ELSEVIER

European Journal of Pharmacology 317 (1996) 83–90

ejp

Increased noradrenaline efflux induced by local infusion of fluoxetine in the rat frontal cortex

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Received 10 June 1996; revised 28 August 1996; accepted 3 September 1996

Abstract

In microdialysis experiments in vivo, local infusion of either the selective serotonin reuptake inhibitor, fluoxetine, or the selective noradrenaline uptake inhibitor, desipramine, increased noradrenaline efflux in rat frontal cortex. Synaptosomal uptake of [^{3}H]noradrenaline was used to test whether inhibition of uptake could contribute to this effect of fluoxetine. Low concentrations of fluoxetine were less effective than desipramine at inhibiting [^{3}H]noradrenaline uptake; both compounds were more potent than the selective serotonin reuptake inhibitor, citalopram. To investigate whether this inhibition of uptake involved an action on noradrenergic neurones, experiments compared the effects of a noradrenergic lesion, induced by the neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), on the inhibition of uptake by fluoxetine, desipramine and citalopram. The lesion reduced [^{3}H]noradrenaline uptake in the presence of fluoxetine and citalopram but increased it in the presence of desipramine. The results suggest both that inhibition of noradrenaline uptake could contribute to the actions of fluoxetine and that a non-noradrenergic mechanism is a target for this action.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; Noradrenaline; Frontal cortex; Microdialysis, in vivo; Synaptosomal uptake

1. Introduction

Inhibition of neuronal uptake of 5-hydroxytryptamine (5-HT, serotonin) is widely thought to underlie the efficacy of the selective serotonin reuptake inhibitors in treatment of depression. Recently, the effects of selective serotonin reuptake inhibitors on the concentration of extracellular noradrenaline in specific brain regions have been investigated. This was achieved by using microdialysis in vivo; test compounds were infused locally via the microdialysis probe. Results from these experiments suggest that, at probe concentrations of between 10–100 μM, fluoxetine increases noradrenaline efflux in the rat medial prefrontal cortex (Jordan et al., 1994), frontal cortex (Hughes and Stanford, 1996) and ventral tegmental area (Chen and Reith, 1994). The selective serotonin reuptake inhibitor, citalopram, had a similar effect in the ventral tegmental area albeit at a higher concentration (100 μM; Chen and Reith, 1994). Since the increase in noradrenaline efflux is evoked by local infusion of fluoxetine or citalopram, this change must involve actions in the terminal field. However, an increase in efflux could be attributed to a change in noradrenaline release and/or reuptake; it is not possible to distinguish between these possibilities on the basis of in vivo microdialysis alone.

There is evidence that increased levels of extracellular 5-HT, acting at heteroceptors, can modify noradrenaline release in the brain (Blandina et al., 1991; Mongeau et al., 1994; Matsumoto et al., 1995). However, the possibility that inhibition of noradrenaline uptake contributes to the increase induced by fluoxetine cannot be excluded. Although this compound is widely regarded as a selective serotonin reuptake inhibitor, its selectivity for inhibition of 5-HT versus noradrenaline uptake into synaptosomes in vitro could be as low as 3-fold (Koe et al., 1983). Moreover, in tissue slices derived from rat frontal cortex, fluoxetine and desipramine were equally effective in inhibiting the uptake of these two monoamines (Harms, 1983).

The present experiments investigated further the changes in the concentration of extracellular noradrenaline caused by local infusion of fluoxetine in the rat frontal cortex. In particular, the concentration and time dependence of any

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PII S0014-2999(96)00715-7

changes were compared with those for desipramine. Desipramine is a preferential inhibitor of noradrenaline uptake: the ratio of the K_i values for inhibiting synaptosomal uptake of noradrenaline versus 5-HT is between 1250–5000 (see: Stanford, 1996). Although desipramine induced the larger increase in noradrenaline efflux, these experiments also exposed a marked increase when fluoxetine was present in the perfusion medium. Subsequent experiments went on to investigate whether inhibition of noradrenaline uptake could contribute to the increase in extracellular noradrenaline caused by fluoxetine. This involved investigation of the effects of fluoxetine on the uptake of [^{3}H]noradrenaline into cortical synaptosomes in vitro. The effects of fluoxetine were compared with those of desipramine and citalopram which also served as active controls.

Finally, to test whether inhibition of uptake could account for this increase, the effects of a lesion of noradrenergic neurones on inhibition of noradrenaline uptake ex vivo were studied. The lesion was induced by systemic injection of the neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4). DSP-4 causes a preferential neuropathy of neurones in the locus coeruleus, including those which innervate the cerebral cortex, but leaves other monoaminergic neurones intact (Lookingland et al., 1986; Fritschy and Grzanna, 1989).

The results of all these experiments suggest fluoxetine, which is widely regarded as a selective serotonin reuptake inhibitor, has marked effects on noradrenaline uptake in rat frontal cortex. This could explain, or contribute to, the increase in extracellular noradrenaline concentration caused by local infusion of this drug. However, it is likely that the noradrenaline transporter on noradrenergic neurones in this brain region is not the sole, or major, target for inhibition of transmitter uptake by selective serotonin reuptake inhibitors.

2. Materials and methods

2.1. Animals

Outbred male Sprague-Dawley rats, derived from a colony at University College London, were used throughout. Subjects were housed in groups of 4 and maintained on a 12 h light/dark cycle (lights on at 07.00 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.

2.2. Intracerebral microdialysis

Microdialysis probes were constructed of 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 kDa. Ringer-primed dialysis probes were implanted vertically, under halothane anaesthesia, into the right frontal cortex (A 3.5, L 1.5, V 5.0 mm; Paxinos and Watson, 1986) of rats (260–350 g). The first series of experiments investigated the effects of infusing increasing concentrations of fluoxetine or desipramine, via the probe, while the animals were maintained under anaesthesia. Immediately after implantation, the probe was perfused at 1.0 μl/min with modified Ringer's solution comprising (mM): NaCl 145, KCl 4, $CaCl_2$ 1.3, (pH 6.1) and dialysates collected at 20 min intervals into 5 μl 0.01 M $HClO_4$. Once stable spontaneous efflux was established, test drugs (fluoxetine or desipramine) were introduced into the perfusion medium as described in Dalley and Stanford (1995a). These were freshly dissolved in the modified Ringer's solution and administered for 80 min at each of 3 concentrations (0.5 μM, 5 μM and 50 μM).

A second series of experiments investigated the time-course of the changes in noradrenaline efflux induced by fluoxetine or desipramine in freely moving rats. Following overnight recovery from the surgery, fluoxetine or desipramine was infused at 5 μM for 3 h. In other respects, all procedures were the same as those described above.

The noradrenaline content of all samples was measured by high pressure liquid chromatography coupled to an electrochemical detector (HPLC-ECD) as described in Dalley and Stanford (1995b). The detection limit for noradrenaline was approximately 5 fmol.

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

Rats (310–400 g) were killed by stunning and cervical dislocation. A crude preparation of synaptosomes was derived from the cerebral cortex as described in Dalley and Stanford (1995b). Briefly, 100 μl aliquots of a resuspended (12 500 × *g*) pellet were preincubated in duplicate, at 37°C or 4°C for 3 min, in modified Tris-Krebs buffer, gassed with 95% O_2/5% CO_2, and comprising (mM): NaCl 136, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, (+)-glucose 10, (+)-ascorbate 1, Tris base 20, pargyline HCl 0.25, pH 7.4. Test drugs were dissolved in buffer and added to the incubation medium in a volume of 100 μl. The assay was started by addition of 100 μl [^{3}H]noradrenaline to give a final concentration of 50 nM in a total volume of 500 μl. The incubation was terminated, after 3 min, by filtration. The protein content of a further aliquot of each resuspended pellet was measured using the method of Lowry et al. (1951). Uptake was calculated as the difference in accumulation of [^{3}H]noradrenaline at 37°C and 4°C and expressed as pmol/mg protein.

2.4. DSP-4 lesion of noradrenergic neurones

Rats (250–420 g) were given a systemic injection of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4, 40

mg/kg i.p.). Control animals were given an equivalent injection of saline vehicle (2 ml/kg). All rats were killed 5 days after the injection. The cerebral cortex was removed, chopped finely, and a sample of this tissue was used to measure noradrenaline, 5-HT and dopamine content by 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. The remainder of the tissue was used to measure synaptosomal uptake of [^{3}H]noradrenaline.

2.5. Statistical analysis

Drug-induced changes in the concentration of noradrenaline in cortical microdialysates were analyzed by split-plot analysis of variance (ANOVA) as described in Dalley and Stanford (1995b). Data were divided into bins of 4 consecutive samples: data from 'basal' samples, collected in the absence of any drug, ('bin 1'); 0–80 min ('bin 2'); 100–160 min ('bin 3'); and 160–240 min ('bin 4') after the onset of drug infusion. Where increasing concentrations of drug were infused, each bin represents an 80 min infusion at each concentration. Raw data from studies of synaptosomal uptake of [^{3}H]noradrenaline were analyzed by the Mann-Whitney *U*-test, one- or two-way ANOVA followed by the Student-Newman-Keuls test, as appropriate.

2.6. Drugs

l-[7-^{3}H]Norepinephrine (specific activity 13.3 Ci/mmol; New England Nuclear) was used. Citalopram HBr was a generous gift from Lundbeck, Copenhagen, Denmark. Desipramine HCl, fluoxetine HCl, pargyline HCl and DSP-4 HCl were purchased from Sigma, UK. Drugs used for systemic injection were dissolved in sterile saline and administered in a volume of 2 ml/kg.

3. Results

3.1. Concentration dependence of changes in noradrenaline efflux in the frontal cortex of anaesthetized rats induced by local infusion of fluoxetine or desipramine

In every experiment, the first 4 dialysis samples were used to evaluate spontaneous efflux of noradrenaline in the anaesthetised rats. The noradrenaline content of these samples was the same in rats destined for subsequent infusion of either fluoxetine (34.9 ± 2.3 fmol/20 μl) or desipramine (38.5 ± 3.7 fmol/20 μl) (Fig. 1). Compared with that in basal samples, fluoxetine significantly increased noradrenaline efflux at both 5 μM ($F(1,10) = 5.77$; $P = 0.037$) and 50 μM ($F(1,10) = 19.48$; $P = 0.001$). Desipramine caused a significantly greater increase in efflux than did fluoxetine at both these concentrations (5 μM: $F(1,9) = 16.3$; $P = 0.003$; 50 μM: $F(1,9) = 13.14$; $P = 0.006$) and significantly increased efflux at 0.5 μM (bin × time interaction: $F(2,14) = 13.3$; $P = 0.001$).

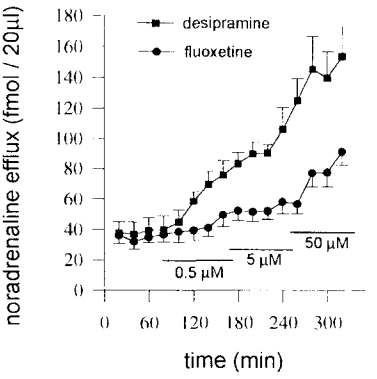

Fig. 1. Effects of local infusion of either fluoxetine ($n = 6$) or desipramine ($n = 5$) on noradrenaline efflux in the frontal cortex of anaesthetised rats. Drugs were dissolved in modified Ringer's solution to give probe concentrations of 0.5, 5, or 50 μM. Each concentration was infused via the probe for 80 min. Results show mean ± S.E. efflux expressed as fmol/20 μl.

3.2. Time dependence of changes in noradrenaline efflux in the frontal cortex of freely moving rats induced by local infusion of fluoxetine or desipramine

Spontaneous efflux of noradrenaline was not significantly different in freely moving rats destined for subsequent infusion of either fluoxetine (24.1 ± 1.3 fmol/20 μl) or desipramine (27.1 ± 1.5 fmol/20 μl) (Fig. 2). Although noradrenaline efflux in the freely moving animals was significantly less than in halothane-anaesthetised rats ($F(1,84) = 22.31$; $P < 0.001$), desipramine or fluoxetine (5 μM) both increased noradrenaline efflux in freely moving rats (Fig. 2). In order to evaluate this increase,

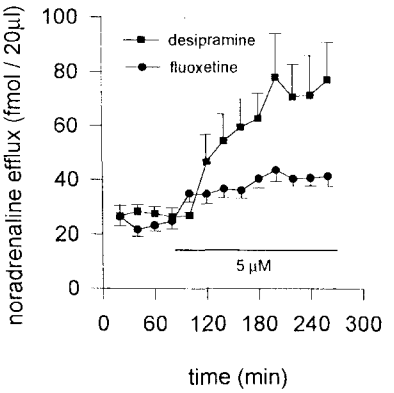

Fig. 2. Effects of local infusion of desipramine ($n = 5$) or fluoxetine ($n = 7$) on noradrenaline efflux in the frontal cortex of freely moving rats. Drugs were dissolved in modified Ringer's solution and delivered via the microdialysis probe at 5 μM for 3 h. Results show mean ± S.E. efflux expressed as fmol/20 μl.

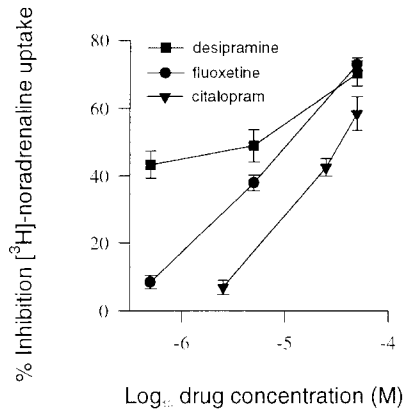

Fig. 3. Inhibition of [^{3}H]noradrenaline uptake into cortical synaptosomes by desipramine ($n = 11$), fluoxetine ($n = 7$) or citalopram ($n = 11$). Test drugs were included in the incubation medium to give final concentrations of 0.5, 5 and 50 μM (desipramine and fluoxetine) or 2.5, 25 and 50 μM (citalopram). Results show mean ± S.E. percentage inhibition of specific uptake.

changes were compared across bins of 4 consecutive samples (each of 80 min). When compared with spontaneous efflux, fluoxetine significantly increased noradrenaline efflux between 0–80 min ($F(1,7) = 8.29$; $P = 0.024$) and 100–160 min ($F(1,8) = 23.6$; $P = 0.001$) of drug administration. However, there was no difference in efflux during these two periods, confirming that the effect of fluoxetine had reached a plateau within the first 80 min.

Similarly, during desipramine administration, noradrenaline efflux was greater than spontaneous efflux during both 0–80 min ($F(1,7) = 5.22$; $P = 0.056$) and 100–160 min ($F(1,8) = 15.9$; $P = 0.004$) of drug infusion. There was no significant difference in efflux during these two periods, indicating that the effects of desipramine were close to maximum within 160 min of administration.

Finally, over the whole time-course, the effect of desipramine on noradrenaline efflux was significantly greater than that of fluoxetine ($F(1,17) = 10.13$; $P = 0.005$).

3.3. Effects of desipramine, fluoxetine or citalopram on uptake of [^{3}H]noradrenaline into cortical synaptosomes in vitro

Desipramine caused a significant and concentration-dependent reduction of [^{3}H]noradrenaline uptake into cortical synaptosomes ($F(3,39) = 19.62$; $P < 0.001$) (Fig. 3). The inhibition by desipramine was possibly biphasic but, since only 3 concentrations were tested, this could not be confirmed statistically.

A reduction in [^{3}H]noradrenaline uptake was also caused by both fluoxetine ($F(3,25) = 35.5$; $P < 0.001$) and citalopram ($F(3,40) = 8.27$; $P < 0.001$). At low (0.5 μM), but not high (50 μM) concentrations, desipramine caused more inhibition of uptake than did fluoxetine. However, fluoxetine was more potent than citalopram at all concentrations studied.

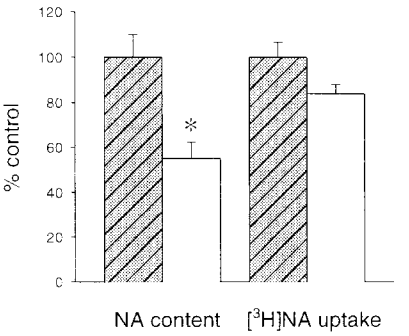

Fig. 4. The effects of a selective lesion of central noradrenergic neurones 5 days after systemic administration of 40 mg/kg DSP-4 i.p. Noradrenaline content of cortical tissue ($n = 21$) and uptake of [^{3}H]noradrenaline into cortical synaptosomes ($n = 18$). Results are expressed as mean ± S.E. percentage of saline-injected controls. * $P < 0.05$ (cf., saline-injected control: Mann-Whitney U-test).

3.4. Effects of a DSP-4 lesion of central noradrenergic neurones on inhibition of synaptosomal uptake of [^{3}H]noradrenaline ex vivo

Five days after systemic injection of DSP-4, the concentration of noradrenaline in the cerebral cortex was reduced by approximately 45% (Table 1) but the concentrations of 5-HT and dopamine were unchanged. Despite this partial lesion, synaptosomal uptake of [^{3}H]noradrenaline was not significantly affected in the absence of test drugs (Fig. 4).

In saline-pretreated tissues, the inhibition of noradrenaline uptake by desipramine was pronounced ($F(3,15) = 58.99$; $P < 0.001$) and statistically significant at 0.5 μM. However, the extent of the inhibition did not change significantly on increasing the concentration of desipramine to 5 or 50 μM. Over the range of concentrations tested, desipramine also caused a significant inhibition of [^{3}H]noradrenaline uptake into synaptosomes from DSP-4-lesioned rats ($F(3,20) = 14.92$; $P = 0.001$) (Fig. 5a). However, in contrast to the unlesioned tissues, the effects of desipramine increased with drug concentration and were significant only at 5 and 50 μM. Moreover, in the pres-

Table 1
Concentrations of noradrenaline, dopamine and 5-HT in the cerebral cortex of saline- or DSP-4-injected rats

	Saline	DSP-4
Content (ng / g wet weight)		
Noradrenaline	412.4 ± 36.0 (22)	224.4 ± 37.4 (21) [a]
Dopamine	328.0 ± 82.0 (8)	252.0 ± 52.0 (8)
5-HT	398.0 ± 22.0 (8)	398.0 ± 46.0 (8)
[^{3}H]Noradrenaline uptake (pmol / mg protein)		
	0.959 ± 0.06 (18)	0.808 ± 0.03 (18)

Data show mean ± S.E. Sample size shown in parentheses. [a] $P < 0.05$ (cf. saline).

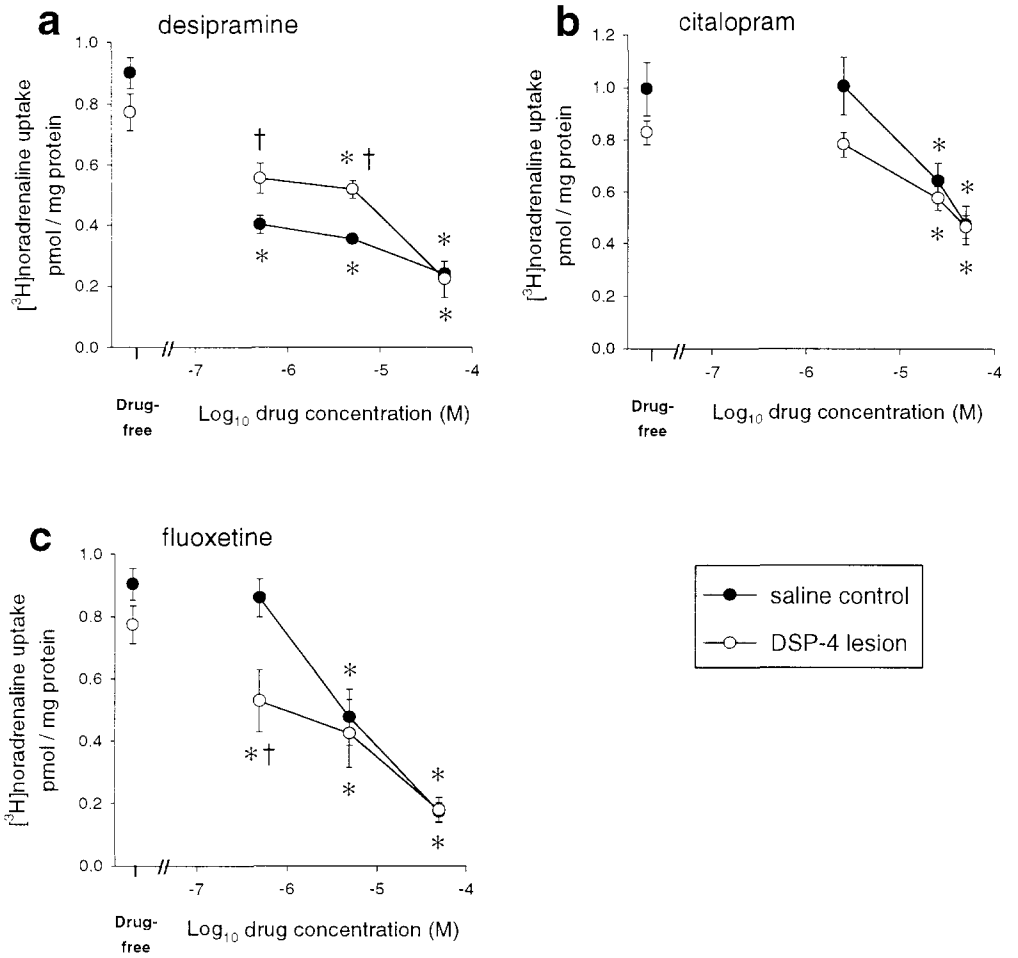

Fig. 5. Inhibition of [^{3}H]noradrenaline uptake into synaptosomes from DSP-4-lesioned and intact cortices by co-incubation with test drugs: (a) desipramine ($n = 4$); (b) citalopram ($n = 10$); and (c) fluoxetine ($n = 5$). Results show mean ± S.E. pmol/mg protein; * $P < 0.05$ cf. drug-free control; † $P < 0.05$ cf. saline (Student-Newman-Keuls test).

ence of desipramine, uptake of [^{3}H]noradrenaline was *greater* in the lesioned tissues; this difference was significant at 0.5 and 5 μM (main effect of lesion: $F(1,16) = 22.06$; $P < 0.001$).

Citalopram significantly reduced [^{3}H]noradrenaline uptake by synaptosomes from saline-pretreated ($F(3,40) = 8.27$; $P < 0.001$) and DSP-4-lesioned ($F(3,39) = 9.74$; $P = 0.001$) rats (Fig. 5b). However, in neither case was there any inhibition of uptake at the lowest concentration tested (2.5 μM). In contrast to desipramine, uptake in the presence of citalopram was significantly less in the lesioned than in intact tissues ($F(1,79) = 4.28$; $P = 0.042$).

Fluoxetine significantly reduced [^{3}H]noradrenaline uptake by synaptosomes from saline- ($F(3,18) = 29.9$; $P = 0.001$) and DSP-4-pretreated ($F(3,21) = 11.21$ $P < 0.001$) rats (Fig. 5c). In the saline-pretreated group, this inhibition was significant at 5 and 50 μM only. However, in synaptosomes from lesioned rats, the inhibition of uptake in the presence of 0.5 μM fluoxetine was statistically significant and greater than that in synaptosomes from intact brains ($F(1,8) = 29.9$; $P = 0.001$).

4. Discussion

The present experiments indicate that local infusion of fluoxetine increased the concentration of extracellular noradrenaline in the frontal cortex of both anaesthetised and freely moving rats. The change in extracellular noradrenaline was concentration dependent in anaesthetised rats, but results from this part of the study suggested that the increase was also dependent on the duration of drug infusion. To test this possibility, the effects of infusing a probe concentration of 5 μM desipramine or fluoxetine were compared. These experiments were carried out in freely moving rats in order to avoid complications of interactions with the anaesthetic agent. Under these conditions, a large increase (maximum attained: 315%) was

induced by infusion of the noradrenaline uptake blocker, desipramine, and this progressive increase was sustained for at least 3 h. Fluoxetine also caused a significant increase in noradrenaline efflux but this reached a plateau (of 180% with respect to the basal samples) within the first hour of infusion.

A previous study found a 2-fold increase in noradrenaline efflux when 10 μM fluoxetine was infused into the medial prefrontal cortex (Jordan et al., 1994). This increase was significantly less than that caused by the tricyclic antidepressant, imipramine. However, although imipramine inhibits 5-HT reuptake, it is metabolised rapidly to desipramine which will cause marked, albeit delayed, inhibition of noradrenaline uptake. It is not clear whether the pharmacokinetic differences between fluoxetine and imipramine (through desipramine), highlighted in the present experiments, were taken into account.

A small (41%), but significant, increase in noradrenaline efflux was also found in the rat ventral tegmental area during local infusion of 10 μM fluoxetine (Chen and Reith, 1994). In contrast, the 5-HT reuptake inhibitor fluvoxamine does not affect noradrenaline efflux in the medial prefrontal cortex (Jordan et al., 1994) and a relatively high concentration of citalopram is required to produce any change in the ventral tegmental area (100 μM, Chen and Reith, 1994). All these findings suggest that fluoxetine can increase the concentration of extracellular noradrenaline in the brain, whereas other selective serotonin reuptake inhibitors tested so far are either not, or weakly, effective in this respect. It remains to be seen whether or not the 5-HT reuptake inhibitors, sertraline and paroxetine, increase noradrenaline efflux also.

It is possible that the increase in extracellular noradrenaline caused by local infusion of fluoxetine is a consequence of activation of heteroceptors in the terminal field. There is some evidence that extracellular 5-HT, which would be increased by fluoxetine, activates 5-HT_{1C} receptors on noradrenergic nerve terminals and augments release of noradrenaline (Blandina et al., 1991). Modulation of noradrenaline release through a 5-HT_{1A} or 5-HT_{2A} receptor-mediated process is also possible (Done and Sharp, 1994). In this respect, it is interesting that the affinity of fluoxetine for 5-HT_{2A} and 5-HT_{2C} receptors is higher than for other selective serotonin reuptake inhibitors (see: Stanford, 1996).

Nevertheless, the question still arises as to whether an inhibition of noradrenaline uptake could contribute to this increase? This should be unlikely because fluoxetine is regarded as a selective serotonin reuptake inhibitor. The most convincing evidence for such selectivity comes from many studies of the effects of fluoxetine on synaptosomal uptake of [^{3}H]noradrenaline in vitro. An uptake selectivity of 50–55-fold is usually reported (e.g. Hyttel, 1994), although it could be as low as 3-fold (Koe et al., 1983). However, one feature of these studies is that comparisons of the effects of fluoxetine on uptake of [^{3}H]5-HT and [^{3}H]noradrenaline were carried out in synaptosomes derived from different brain regions (e.g. Koe et al., 1983; Bolden-Watson and Richelson, 1993) despite evidence that uptake in different brain regions can vary (Kimelberg and Katz, 1986). When the effects of these drugs were studied in synaptosomes from the same brain region, a somewhat lower selectivity of 20-fold was reported (Richelson and Pfenning, 1984; Thomas et al., 1987). Another study of inhibition of uptake into slices of rat cerebral cortex found no evidence for selectivity of inhibition of 5-HT versus noradrenaline by fluoxetine (Harms, 1983).

Regardless of their selectivity, the present results suggest that both fluoxetine and citalopram inhibit [^{3}H]noradrenaline uptake to an appreciable extent, but fluoxetine was more potent than citalopram. This finding is consistent with evidence that citalopram, the most selective of the selective serotonin reuptake inhibitors, is the least potent in inhibiting [^{3}H]noradrenaline uptake in vitro (see: Stanford, 1996). However, as in a previous report (Hughes and Stanford, 1995), fluoxetine and desipramine were more potent in this respect.

The findings that both fluoxetine and, to a lesser extent, citalopram, inhibit noradrenaline uptake in vitro prompt the question of whether this action involves exclusively noradrenergic neurones? This was investigated in experiments comparing [^{3}H]noradrenaline uptake by cortical synaptosomes derived from DSP-4-lesioned and vehicle-injected rats. The DSP-4 lesion reduced the noradrenaline content of the cortex by 45% but left cortical 5-HT and dopamine stores intact. Despite this selective loss of noradrenergic neurones, there was no significant reduction in synaptosomal uptake of [^{3}H]noradrenaline ex vivo. This could indicate a compensatory increase in uptake by the noradrenaline transporter on surviving neurones, but it would then be hard to explain why the inhibitory effect of desipramine on uptake was *diminished* by the lesion.

If another, non-noradrenergic, site is a target for fluoxetine then this site would be predicted to assume greater importance after a noradrenergic lesion. This is because relatively fewer noradrenergic synaptosomes will be available to contribute to the sequestration of noradrenaline. This prediction is borne out by the present findings: uptake in the presence of citalopram and fluoxetine was consistently less in synaptosomes from DSP-4-lesioned tissues than in intact controls. This is underlined by the finding that 0.5 μM fluoxetine inhibited [^{3}H]noradrenaline uptake by synaptosomes from lesioned, but not intact, cortices.

The identity of this non-noradrenergic site is unknown but is unlikely to be the 5-HT transporter. This is because citalopram has a higher affinity for inhibition of 5-HT uptake (K_i: 1–6 nM) than fluoxetine (K_i: 12–55 nM) yet inhibited [^{3}H]noradrenaline uptake only at concentrations in excess of 25 μM. This is at least 5-fold greater than an effective concentration of fluoxetine. A transporter on dopaminergic neurones is a more likely target since it has already been suggested that there is extensive uptake of

noradrenaline by dopaminergic neurones (Michel et al., 1984; see also: Wood and Wyllie, 1983). Consistent with this, and the present results, the K_i for inhibition of dopamine uptake by citalopram (28 μM) is somewhat greater than that for fluoxetine (1.6 μM) and desipramine (5.2 μM; Richelson and Pfenning, 1984). However, uptake into glial cells should also be considered (Kimelberg and Katz, 1986).

It must be borne in mind that, regardless of its site of action, inhibition of noradrenaline uptake by fluoxetine in vivo will occur only if the drug concentrations used in the present study resemble extracellular concentrations of fluoxetine attained in the clinical context. It is not possible to determine the extracellular concentration of fluoxetine when this is infused via a microdialysis probe. This is because the drug concentration will form a gradient, decreasing with increasing distance from the probe. However, even when infusing the lowest concentration of fluoxetine which significantly inhibited [^{3}H]noradrenaline uptake (5 μM), and assuming a probe efficiency of only 10%, the concentration of fluoxetine surrounding the probe will be well within the range of the K_i for inhibition of uptake (0.1–10 μM, see: Stanford, 1996). This is supported by findings that extracellular concentrations of fluoxetine, similar to the K_i for inhibition of noradrenaline uptake, are achieved after systemic administration of doses which produce 5-HT-related anticonvulsant effects (Dailey et al., 1992). Moreover, it has already been acknowledged that, when plasma levels of citalopram in rats match those which are clinically effective in humans, the concentration in the brain is 1000-fold greater than those which inhibit uptake of 5-HT in vitro (Hyttel et al., 1984). This raises the question of whether fluoxetine should be regarded as a selective serotonin reuptake inhibitor, or whether its therapeutic actions might more closely resemble those of the nonselective reuptake inhibitor, venlafaxine (Bolden-Watson and Richelson, 1993).

Collectively, these results suggest that at extracellular concentrations of fluoxetine, similar to those attained after systemic administration of this drug, there is significant inhibition of noradrenaline uptake in the rat cerebral cortex. It is possible that this inhibition involves one, or more, non-noradrenergic sites, possibly a transporter on dopaminergic neurones. Such an action could explain, or contribute to, the increase in the concentration of extracellular noradrenaline caused by intracortical administration of fluoxetine. This route for sequestration of noradrenaline seems to be an especially important target for fluoxetine when uptake by noradrenergic neurones is compromised.

Acknowledgements

Z.A.H. is an MRC scholar.

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