British Journal of Pharmacology (1998) 124, 1141–1148
http://www.stockton-press.co.uk/bjp

Evidence from microdialysis and synaptosomal studies of rat cortex for noradrenaline uptake sites with different sensitivities to SSRIs

[1,2]Zoë A. Hughes & [1,3]S. Clare Stanford

[1]Department of Pharmacology, University College London, Gower Street, London WC1E 6BT

1 Microdialysis of the frontal cortex of freely-moving rats and uptake of [^{3}H]noradrenaline into cortical synaptosomes were used to evaluate changes in efflux of noradrenaline *in vivo* and uptake of [^{3}H]noradrenaline *in vitro*, respectively, induced by the selective serotonin reuptake inhibitors (SSRIs), fluoxetine and citalopram, and the tricyclic antidepressant, desipramine.

2 Noradrenaline efflux was increased during local infusion into the cortex of each of these drugs. All three agents also inhibited synaptosomal uptake of [^{3}H]noradrenaline; this inhibition was unaffected by a substantial (50%) lesion of central 5-hydroxytrytaminergic neurones induced by intracerebroventricular infusion of 5,7-DHT (150 μg).

3 A noradrenergic lesion (70%), induced by pretreatment with the selective neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4, 40 mg kg^{-1} i.p.), 5 days earlier, abolished the increase in noradrenaline efflux caused by local infusion of fluoxetine. In contrast, the desipramine-induced increase in efflux was greater than in non-lesioned rats whereas the effect of citalopram on noradrenaline efflux was unaffected by DSP-4 pretreatment.

4 The combined results of all these experiments suggest that there could be more than one, functionally distinct, noradrenaline uptake site in rat frontal cortex which can be distinguished by their different sensitivities to desipramine and the SSRIs, fluoxetine and citalopram.

Keywords: citalopram; desipramine; 5,7,-DHT; DSP-4, fluoxetine; frontal cortex; microdialysis; noradrenaline; selective serotonin reuptake inhibitor (SSRI); synaptosomal uptake

Introduction

Low micromolar concentrations of fluoxetine inhibit the uptake of [^{3}H]noradrenaline by synaptosomes from rat cerebral cortex. Citalopram has a similar effect, albeit at approximately 10 fold higher concentrations (Hughes & Stanford, 1996). Given that fluoxetine and citalopram are regarded as selective, high-affinity inhibitors of the 5-hydroxytryptamine (5-HT) transporter (*reviewed by*: Stanford, 1996), the target for the inhibition of [^{3}H]noradrenaline uptake by these drugs is unclear.

Previous experiments have already tested whether selective inhibitors of 5-HT uptake (SSRIs) could act at a site on central noradrenergic nerve terminals (Hughes & Stanford, 1996). This was investigated by evaluating the effects of a lesion of noradrenergic neurones on the inhibition by these drugs of [^{3}H]noradrenaline uptake *in vitro*. The lesion was induced by intraperitoneal (i.p.) injection of the selective neurotoxin *N*-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) (Fritschy & Grzanna, 1989; Fritschy *et al*., 1990; Cheetham *et al*., 1996). This procedure modified the inhibition of synaptosomal uptake of [^{3}H]noradrenaline by desipramine and fluoxetine in different ways, but the effects of citalopram were unaltered (Hughes & Stanford, 1996).

All these findings provide some evidence that a site on noradrenergic neurones might be a target for fluoxetine and desipramine at least. However, the possibility that both fluoxetine and citalopram might prevent uptake of [^{3}H]noradrenaline by sites associated with 5-hydroxytryptaminergic neurones should be considered also. By definition, SSRIs show preferential selectivity for 5-HT uptake sites and evidence suggests that 5-HT transporters are found on 5-hydroxytryptaminergic, but not noradrenergic neurones (Austin *et al*., 1994; Zhou *et al*., 1996). However, the possibility that some other mechanism effects transport of noradrenaline into 5-HT neurones cannot be ruled out. If this occurs, inhibition of synaptosomal uptake of [^{3}H]noradrenaline by SSRIs, and possibly total [^{3}H]noradrenaline uptake, should be diminished by a lesion of central 5-hydroxytryptaminergic neurones.

The first of the present experiments investigated this possibility by testing whether a selective lesion of central 5-HT-releasing neurones, induced by intracerebroventricular (i.c.v.) injection of the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) (Baumgarten *et al*., 1973; Björklund *et al*., 1975), affected the inhibition of synaptosomal uptake of [^{3}H]noradrenaline by fluoxetine or citalopram. The selective noradrenaline uptake blocker, desipramine, was used routinely as an active control. However, this still leaves open the question of whether inhibition of noradrenaline uptake into noradrenergic neurones contributes to an increase in the concentration of extracellular noradrenaline in the frontal cortex. To explore this further, the present study went on to investigate the effects of a noradrenergic lesion on the changes in efflux of noradrenaline in this brain region caused by local infusion of SSRIs or desipramine. In these experiments, microdialysis in freely-moving rats was used to compare efflux of noradrenaline in the frontal cortex of rats treated with either DSP-4 or vehicle (non-lesioned) 5 days earlier. Finally, to test whether any changes in noradrenaline efflux were secondary to an increase in the extracellular concentration of 5-HT, the effect of local infusion of 5-HT itself on noradrenaline efflux was also assessed.

[2] Present address: University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.
[3] Author for correspondence.

Methods

Animals

Outbred male Sprague-Dawley rats (280–320 g), derived from a colony at University College London, were used throughout. Rats were housed in groups of four and maintained on a 12 h light/dark cycle (lights on at 08.00 h) with unlimited access to food and water. Drug-naïve animals were used for every experiment and all procedures complied with the U.K. Scientific Procedures (Animals) Act, 1986.

5,7-DHT lesion of 5-hydroxytryptaminergic neurones

Rats were injected with desipramine (25 mg kg^{-1} i.p.). Halothane anaesthesia was induced 40 min later, after which rats were placed in a stereotaxic frame. The skull was exposed to enable i.c.v. injection of 5,7-DHT (150 μg 5,7-DHT dissolved in 10 μl sterile saline containing 0.02% ascorbate), or vehicle (for non-lesioned rats), into the left or right lateral ventricle (AP 0.8; ML $\pm$1.4; DV 3.9 mm from bregma; Paxinos & Watson, 1986). The rats were killed 7 days later and their brains dissected for preparation of cerebral cortical synaptosomes. An aliquot of cortical tissue was frozen for subsequent measurement of tissue 5-HT, noradrenaline and dopamine content using HPLC-ECD.

Uptake of [^{3}H]noradrenaline into cortical synaptosomes

Seven days after surgery, 5,7-DHT-pretreated and non-lesioned rats were killed by stunning and cervical dislocation. A crude preparation of synaptosomes was derived from the cerebral cortex as described in Dalley & Stanford (1995a). Briefly, 100 μl aliquots of a resuspended (12,500 $\times$ *g*) pellet were preincubated in duplicate, at 37°C or 4°C, for 3 min in modified Tris-Krebs buffer comprising (mM): NaCl 136, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, (+)–glucose 10, (+)–ascorbate 1, Tris base 20 and pargyline HCl 0.25. The buffer was gassed with 95% O_2/5% CO_2 for 30 min and its pH adjusted to 7.4 at 37°C before use. Test drugs were dissolved in the buffer solution and added to the incubation medium, in a volume of 100 μl buffer, to give the following final concentrations: desipramine or fluoxetine, 0.5, 5.0 and 50 μM; citalopram, 2.5, 25 or 50 μM. After 5 min, the uptake assay was started by addition of 100 μl [^{3}H]noradrenaline in a total volume of 500 μl (final concentration in incubate: 50 nM). The incubation was terminated by filtration, 3 min later. Non-specific uptake was defined as the amount of [^{3}H]noradrenaline which accumulated in synaptosomes incubated at 4°C. Specific uptake of [^{3}H]noradrenaline was expressed as pmol mg^{-1} protein. The protein content of an aliquot of each resuspended pellet was measured using the method of Lowry *et al.* (1951).

DSP-4 lesion of noradrenergic neurones

Rats were injected systemically with DSP-4 (40 mg kg^{-1} i.p.); control (non-lesioned) animals were given an equivalent injection of saline vehicle (2 ml kg^{-1}). Rats from both groups were used for microdialysis experiments 5 days later. After completion of these experiments, rats were killed and their brains removed. The frontal cortex (on the side of the brain contralateral to where the probe was implanted) was dissected for measurement of tissue noradrenaline, 5-HT and dopamine content by HPLC-ECD.

Intracerebral microdialysis

Microdialysis probes were constructed from Filtral 12 membrane (Hospal Industrie, France) with a 5 mm conducting zone; inner diameter 200 μm, outer diameter 300 μm with relative molecular mass cut-off at 20 kD. Four days after pretreatment of rats with either DSP-4 or saline, Ringer-primed dialysis probes were implanted vertically, under halothane anaesthesia, into the right or left frontal cortex (AP 3.5, ML $\pm$1.5, DV 5.0 mm; Paxinos & Watson, 1986). On the following day, after recovery from the surgery (and 5 days after the pretreatment), the probe was reconnected and perfused, at 1.0 μl min^{-1} with modified Ringer's solution comprising (mM): NaCl 145, KCl 4, $CaCl_2$ 1.3, (pH 6.6). Dialysates were collected into 5 μl 0.01 M perchloric acid, at 20 min intervals, starting 90 min after reconnection of the probes. Once stable efflux of noradrenaline was obtained, four samples of dialysate were collected in order to establish spontaneous ('basal') noradrenaline efflux. After collection of the fourth basal sample, a perfusion medium containing either desipramine, fluoxetine or citalopram was infused. Test drugs were freshly dissolved in modified Ringer's (composition as above) and administered by reverse dialysis for 3 h. The probe concentrations of each drug were: desipramine, 5 μM; fluoxetine, 5 or 50 μM; and citalopram 50 μM. In a final set of experiments, 5-HT (5 μM) was dissolved in the same medium and infused, for 80 min, *via* the probe.

The noradrenaline content of the sample dialysates was measured by reverse-phase HPLC with electrochemical detection (Coulochem II; ESA) (Dalley & Stanford, 1995b). Samples were injected in a loop volume of 20 μl. Separation was achieved on a Hypersil ODS 5 μm column (25 cm) using mobile phase of the following composition: sodium dihydrogen orthophosphate (83 mM); sodium octanesulfonic acid (2.77 mM), EDTA (0.85 mM), methanol (12%); pH 3.4 (adjusted with orthophosphoric acid). Noradrenaline was detected at an oxidising potential of 180 mV and chromatograms were relayed to an on-line integrator where peak height was used to determine content. The detection limit for noradrenaline was approximately 2 fmoles. Each rat was used to study the effects of only one drug at a single concentration.

Measurement of monoamine content of cortical tissue

Cortical tissue was homogenised in 0.01 M perchloric acid at a concentration of 100 mg ml^{-1} and spun at 9,500 $\times$ *g* for 5 min. The supernatant was analysed for noradrenaline, dopamine and 5-HT content using HPLC-ECD as described in Hughes & Stanford (1996). Briefly, 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 by amperometric detection using a glassy carbon electrode at an oxidising potential of 600 mV.

Statistical analysis

Drug-induced changes in the concentration of noradrenaline in cortical microdialysates were analysed by split-plot analysis of variance (ANOVA) of orthonormalised raw data, as described in Dalley & Stanford (1995b), with 'time' and 'bin' (clusters of three or four consecutive samples, using a balanced design) as 'within-subjects' factors and 'treatment' as the 'between-subjects' factor. Data from uptake experiments were analysed using 1- and 2-way ANOVA with *post-hoc* Duncan Test. The Mann–Whitney U-Test was used to compare differences in the

monoamine content of cortical tissues. Criterion for statistical significance was set at $P<0.05$.

Drugs

l-[7-^{3}H]norepinephrine (specific activity 13.3 Ci mmol^{-1}; New England Nuclear) was used. Desipramine HCl, fluoxetine HCl, 5-hydroxytryptamine creatinine sulphate and 5,7-DHT creatinine sulphate were purchased from Sigma Chemical Co., U.K., and DSP-4 HCl from Research Biochemicals International. Citalopram HBr was a generous gift from Lundbeck, Copenhagen, Denmark. Drugs for i.p. injection were made up in sterile saline (0.9%) and injected in a volume of 2 ml kg^{-1}.

Results

Cortical monoamine content after 5,7-DHT pretreatment

The 5-HT content of the cerebral cortex, 7 days after 5,7-DHT pretreatment was 54% lower than in non-lesioned rats ($P<0.001$), indicating a substantial lesion of 5-hydroxytryptaminergic neurones. In contrast, there was no statistically significant change in either noradrenaline or dopamine content (Table 1).

[^{3}H]noradrenaline uptake into synaptosomes from non-lesioned or 5,7-DHT-injected rats

In the absence of test drugs, there was no difference in synaptosomal uptake of [^{3}H]noradrenaline in tissues from 5,7-DHT-pretreated and non-lesioned rats: (pmol mg^{-1} protein: non-lesioned, 1.11 ± 0.11 ($n=10$); 5,7-DHT, 1.02 ± 0.1 ($n=11$)).

Addition of fluoxetine (0.5–50 μM) to the incubation medium reduced the uptake of [^{3}H]noradrenaline into synaptosomes from both non-lesioned ($F=37.6$; d.f. 3,13; $P<0.001$) and 5,7-DHT-pretreated rats ($F=9.23$; d.f. 3,15; $P=0.002$) (Figure 1a). This inhibition by fluoxetine was unaffected by 5,7-DHT pretreatment (drug concentration × lesion interaction: $F=0.85$; d.f. 3,29; $P=0.48$).

Citalopram also inhibited [^{3}H]noradrenaline uptake into synaptosomes prepared from non-lesioned ($F=3.07$; d.f. 3,21; $P<0.05$) or 5,7-DHT-treated rats ($F=9.04$; d.f. 3,25; $P<0.001$; Figure 1b). This was evident at concentrations of 25 or 50, but not 2.5 μM. Like fluoxetine, the inhibition of [^{3}H]noradrenaline uptake by citalopram was not affected by treatment with 5,7-DHT (drug × lesion interaction; $F=0.95$; d.f. 3,47; $P=0.43$).

Finally, incubation with desipramine inhibited [^{3}H]noradrenaline uptake in tissues from non-lesioned ($F=14.3$; d.f. 3,23; $P<0.001$) and 5,7-DHT-treated rats ($F=17.9$; d.f. 3.23; $P<0.001$) (Figure 1c). In both treatment groups, this inhibition was statistically significant at all concentrations tested (0.5–50 μM). As with fluoxetine and citalopram, the pretreatment with 5,7-DHT did not affect the inhibition of uptake by desipramine ($F=0.02$; d.f. 3,47; $P=0.99$).

Cortical monoamine content after DSP-4 pretreatment

At 5 days post-injection, the noradrenaline content of the frontal cortex of DSP-4 pretreated rats was reduced by 70% when compared with non-lesioned controls ($P<0.001$). In contrast, neither the concentration of 5-HT nor dopamine was significantly affected by DSP-4 (Table 2).

Table 1 Concentrations of noradrenaline, dopamine and 5-HT of the cerebral cortex of non-lesioned or 5,7-DHT-treated rats

	Concentration (ng g^{-1}) *non-lesioned*	*5,7-DHT*
Noradrenaline	447.4±41.5 (14)	416.8±36.0 (17)
Dopamine	197.0±32.0 (12)	304.1±57.8 (17)
5-HT	379.1±50.7 (14)	174.7±16.2 (17)*

Data show mean±s.e.mean ng g^{-1} wet tissue weight with sample size in parentheses. *$P<0.001$ (*c.f.* non-lesioned group).

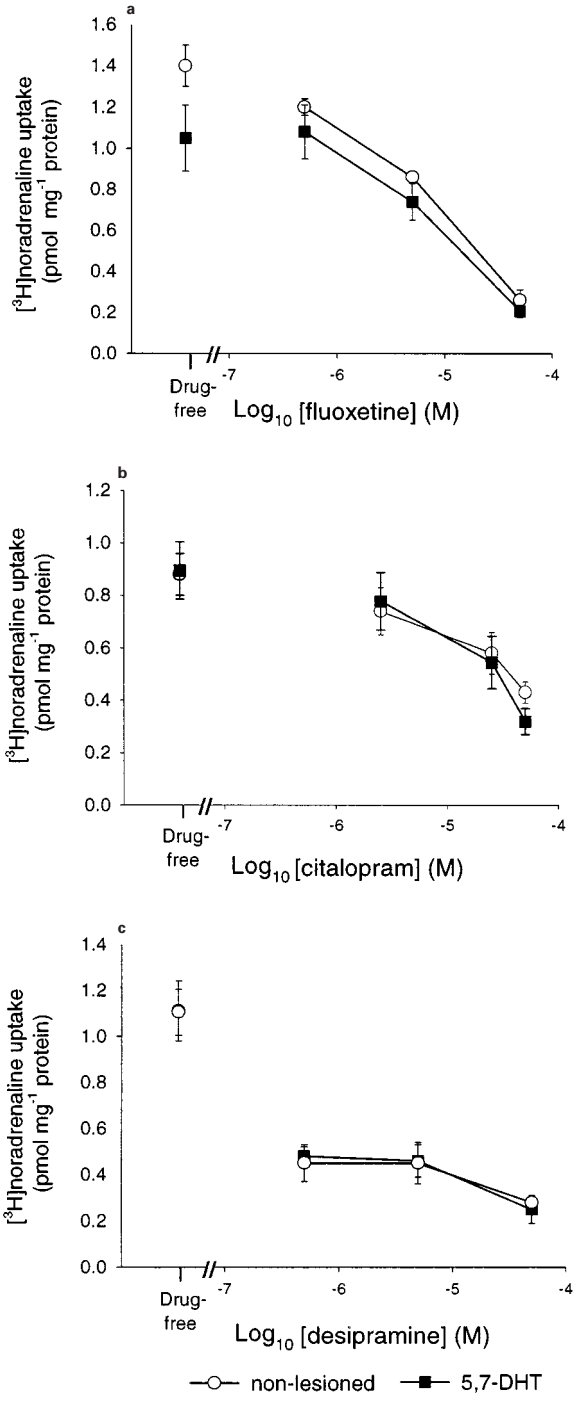

Figure 1 Synaptosomal [^{3}H]noradrenaline uptake in the presence of (a) fluoxetine, (b) citalopram and (c) desipramine. Graphs show uptake into synaptosomes prepared from rats injected (i.c.v) with either vehicle- (non-lesioned) or 5,7-DHT, 7 days earlier ($n=4-7$).

Noradrenaline efflux during local infusion of desipramine

Infusion of 5 μM desipramine increased noradrenaline efflux in both non-lesioned and DSP-4-pretreated rats (non-lesioned: $F=8.16$; d.f. 2,11; $P=0.007$; DSP-4: $F=6.02$; d.f. 2,22; $P=0.008$; Figure 2a). However, one factor which might confound these results is the efflux of noradrenaline in the basal samples from DSP-4 pretreated rats which was approximately 2 fold greater than in the non-lesioned animals (DSP-4: 50.2 ± 2.2; non-lesioned: 29.6 ± 1.8 fmol 20 min^{-1}; $P<0.001$). To compensate for this increase, 'net' efflux of noradrenaline was calculated. This involved subtracting the mean basal efflux for each rat from every individual point of the time course, followed by calculation of the mean$\pm$s.e.mean of the ensuing differences.

When the net increase in efflux of noradrenaline was calculated, it was evident that during the second and third hour of desipramine infusion, this was approximately 2 fold greater in DSP-4 pretreated rats than in the non-lesioned controls ($F=5.92$; d.f. 1,12; $P=0.03$) (Figure 2b).

Table 2 Concentrations of noradrenaline, dopamine and 5-HT of the frontal cortex of non-lesioned or DSP-4-injected rats

	Concentration (ng g^{-1}) *non-lesioned*		*DSP-4*
Noradrenaline	579.4$\pm$77.9	(9)	174.7$\pm$11.9 (20)*
Dopamine	25.8$\pm$7.2	(9)	81.1$\pm$19.2 (20)
5-HT	171.8$\pm$48.8	(9)	151.0$\pm$19.5 (20)

Data show mean$\pm$s.e.mean ng g^{-1} wet tissue weight with sample size in parentheses. *$P<0.001$ (*c.f.* non-lesioned group).

Noradrenaline efflux during local infusion of citalopram

During the first hour of infusion, citalopram (50 μM) increased noradrenaline efflux in both non-lesioned ($F=6.25$; d.f. 1,7; $P=0.04$) and DSP-4 ($F=4.96$; d.f. 1, 13; $P=0.04$) injected rats (Figure 3a). Because, as before, the basal level of efflux was greater in the DSP-4 pretreated group, net noradrenaline efflux was calculated in order to expose a clearer comparison of the drug-induced changes in efflux. This procedure showed that the net increase in efflux induced by citalopram over the 3 h of infusion was the same in the non-lesioned and DSP-4 pretreated rats (approximately 15 fmol 20 min^{-1}; $F=0.12$; d.f. 1,26; $P=0.73$) (Figure 3b).

Noradrenaline efflux during local infusion of fluoxetine

Local infusion of either 5 μM (Figure 4; $F=9.25$; d.f. 2,13; $P=0.003$) or 50 μM (Figure 5; $F=10.9$; d.f. 2,14; $P=0.001$) fluoxetine increased noradrenaline efflux in non-lesioned rats.

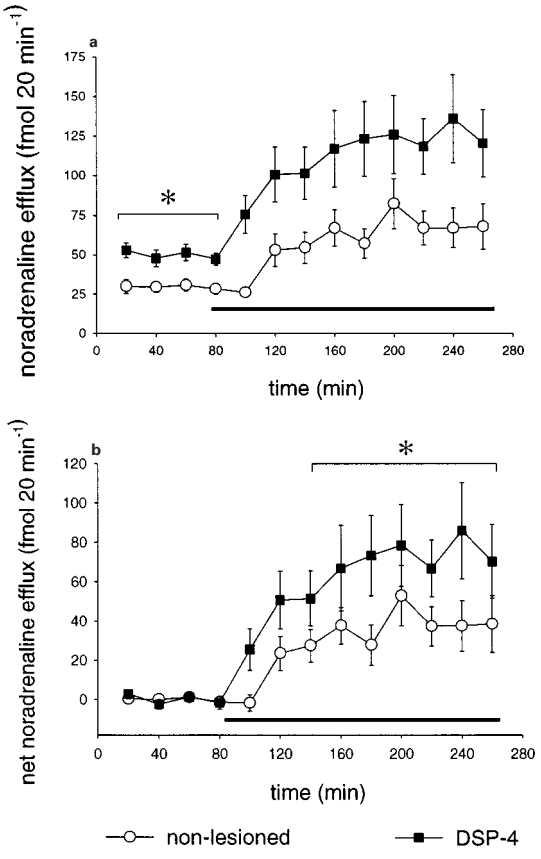

Figure 2 The effect of desipramine (5 μM) infusion on noradrenaline efflux in the frontal cortex of freely-moving vehicle (non-lesioned) or DSP-4 pretreated rats. — indicates duration of drug infusion. Graphs show mean$\pm$s.e.mean ($n=5-9$) noradrenaline efflux expressed as fmol 20 min^{-1}. *$P<0.05$ (*c.f.* non-lesioned controls). (a) Absolute levels of noradrenaline efflux. (b) Noradrenaline concentration of dialysates expressed as net noradrenaline efflux.

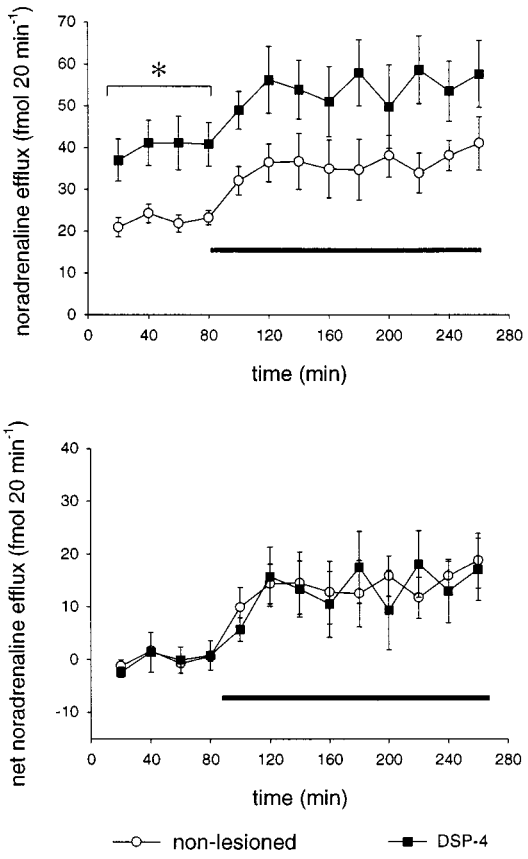

Figure 3 The effect of infusion of 50 μM citalopram on noradrenaline efflux in the frontal cortex of freely-moving vehicle- (non-lesioned) or DSP-4 pretreated rats. — indicates duration of drug infusion. Graphs show mean$\pm$s.e.mean ($n=5-7$) noradrenaline efflux expressed as fmol 20 min^{-1}. *$P<0.05$ (*c.f.* non-lesioned controls). (a) Absolute levels of noradrenaline efflux. (b) Noradrenaline concentration of dialysates expressed as net noradrenaline efflux.

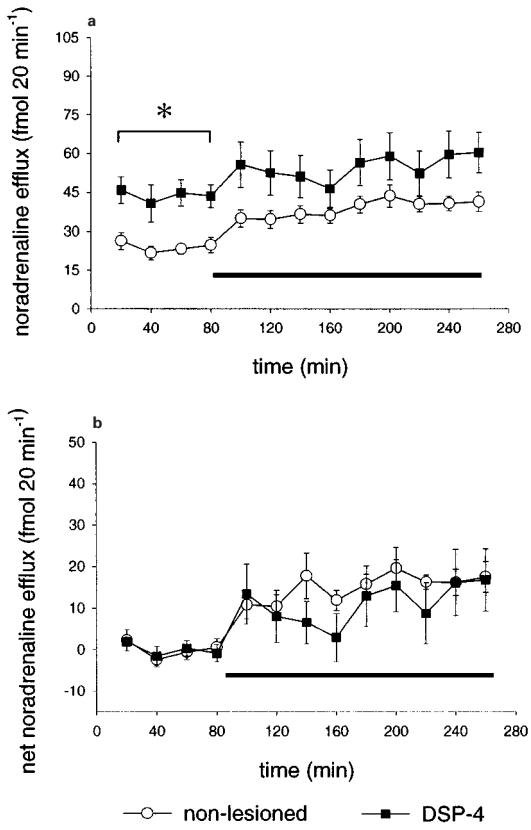

Figure 4 The effect of infusion of fluoxetine (5 μM) on noradrenaline efflux in the frontal cortex of freely-moving vehicle- (non-lesioned) or DSP-4 pretreated rats. — indicates duration of drug infusion. Graphs show mean $\pm$ s.e.mean ($n=7-9$) noradrenaline efflux expressed as fmol 20 min^{-1}. $^{*}P<0.05$ (*c.f.* non-lesioned controls). (a) Absolute levels of noradrenaline efflux. (b) Noradrenaline concentration of dialysates expressed as net noradrenaline efflux.

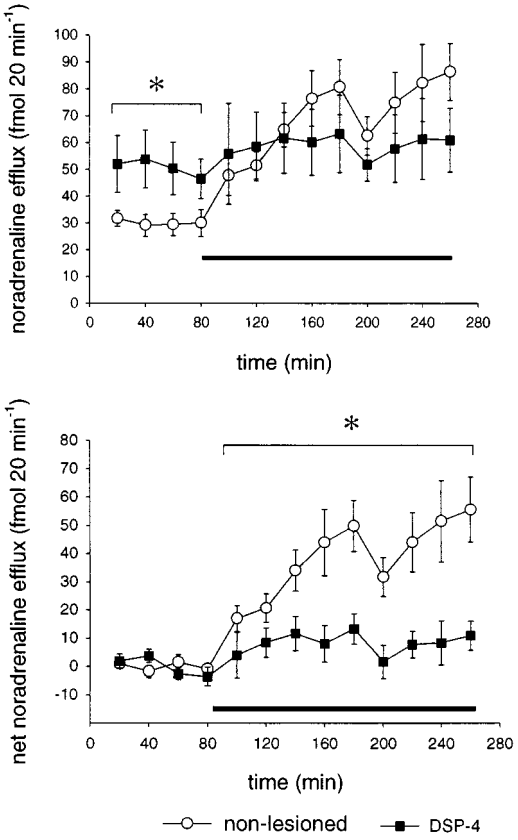

Figure 5 The effect of infusion of 50 μM fluoxetine on noradrenaline efflux in the frontal cortex of freely-moving vehicle- (non-lesioned) or DSP-4 pretreated rats. — indicates duration of drug infusion. Graphs show mean $\pm$ s.e.mean ($n=6-8$) noradrenaline efflux expressed as fmol 20 min^{-1}. $^{*}P<0.05$ (*c.f.* non-lesioned controls). (a) Absolute levels of noradrenaline efflux. (b) Noradrenaline concentration of dialysates expressed as net noradrenaline efflux.

The higher concentration caused an approximately 3 fold increase in efflux in these rats. In contrast, neither concentration of fluoxetine increased noradrenaline efflux in DSP-4 pretreated rats (5 μM: $F=0.51$; d.f. 2,8; $P=0.62$; 50 μM: $F=0.48$; d.f. 2,15; $P=0.631$). The attenuation of the increase in noradrenaline efflux induced by infusion of fluoxetine (50 μM) was statistically significant ($F=17.31$; d.f. 1,8; $P=0.003$; Figure 5b).

Noradrenaline efflux after infusion of 5-HT

Infusion of 5-HT (5 μM) *via* the probe for 80 min had no effect on the concentration of noradrenaline in the dialysis samples ($F=0.78$; d.f. 2,7; $P=0.527$) (Figure 6).

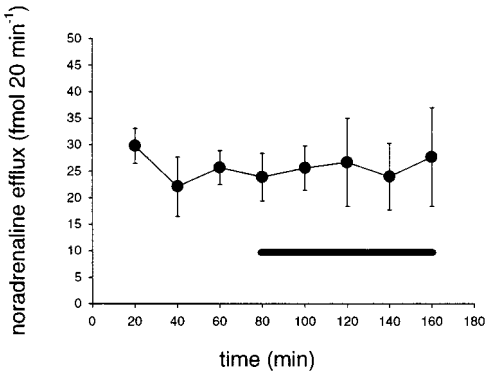

Figure 6 The effect of 5-HT (5 μM) infusion on noradrenaline efflux in the frontal cortex of freely-moving rats ($n=3$). Graphs show mean $\pm$ s.e. mean noradrenaline efflux expressed as fmol 20 min^{-1}. — indicates the duration of infusion.

Discussion

Although noradrenaline efflux is stable during continuous infusion of modified Ringer's solution (Dalley & Stanford, 1995b), the results of this study show that there is an increase in the extracellular concentration of noradrenaline in the frontal cortex when fluoxetine and citalopram are included in the medium. They also confirm our earlier finding that these SSRIs inhibit uptake of [^{3}H]noradrenaline into rat cortical synaptosomes *in vitro* (Hughes & Stanford, 1996). The increase in noradrenaline efflux does not seem to be a result of competition for uptake between noradrenaline and 5-HT, which will accumulate in the presence of SSRIs, because infusion of 5-HT itself (5 μM) did not affect noradrenaline efflux. This is further supported by the evidence that fluoxetine caused a greater increase in noradrenaline efflux than did citalopram, despite the lower potency of fluoxetine as an inhibitor of 5-HT uptake (Richelson & Pfenning, 1984; *see also*: Stanford, 1996).

Transporters for 5-HT are unlikely to be the target for the inhibition of noradrenaline uptake by SSRIs. Evidence

suggests that these transporters are found in association with 5-hydroxytryptaminergic neurones, only (Zhou *et al*., 1996; McLaughlin *et al*., 1996). Yet the inhibition of noradrenaline uptake caused by the SSRIs was not altered by the substantial lesion of 5-hydroxytryptaminergic neurones induced by pretreatment of rats with 5,7-DHT. This supports evidence that 5-HT transporters do not take up noradrenaline (Paczkowski *et al*., 1996) and findings that noradrenaline does not displace [^{3}H]paroxetine binding from solubilized placental 5-HT transporters (Ramamoorthy *et al*., 1993). Interestingly, this contrasts with evidence that 5-HT transporters can take up dopamine, and that this is prevented by SSRIs (Faraj *et al*., 1994). The lack of effect of the 5,7-DHT lesion also suggests that it is equally unlikely that inhibition of a noradrenaline transporter on 5-hydroxytryptaminergic neurones accounts for the increased noradrenaline efflux caused by fluoxetine or citalopram. This is consistent with evidence that [^{3}H]nisoxetine bindings sites are found only on noradrenergic neurones in the rat (Tejani-Butt *et al*., 1990) and that, in the human brainstem, mRNA for noradrenaline transporters is found in noradrenergic neurones, but not 5-hydroxytryptaminergic or dopaminergic neurones (Eymin *et al*., 1995). In view of all this evidence, monoamine transporters on 5-hydroxytryptaminergic neurones do not seem to account for inhibition of noradrenaline uptake by SSRIs.

Nevertheless, there are several lines of evidence to suggest that SSRIs can inhibit noradrenaline transporters. For instance, these drugs displace binding of [^{3}H]nisoxetine, a radioligand used as a marker for the noradrenaline transporter (Jayanthi *et al*., 1993). More recently, it has been suggested that there is a binding domain for SSRIs on the noradrenaline transporter; this is thought to induce allosteric changes which modify the binding of other ligands to other domains on the transporter (Plenge & Mellerup, 1997). If SSRIs can affect uptake by noradrenaline transporters, the next question is: are these transporters on noradrenergic neurones? Since both *in situ* hybridisation of noradrenaline transporter mRNA (Eymin *et al*., 1995) and immunocytochemistry (Lorang *et al*., 1994) suggest that, in the brainstem, the noradrenaline transporter is associated only with noradrenergic neurones, it would be predicted that a selective lesion of these neurones would diminish the increase in noradrenaline efflux caused by SSRIs. However, the present results exposed marked differences in the effects of fluoxetine, citalopram and desipramine on noradrenaline efflux in DSP-4 pretreated rats. These differences cannot be explained in terms of all these compounds acting at the same site.

Considering first, the effects of desipramine: noradrenaline efflux in both non-lesioned and DSP-4 pretreated rats was increased by local infusion of this antidepressant. In fact, the increase in efflux was even greater in the latter group. This means that, despite a 70% reduction in noradrenaline content after DSP-4 pretreatment, uptake of noradrenaline was actually increased after the lesion. Since basal efflux was also increased in these rats, this suggests that there is also an increase in release of noradrenaline from neurones which survive exposure to DSP-4 (*see also*: Hughes & Stanford, 1998). However, of greater relevance to the present study, is the finding that there are noradrenaline uptake sites which survive DSP-4 and which are inhibited by desipramine.

Although the regimen for administration of DSP-4 used here did not completely eliminate noradrenergic neurones in the cortex, there are reports of binding sites for [^{3}H]desipramine which survive exposure to DSP-4. In brief, there is a loss of high-, but not low-, affinity [^{3}H]desipramine binding sites (Lee *et al*., 1982; Bäckström *et al*., 1989). It is thought that the latter are not functional noradrenaline uptake sites (Lee *et al*., 1982; Bäckström *et al*., 1989). However, our previous studies of [^{3}H]noradrenaline uptake *in vitro* indicated that inhibition by low concentrations of desipramine (*i.e* high affinity sites) was diminished after DSP-4 pretreatment, whereas the inhibition at higher concentrations was not (Hughes & Stanford, 1996). It remains to be seen how these DSP-4 resistant uptake sites, with a low affinity for desipramine, relate to the low affinity [^{3}H]desipramine binding sites described in earlier studies.

Quite different results were found with citalopram in that neither the inhibition of [^{3}H]noradrenaline uptake (Hughes & Stanford, 1996), nor the increased noradrenaline efflux caused by this drug (present study) were affected by the DSP-4 lesion. This could suggest that citalopram acts at sites found only on cells which are resistant to DSP-4. In view of the obvious presence of desipramine-sensitive uptake sites on cells which survive exposure to DSP-4, these could be the target for citalopram. The relatively small increase in efflux caused by citalopram, even at high concentrations, compared with that caused by desipramine or fluoxetine, would be consistent with the lower potency of citalopram for inhibition of [^{3}H]noradrenaline uptake and its preferential selectivity for inhibition of 5-HT uptake (*see*: Stanford, 1996). In view of the small increase in efflux of noradrenaline caused by citalopram, it would be hard to distinguish whether this was any greater in DSP-4 pretreated rats, as predicted by the experiment with desipramine. Alternatively, citalopram might bind to a separate domain on desipramine-sensitive transporters and non-competitively reduce uptake of noradrenaline (see: Plenge & Mellerup, 1997). If neither of these is the case, it must be inferred that citalopram targets a different transporter altogether. However, this would lead to the question of how inhibition of one uptake site by citalopram could result in an increase in noradrenaline efflux when another uptake mechanism, which evidently has a large capacity for sequestration of extracellular noradrenaline, remains functionally intact?

Fluoxetine (5 and 50 μM) also increased the efflux of noradrenaline but this was evident only in non-lesioned rats. This is underlined by the finding that the attenuation by the lesion of the increase in noradrenaline efflux induced by the higher concentration of fluoxetine was statistically significant. Yet the lesion did not blunt on the increase in noradrenaline efflux caused by either desipramine or citalopram. An obvious explanation for this finding is that fluoxetine targets the desipramine-sensitive transporter on noradrenergic neurones which are eliminated by DSP-4. However, an argument against this rests on our earlier finding that the amount of [^{3}H]noradrenaline uptake *in vitro* which was inhibited by low concentrations of fluoxetine was increased, rather than reduced, after DSP-4 (Hughes & Stanford 1996) and the effects of higher fluoxetine concentrations were not affected at all. This finding rules out the possibility that fluoxetine targets a separate binding domain on a single transporter protein. It is also unlikely that fluoxetine targets the desipramine-sensitive sites which survive exposure to DSP-4. This is because the increase in efflux caused by fluoxetine, unlike that caused by either desipramine or citalopram, was abolished in DSP-4 pretreated rats.

Collectively, the findings described so far suggest that fluoxetine targets noradrenaline uptake sites *in vivo* which are not the same as those inhibited by desipramine or citalopram. However, it remains to be explained why fluoxetine-sensitive sites are evident in synaptosomes prepared from DSP-4 pretreated rats, but not in lesioned rats *in vivo*. This apparent anomaly suggests that the contribution to clearance of extracellular noradrenaline, by uptake sites which are sensitive

to fluoxetine, is masked *in vivo*. Such masking could arise if the DSP-4 resistant, desipramine-sensitive sites prevent any increase in noradrenaline efflux by sequestering extracellular noradrenaline which would otherwise accumulate after fluoxetine infusion. This would not occur *in vitro* because inhibition of uptake by fluoxetine would not lead to accumulation of noradrenaline outside the synaptosomes. Yet, such masking would also be expected to occur in unlesioned rats unless there is a contextual difference which influences transporter function in these two conditions. For instance, and drawing parallels with inferences from immunocytochemical studies of dopamine transporters (Pickel *et al*., 1996), there could be 'spatial buffering' of noradrenaline. Thus, fluoxetine could increase noradrenaline efflux in unlesioned, but not lesioned, rats if desipramine-sensitive sites are ubiquitously distributed whereas those targeted by fluoxetine are clustered near, and sequester noradrenaline mainly released from, DSP-4 sensitive neurones.

The suggestion that there is more than one type of transporter for noradrenaline is not new. It has been known for many years that noradrenaline released from sympathetic neurones is inactivated by two distinct uptake processes, known as 'uptake1' and 'uptake2'. That these are entirely different transporter systems is indicated by their different transport kinetics (Iversen, 1965) and susceptibility to antagonists (Russ *et al*., 1996; Eisenhofer *et al*., 1996). Recently, a third uptake process, which is resistant to inhibition by desipramine, has been characterised in hepatocytes (Martel *et al*., 1994). Less attention has been devoted to the possibility of multiple noradrenaline transporter systems in the brain, although it has long been suggested that the dopamine transporter could be a second uptake site for noradrenaline in the CNS (Michel *et al*., 1984). Indeed, this is supported by recent evidence that both noradrenaline and dopamine transporters discriminate poorly between these catecholamines (Gu *et al*., 1994; Giros *et al*., 1994). Furthermore, Northern blot analysis of brain tissue has confirmed that there are two mRNAs (5.8 and 3.6 kb) for noradrenaline transporters and it has been suggested that the 3.6-kb mRNA could encode a glial-specific protein (Pacholczyk *et al*., 1991). This is consistent with recent evidence that a transporter responsible for 'uptake2' is found on glial cells in the brain (Russ *et al*., 1996).

Alternative explanations, not resting on the speculation that there are multiple uptake sites for noradrenaline must be considered. In particular, it should be borne in mind that an increase in release of noradrenaline could contribute to the increase in the concentration of extracellular noradrenaline induced by SSRIs. There is ample evidence that 5-HT modulates noradrenaline release through activation of 5-HT receptors on noradrenergic neurones in the locus coeruleus (Done & Sharp, 1994). However, unless there is polysynaptic activation of noradrenaline release, this is unlikely to be relevant here because test drugs were administered locally *via* the microdialysis probe. In any case, this could not explain the difference in the effects of the lesion on fluoxetine and citalopram.

There is also evidence that 5-HT can directly increase release of noradrenaline through activation of heteroceptors on noradrenergic nerve terminals (Mongeau *et al*., 1994). This means that an increase in extracellular 5-HT, resulting from local infusion of SSRIs, could cause an increase in noradrenaline release. However, there are several reasons to exclude this as an explanation for the present results. First, as mentioned above, local infusion of 5-HT itself had no effect on noradrenaline efflux in the frontal cortex. Secondly, citalopram was considerably less potent than fluoxetine in increasing noradrenaline efflux despite its greater affinity for inhibition of 5-HT uptake (Richelson & Pfenning, 1984; *see*: Stanford, 1996). Finally, indirect activation of 5-HT heteroceptors, through accumulation of 5-HT, could not explain why the effects of fluoxetine, but not those of citalopram, were abolished by DSP-4 pretreatment.

Whatever accounts for the present results it is clear that the SSRIs, fluoxetine and citalopram, increase extracellular noradrenaline *in vivo*. This finding has obvious implications for the mechanisms underlying the antidepressant actions of these drugs. It also seems that these two SSRIs affect noradrenaline efflux through different mechanisms and that neither drug relies on processes involving 5-HT neurones. The questions arising from this study merit further investigation in that they suggest that either the contribution of the noradrenaline transporter to noradrenaline uptake depends on its proximity to the site of transmitter release and/or there is more than one, and possibly even a family of, uptake site(s) for noradrenaline which might be distinguished by their different sensitivities to antidepressants.

Z.A. Hughes is an MRC PhD scholar.

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(Received November 14, 1997
Revised March 13, 1998
Accepted April 20, 1998)