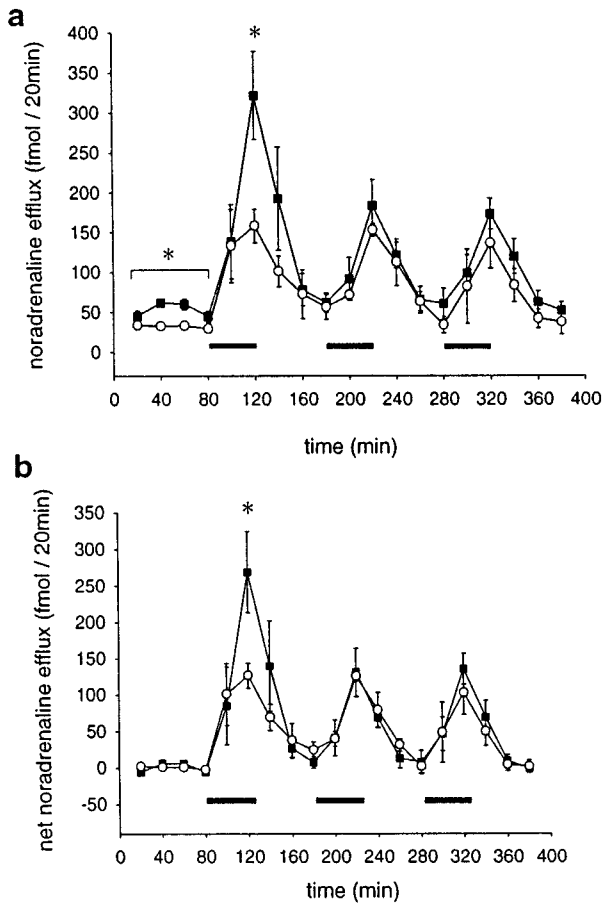

Fig. 3 a,b The effects on noradrenaline efflux of infusion of three consecutive 40-min depolarising pulses of Ringer's containing 80 mM K^+ in saline- (○) ($n = 5$) and DSP-4-(■) ($n = 4$) pretreated rats. *Solid bar* indicates infusion of K^+; at all other times normal Ringer's was infused. Data expressed as mean ± SEM noradrenaline efflux (fmol/20 min). *$P < 0.05$ cf. saline controls (ANOVA). **a** Results show absolute levels of noradrenaline in dialysates. **b** The change in noradrenaline concentration of dialysates expressed as "net" noradrenaline efflux

calculated (see above). In both DSP-4 and saline-pretreated rats, all three depolarising pulses of K^+ caused a rapid increase in noradrenaline efflux (Fig. 3b). The increase caused by the first K^+ pulse in the DSP-4-pretreated rats was considerably greater than that in the saline controls ($F_{1,8} = 7.3$; $P = 0.03$). Also, in the DSP-4-pretreated group, the increase in noradrenaline efflux induced by the first K^+ pulse was statistically significantly greater than that due to the second and third pulses (270 fmol/20 min; $P < 0.05$).

Discussion

The noradrenaline content of the frontal cortex was reduced by approximately 75% in rats which had been treated with DSP-4 5 days earlier. At this time, a marked reduction in dopamine-β-hydroxylase in the terminal vesicles of noradrenergic neurones in the brain has been reported (Fritschy et al. 1990). This reduction, which is regarded as a reliable indicator of axon degeneration, is profound within 5 days of DSP-4 treatment. Together with the depletion of noradrenaline stores, these findings suggest that, in the present experiment, DSP-4 caused a partial lesion of noradrenergic neurones in the frontal cortex.

Despite this, the concentration of extracellular noradrenaline was nearly two-fold (20 fmol/20 min) *greater* in the DSP-4-pretreated rats than in the saline controls. Kask and colleagues (1997) have measured extracellular noradrenaline in anaesthetised rats 7 days after DSP-4 administration, but did not find any change in basal efflux of noradrenaline. This could be attributed to either the use of anaesthesia or, more importantly, the presence of an uptake blocker (nomifensine) in the perfusion medium. It is difficult to predict how either of these factors might affect spontaneous noradrenaline release after a lesion. Interestingly, Abercrombie and Zigmond (1989) found that a 6-OHDA-induced lesion of noradrenergic neurones in the hippocampus was not paralleled by a reduction in the extracellular concentration of noradrenaline.

An increase in the concentration of extracellular noradrenaline after DSP-4 treatment could be explained by either an increase in the amount of noradrenaline released from, and/or a decrease in the amount of noradrenaline taken up by, surviving neurones. Evidence from the present experiments suggests that neurones which survive exposure to the neurotoxin are still able to take up extracellular noradrenaline. This is because local infusion of the noradrenaline uptake blocker, desipramine, increased extracellular noradrenaline in both saline- and DSP-4-treated rats. In fact, the net increase in efflux was greater in the rats which had developed a partial lesion of noradrenergic neurones, suggesting that uptake is increased, rather than reduced under these conditions.

This finding would seem to be at variance with results from experiments looking at synaptosomal uptake of [^{3}H]noradrenaline in vitro, in which the inhibition of uptake by low concentrations of desipramine was reduced by DSP-4 pretreatment (Hughes and Stanford 1996). However, the maximum inhibition of uptake, at higher concentrations of desipramine, was unchanged. This suggests that there could be two components of the uptake of noradrenaline and only one, a high affinity component, is diminished by treatment with DSP-4. This possibility is consistent with results from an earlier study (Lee et al. 1982) which showed that [^{3}H]desipramine binding to rat brain membranes has two components distinguished by their affinity for [^{3}H]desipramine. The high, but not the low, affinity site is abolished by a DSP-4 lesion. It is likely, therefore, that the desipramine-induced increase in noradrenaline efflux, seen in the present experiments is

due to inhibition of noradrenaline uptake via the low affinity uptake site. Since microdialysis showed that the desipramine-induced increase in noradrenaline efflux was greater in DSP-4-treated rats than in the controls, this low affinity site evidently has a large capacity for noradrenaline uptake.

Nevertheless, it is obvious that an increase in noradrenaline uptake cannot cause an increase in its extracellular concentration. This means that, as well as an increase in uptake, the rate of release of noradrenaline must also be increased despite the partial lesion of noradrenergic neurones. Approximately 75% of spontaneous noradrenaline efflux measured in the DSP-4-treated rats was dependent on the presence of extracellular Ca^{2+}. This suggests that the extracellular noradrenaline is largely derived from Ca^{2+} dependent exocytotic release of transmitter from surviving neurones. The possibility that release rate is increased despite depletion of noradrenaline is further supported by the effects of a depolarising pulse of K^+ on noradrenaline efflux: the increase in efflux in the DSP-4-pretreated group was more than twice that caused by the same challenge in control rats. Interestingly, on repeating the K^+ challenge, the exaggerated noradrenaline response was no longer evident: i.e. the increase in efflux was the same as in the controls. It appears that neurones which survive the DSP-4 treatment cannot sustain a high rate of transmitter release.

An increase in noradrenaline release after DSP-4 treatment is consistent with results from two early studies. These showed that noradrenaline turnover was increased, but only in brain regions where tissue noradrenaline content was greatly depleted (cortex, hippocampus: Hallman and Jonsson 1984; Logue et al. 1985). Also, Chiodo et al. (1983) found a four-fold increase in the firing rate of locus coeruleus noradrenergic neurones which survived a 6-OHDA lesion. These authors speculated that the increase in neuronal activity is a compensatory response to the lesion.

In conclusion, the present experiments indicate that, 5 days after administration of DSP-4, when there is a partial lesion of noradrenergic neurones, the concentration of extracellular noradrenaline in the rat frontal cortex is increased. This paradoxical finding involves an increase in both the rate of reuptake and release of noradrenaline in the DSP-4 treated rats. However, an imbalance in these changes, possibly arising from loss of a high affinity noradrenaline uptake site, means that uptake cannot keep pace with release. These results challenge the widely held assumption that a reduction in noradrenaline content after treatment with DSP-4 is invariably paralleled by a reduction in noradrenergic transmission in the brain.

Acknowledgement Z.A.H. is an MRC scholar.

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