British Journal of Pharmacology (2001) 132, 183–188

www.nature.com/bjp

Chronic treatment with reboxetine by osmotic pumps facilitates its effect on extracellular noradrenaline and may desensitize α_2-adrenoceptors in the prefrontal cortex

[1]R. W. Invernizzi, [1]S. Parini, [1]G. Sacchetti, [2]C. Fracasso, [2]S. Caccia, [3]K. Annoni & *,[1]R. Samanin

[1]Laboratory of Neuropharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157, Milano, Italy; [2]Laboratory of Drug Metabolism, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157, Milano, Italy and [3]Pharmacia & Upjohn, Medical Department, Milano, Italy

1 This study investigated the effect of acute (2 days) and chronic (14 days) treatment with a selective inhibitor of noradrenaline uptake, reboxetine (10 mg kg^{-1} day^{-1}) by osmotic pumps, on extracellular noradrenaline and the sensitivity of α_2-adrenoceptors in the prefrontal cortex of rats.

2 The effect of continuous infusion of reboxetine for 14 days on cortical extracellular noradrenaline was significantly higher (599% of vehicle levels) than after 2 days (263% of vehicle levels).

3 Brain concentrations of reboxetine after 2 and 14 days of infusion were 37.9 ± 17.8 and 37.1 ± 7.7 ng g^{-1}, respectively.

4 Reboxetine infused for 2 and 14 days significantly increased extracellular dopamine in the prefrontal cortex, to a similar extent (257 and 342% of vehicle levels, respectively), whereas extracellular 5-HT was not modified by either treatment.

5 Clonidine (10 and 30 μg kg^{-1} i.p.) reduced cortical extracellular noradrenaline similarly in animals treated with reboxetine or vehicle for 2 days whereas the effects in rats infused with reboxetine for 14 days were markedly less than in vehicle-treated animals.

6 Clonidine (0.05 and 0.2 μM), infused through the dialysis probe into the prefrontal cortex, reduced cortical extracellular noradrenaline much less in rats treated with reboxetine for 14 days than in vehicle-treated animals.

7 Reboxetine's effect on extracellular noradrenaline in the prefrontal cortex was greater after chronic treatment and could be associated with desensitization of terminal α_2-adrenoceptors that normally serve to inhibit noradrenaline release.

British Journal of Pharmacology (2001) **132,** 183–188

Keywords: Microdialysis; chronic treatment; reboxetine; prefrontal cortex; noradrenaline; serotonin; dopamine; α_2-adrenoceptors

Abbreviations: aCSF, artificial cerebrospinal fluid; AUC, area under the curve; DA, dopamine; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; NA, noradrenaline

Introduction

There is evidence that after acute administration of antidepressant drugs that inhibit noradrenaline (NA) uptake, their effect on extracellular NA in terminal regions is attenuated by the fact that they cause increases in endogenous NA that are sufficient to activate inhibitory somatodendritic α_2-adrenoceptors. Thus, cortical extracellular NA can be increased to greater extents when an acute dose of desipramine is injected concomitantly with an α_2-adrenoceptor antagonist into the locus coeruleus (Mateo *et al*., 1998). This mechanism, which limits the increase of extracellular NA in terminal regions after an acute dose of desipramine alone, may contribute to the slow onset of its antidepressant action. This had led to the suggestion that combining an α_2-adrenoceptor antagonist with a NA reuptake inhibitor may improve efficacy in depressed patients (Palij & Stamford, 1996).

*Author for correspondence; E-mail: samanin@irfmn.mnegri.it

NA release is also modulated by α_2-adrenoceptors on nerve terminals (Dennis *et al*., 1987). The appearance of the beneficial effects of antidepressant drugs that inhibit NA reuptake after a few weeks may involve adaptive changes in these receptors. Electrophysiological studies suggest that terminal α_2-adrenoceptors in the dorsal hippocampus become subsensitive during long-term treatment with desipramine (Lacroix *et al*., 1991). We found recently that three concentrations of clonidine (0.05, 0.5 and 1 μM), infused into the dorsal hippocampus through the microdialysis probe, significantly lowered extracellular NA, to a similar extent in rats chronically treated with desipramine (10 mg kg^{-1} i.p. once daily for 14 days) or saline (Sacchetti *et al*., 2000). Further, clonidine's inhibitory effect on NA release in hippocampal slices is not modified by long-term treatment with desipramine (Campbell & McKernan, 1986; Schoffelmeer & Mulder, 1982). It is therefore not clear whether long-term treatment with NA uptake inhibitors facilitates their elevating effect on extracellular NA in terminal regions by desensitizing inhibitory α_2-adrenoceptors.

Reboxetine is a novel antidepressant drug that potently and selectively inhibits NA uptake. Unlike tricyclic antidepressant drugs, it has no appreciable action on muscarinic cholinergic or α_1-adrenergic receptors (Wong *et al*., 2000). We found recently that an acute dose of reboxetine raised extracellular NA in the prefrontal cortex and dorsal hippocampus of rats and these effects were markedly potentiated by concomitant administration of the α_2-adrenoceptor antagonist, idazoxan (Sacchetti *et al*., 1999). In the same study, long-term treatment with reboxetine (15 mg kg^{-1} once daily for 14 days) did not cause significant adaptive changes in noradrenergic transmission that might be reflected by changes in the drug's effect on extracellular NA in terminal regions (Sacchetti *et al*., 1999).

In view of the rapid clearance of reboxetine in rats (Dostert *et al*., 1997), it was suggested that experiments using osmotic pumps to deliver continuous adequate drug levels to rats may be closer to the results in depressed patients, in whom reboxetine's half-life is considerably longer than in rats (Edwards *et al*., 1995; Caccia, 1998 for review).

The main objectives of the present study were: (1) to examine whether long-term treatment with reboxetine, delivered through osmotic pumps, facilitated the elevation of extracellular concentrations of NA in the prefrontal cortex, a region that has been involved in the pathogenesis of depressive disorders (Kapur & Mann, 1992); (2) to assess the sensitivity of α_2-adrenoceptors regulating NA release in the prefrontal cortex by studying whether the α_2-adrenoceptor agonist clonidine, administered systemically or locally, reduced cortical extracellular NA in rats treated chronically with reboxetine and (3) to assess the selectivity of reboxetine's effect on the noradrenergic system after chronic treatment by examining how it affects extracellular serotonin (5-HT) in the prefrontal cortex. Finally, since an acute dose of NA reuptake inhibitors raises extracellular levels of dopamine (DA) in the prefrontal cortex (Carboni *et al*., 1990; Pozzi *et al*., 1994), we also examined whether chronic reboxetine increased cortical extracellular DA.

Methods

Animals

Male Sprague-Dawley rats (CD-COBS, Charles River, Italy) weighing between 200 and 350 g were used. They were housed at constant room temperature ($22 \pm 2^{\circ}C$) and relative humidity ($60 \pm 5\%$), with a regular 12/12 h light/dark cycle. Food and water were available *ad libitum*. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare N° 8, G.U., 14 Luglio, 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). All efforts were made to minimize suffering and to limit the number of animals used.

Treatments

Because of its rapid clearance in rats (Dostert *et al*., 1997), reboxetine was infused by osmotic pumps (mod. 2ML2 delivering 5 μl h^{-1}, 14 days; ALZET, Palo Alto, CA, U.S.A.) filled with 27.1 mg ml^{-1} reboxetine methansulphonate or vehicle (0.9% NaCl). Rats were anaesthetized with 3 ml kg^{-1} Equithesin (1.2 g pentobarbital, 5.3 g chloral hydrate, 2.7 g $MgSO_4$ hexahydrate, 49.5 ml propylene glycol, 12.5 ml ethanol and 58 ml distilled water), the skin was shaved and washed with antiseptic solution (Citrosil®, Manetti-Roberts Sanitas S.p.A., Italy); an incision of about 2 cm, was made between the scapulae and a haemostat was inserted to create a suitable pocket. The filled pump was inserted into the pocket and the wound closed with two clips and disinfected. All the studies were done with the osmotic pump in place 2 or 14 days after implantation. Doses of reboxetine were calculated for a mean body weight of 250 g.

In one experiment, basal extracellular concentrations of NA, 5-HT and DA were measured in the prefrontal cortex of freely-moving rats given 10 mg kg^{-1} day^{-1} reboxetine or vehicle for 2 and 14 days. Osmotic pumps were implanted on day 0 and dialysis probes on day 1 and 13 respectively. Probe perfusion started on days 2 and 14, about 20 h after probe implantation. After 1 h washout, 30-min samples of perfusate were collected until basal levels of extracellular neurotransmitters were stable (usually 1 or 2 h after the start of perfusion).

In another experiment, rats were given vehicle or 10 mg kg^{-1} day^{-1} reboxetine for 2 or 14 days. On days 2 and 14, once the release of NA was stable, they received 10 and 30 μg kg^{-1} clonidine intraperitoneally. Finally, another group of rats treated with vehicle or 10 mg kg^{-1} day^{-1} reboxetine for 14 days received clonidine (0.05 and 0.2 μM) through the probe in the prefrontal cortex.

Drugs

Reboxetine methansulphonate (Pharmacia & Upjohn, Milano, Italy) and clonidine hydrochloride (Boehringer Ingelheim, Milano, Italy) were dissolved in saline. Reboxetine was infused subcutaneously by osmotic pumps and clonidine was injected intraperitoneally (2 ml kg^{-1}). Doses refer to the free base. The reboxetine dose was selected on the basis of preliminary experiments showing that 1, 3, 10 and 30 mg kg^{-1} day^{-1} infused for 2 days dose-dependently raised extracellular NA in the prefrontal cortex, the highest dose having a supramaximal effect.

Microdialysis procedures

The day before the microdialysis experiment, rats were deeply anesthetized with 3 ml kg^{-1} i.p. Equithesin. On days 1 and 13 of reboxetine or vehicle infusion, rats were placed on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.) and a vertical dialysis probe (4 mm long) made of Cuprophan (Sorin Biomedica, Italy; 200 μm inner diameter with 3000 Da molecular weight cutoff) essentially as described by Robinson & Wishaw (1988) was implanted in the prefrontal cortex at the following stereotaxic coordinates (from bregma and skull surface): $AP = +4.2$; $L = \pm 0.7$; $V = -5.5$ (Paxinos & Watson, 1982). About 20 h after the surgical procedure, the dialysis probes were perfused with an aCSF (containing mM): NaCl 145, $CaCl_2$ 1.26, KCl 3, $MgCl_2$ 1 at pH 7.4 with sodium phosphate buffer at a constant flow rate of 1 μl min^{-1}, using a microperfusion pump (CMA/100,

CMA Microdialysis, Sweden). After 1 h washout, samples were collected at 30-min intervals and directly injected into the high-performance liquid chromatograph (HPLC).

Analytical procedures

Extracellular concentrations of NA, DA and 5-HT were measured in separate samples by HPLC coupled to an electrochemical detector (Coulochem II, ESA, Bedford, MA, U.S.A.). For NA, the first electrode was set at -0.2 V and the second at $+0.25$ V. NA was quantified from the second electrode output signal. Separation was obtained using a reverse-phase column (Hypersil-ODS 5 μm, 125×3.1 mm, Bischoff). The mobile phase, consisting of (mM): citric acid 25, sodium acetate 24, sodium octyl sulfate 1.55, Na_2EDTA 0.1 and 80 ml l^{-1} CH_3OH, was pumped at a constant flow rate of 1 ml min^{-1} with a ESA 580 pump (ESA, Bedford, MA, U.S.A.). DA was separated through a 150×4.6 mm column (Supelcosil LC18-DB, 5 μm, Supelco, Bellefonte, PA, U.S.A.) using a mobile phase containing sodium acetate 0.1 M, sodium octyl sulfate 0.34 mM, Na_2EDTA 0.1 mM, 60 ml l^{-1} CH_3OH, pH 4.2 with acetic acid. A constant flow rate of 1 ml min^{-1} was maintained by a Constametric 3200 pump (LDC/Milton Roy, Riviera Beach, FL, U.S.A.).

For DA, the first electrode was set at $+350$ mV and the second at -300 mV. DA was quantified from the second electrode output signal. 5-HT was measured as described by Invernizzi *et al*. (1992).

Reboxetine was determined in plasma and brain tissue by HPLC as described by Frigerio *et al*. (1994), with minor modifications, using viloxazine (ICI, UK) as internal standard (I.S.). Briefly, to 1.5 ml of plasma, 25 μl of a methanolic solution of the I.S. (10 μg ml^{-1}) and 1.5 ml of 0.2 M TRIS buffer, pH 9.1, were added. The samples were shaken with 10 ml of diethyl ether. After centrifugation, the organic phase was separated and back-extracted with 0.4 ml of TRIS buffer, pH 9.1. The aqueous phase was washed with 2 ml hexane. After centrifugation, 200 μl of the aqueous phase were analysed by HPLC with UV detection (210 nm).

Brain tissue was homogenized (5 ml g^{-1}) in TRIS buffer, pH 9.1, and a volume (2 ml) containing approximately 400 mg of tissue was processed as described for plasma. Separation was done on a μBondapack C18 Column (300×3.9 mm, 10 μm particle size, Waters, Italy) at room temperature. The mobile phase was 0.016 M KH_2PO_4:CH_3CN (70 : 30 v v^{-1}), adjusted to pH 2.85 with H_3PO_4, delivered at a flow rate of 1 ml min^{-1}. The retention times were approximately 15 min for reboxetine and 5.7 min for the I.S.

Reagents were of analytical grade and were purchased from Merck (Bracco, Milan, Italy) or Sigma-Aldrich (Milan, Italy).

Histological procedure

At the end of the experiments, rats were killed by decapitation, their brains immediately removed and the correct placement of the probes checked by examining the probe tracks on 40-μm frozen slices. Only rats with correct probe placement (more than 90%) were considered in the results.

Data calculation and statistics

Basal values of extracellular neurotransmitters were defined as the means of two consecutive stable samples (at least two consecutive samples not differing by more than 15%) and were expressed as fmol 30 μl^{-1} (not corrected for recovery). The last of the two consecutive stable samples was used for the statistical analysis.

The effects of 2 and 14 day infusion of reboxetine and vehicle on extracellular NA, DA and 5-HT were compared by two-way ANOVA with treatment and days as main factors. *Post-hoc* comparisons were made by Tukey-Kramer's test. Brain concentrations of reboxetine after 2 and 14 days of infusion were compared by Student's *t*-test. The overall effect of clonidine on extracellular NA in rats given vehicle or reboxetine for 14 days was estimated from the area under the curve (AUC) calculated as a percentage of basal values for each rat. The resulting data were compared by Mann-Whitney's test.

Results

Extracellular NA in the prefrontal cortex of rats given reboxetine for 2 and 14 days

Reboxetine given at 10 mg kg^{-1} s.c. daily *via* osmotic pump for 2 and 14 days raised extracellular NA in the prefrontal cortex by 263 and 599% of vehicle levels (Table 1). The difference between the two treatments was significant ($Fdays_{(1,24)} = 2.1$, $P > 0.05$; $Ftreatment_{(1,24)} = 71.9$, $P < 0.001$; $Fdays \times treatment_{(1,24)} = 16.3$, $P < 0.001$; two-way ANOVA).

Reboxetine in the brain and plasma of rats treated with reboxetine for 2 and 14 days

No significant differences were found between the concentrations of reboxetine in the brain and plasma of rats given 10 mg kg^{-1} of the drug daily for 2 and 14 days (Table 2).

Extracellular NA in the prefrontal cortex of rats treated for 2 and 14 days with reboxetine and given clonidine intraperitoneally

Injections of 10 and 30 μg kg^{-1} clonidine reduced extracellular NA by 30 and 60% respectively in rats infused with vehicle for 14 days (Figure 1). The inhibitory effects of 10 and 30 μg kg^{-1} clonidine on NA release were significantly less in rats given 10 mg kg^{-1} reboxetine daily for 14 days ($AUC_{0-150\ min}$: vehicle 9825 ± 411, reboxetine 12358 ± 858, $P < 0.05$; $AUC_{150-300\ min}$: vehicle 7768 ± 423, reboxetine 10393 ± 505, $P < 0.02$, Mann-Whitney test) (Figure 1). Ten and 30 μg kg^{-1} clonidine reduced extracellular NA similarly in rats given vehicle for 14 days or 10 mg kg^{-1} daily reboxetine for 2 days ($AUC_{0-150\ min}$: vehicle 9825 ± 411, reboxetine 10862 ± 451, $P > 0.05$; $AUC_{150-300\ min}$: vehicle 7768 ± 423, reboxetine 8493 ± 307, $P > 0.05$, Mann-Whitney test).

Extracellular NA in the prefrontal cortex of rats given clonidine through the dialysis probe

Administered through the dialysis probe, 0.05 and 0.2 μM clonidine reduced extracellular NA by respectively 31 and

Table 1 Extracellular NA in the prefrontal cortex of rats infused with 10 mg kg^{-1} day^{-1} reboxetine for 2 or 14 days

	Extracellular NA (fmol 30 μl^{-1})	
Days of infusion	*Vehicle*	*Reboxetine*
2	9.8±1.0 (6)	25.8±3.0* (9)
14	7.1±0.9 (7)	42.5±4.6*† (6)

Rats were infused with vehicle or 10 mg kg^{-1} day^{-1} reboxetine by subcutaneous osmotic pumps for 2 or 14 days. Data are means±s.e.mean. The number of rats is shown in parentheses. *$P<0.05$ vs vehicle; †$P<0.05$ vs 2-days (Tukey–Kramer's test).

Table 2 Plasma and brain concentrations of reboxetine in rats infused with 10 mg kg^{-1} day^{-1} reboxetine for 2 or 14 days

	Plasma	*Brain*
Days of infusion	(ng ml^{-1})	(ng g^{-1})
2	52.2±14.5 (3)	37.9±17.8 (3)
14	52.7±7.1 (7)	37.1±7.7 (7)

Mean±s.e.mean. The number of rats is shown in parentheses.

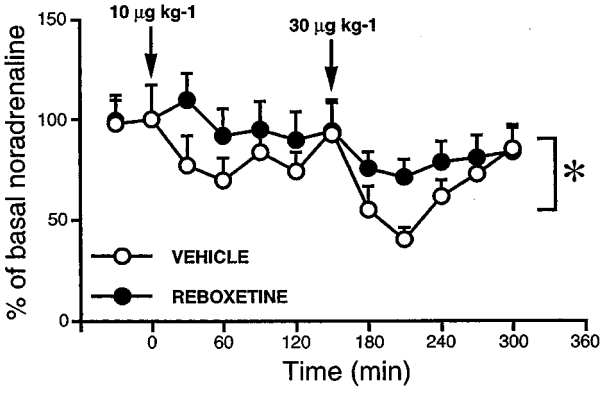

Figure 1 Effects of 10 and 30 μg kg^{-1} clonidine administered intraperitoneally on extracellular NA in rats given vehicle ($n=6$) or 10 mg kg^{-1} day^{-1} reboxetine ($n=7$) continuously for 14 days. Mean±s.e.mean. Arrows indicate the clonidine injection. *$P<0.05$ vs vehicle (AUC; Mann-Whitney).

67% in rats infused with vehicle for 14 days (Figure 2). The effect of both concentrations of clonidine in rats infused with 10 mg kg^{-1} reboxetine daily for 14 days was significantly less than in vehicle treated animals ($AUC_{30-90\ min}$: vehicle 3504±246, reboxetine 5382±457, $P<0.01$; $AUC_{120-180\ min}$: vehicle 2826±483, reboxetine 4119±451, $P<0.05$, Mann-Whitney test).

Extracellular 5-HT and DA in the prefrontal cortex of rats treated with reboxetine for 2 and 14 days

Figure 3 shows the effect of the infusion of 10 mg kg^{-1} day^{-1} reboxetine or vehicle on basal extracellular concentrations of 5-HT and DA measured during the phase of stable release (24 h after probe implantation) during 60-min. Infusion of

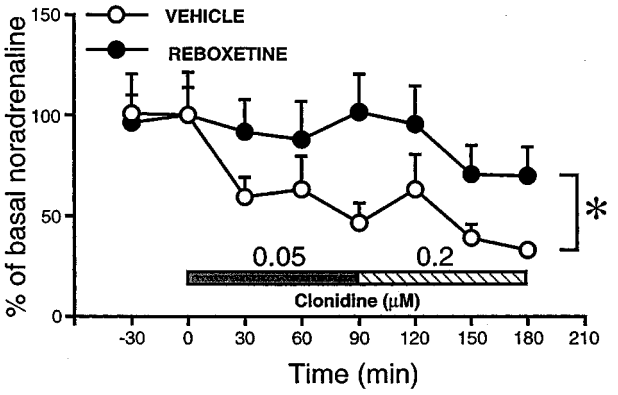

Figure 2 Effects of 0.05 and 0.2 μM clonidine administered through the probe on extracellular NA in rats given vehicle ($n=5$) or 10 mg kg^{-1} day^{-1} reboxetine ($n=5$) continuously for 14 days. Mean±s.e.mean. Horizontal bars indicate the duration of clonidine infusion. *$P<0.05$ vs vehicle (AUC; Mann-Whitney).

reboxetine for 2 or 14 days had no significant effects on basal extracellular 5-HT in the prefrontal cortex ($Fdays_{(1,21)}=0.06$, $P>0.05$; $Ftreatment_{(1,21)}=0.6$, $P>0.05$; $Fdays\times treatment_{(1,21)}=1.7$, $P>0.05$; two-way ANOVA). Infusion of reboxetine for 2 and 14 days raised extracellular DA respectively by 257 and 342% of vehicle levels. Reboxetine had similar effects on extracellular DA at 2 and 14 days ($Fdays_{(1,23)}=0.07$, $P>0.05$; $Ftreatment_{(1,23)}=43.4$, $P<0.001$; $Fdays\times treatment_{(1,23)}=0.5$, $P>0.05$; two-way ANOVA).

Discussion

It is generally assumed that the beneficial effects of prolonged treatment with selective serotonin reuptake inhibitors in depression are due to adaptive neuronal changes, particularly desensitization of the autoreceptors inhibiting transmitter release (Blier & de Montigny, 1994). The present study suggests that a similar mechanism may help explain the latency in the appearance of beneficial effects of antidepressant drugs that inhibit noradrenaline uptake. Continuous infusion of reboxetine (10 mg kg^{-1} day^{-1}) by means of osmotic pumps for 14 days facilitated its effect on extracellular NA in the prefrontal cortex, an action that may be associated with desensitization of α_2-adrenoceptors in that brain region.

The fact that repeated daily injections of reboxetine (15 mg kg^{-1} i.p.) for 14 days did not produce such effects (Sacchetti *et al*., 1999) underlines the importance of delivering stable drug levels to rats to mimic as close as possible the drug's kinetics in man. Daily multiple dosing may cause fluctuations from high to very low plasma levels of reboxetine because of its relatively rapid clearance in rats (Dostert *et al*., 1997) and this could prevent the development of adaptive changes.

The effect of 14 days' reboxetine on extracellular NA was significantly greater than after 2 days of infusion. Since brain levels of reboxetine on day 14 were not different from those on day 2, it can be excluded that changes in drug kinetics contributed to this effect. Ten and 30 μg kg^{-1} clonidine i.p. significantly reduced cortical extracellular NA to similar

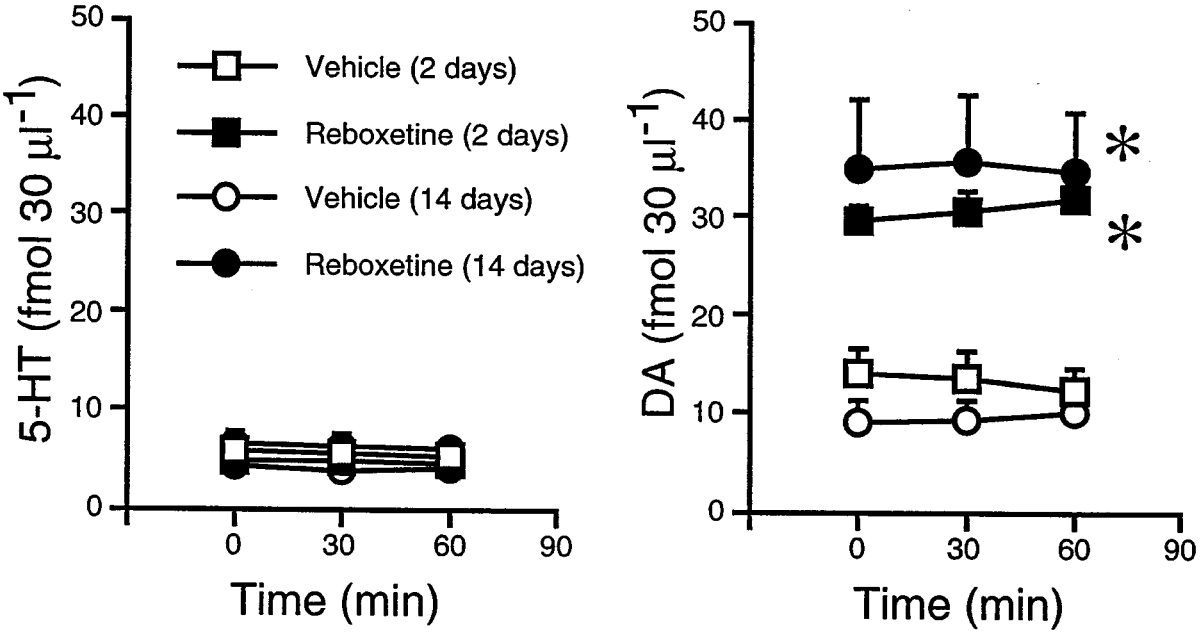

Figure 3 Basal extracellular 5-HT and DA in the prefrontal cortex of rats infused with vehicle ($n=6-8$) or 10 mg kg^{-1} day^{-1} reboxetine ($n=5-7$) continuously for 2 or 14 days. Extracellular concentrations of 5-HT and DA were measured 24 h after probe implantation, during the phase of stable release. Mean$\pm$s.e.mean. *P$<$0.05 vs respective vehicle (Tukey-Kramer's test).

extents in rats which had received vehicle for 14 days or reboxetine for 2 days, whereas the effects of both clonidine doses in animals infused with reboxetine for 14 days were markedly less than in vehicle-treated animals. Since this attenuation may be the expression of adaptive changes in somatodentritic and/or terminal autoreceptors, we infused clonidine (0.05 and 0.2 μM) through the dialysis probe into the prefrontal cortex. The effect on extracellular NA was markedly less in rats treated chronically with reboxetine than in vehicle-treated animals.

Although the changes in the effect of systemically administered clonidine may involve adaptations in somatodendritic autoreceptors, the present findings suggest that desensitization of terminal α_2-adrenoceptors in the cerebral cortex may occur in rats chronically treated with reboxetine. Behavioural studies in mice have shown that the effect of clonidine on presynaptic α_2-adrenoceptors was attenuated by an antidepressant which increase synaptic NA (Heal *et al*., 1991). However, it is not clear to what extent these findings are related to a reduced effect of clonidine on cortical α_2-adrenoceptors controlling NA release in rats given reboxetine chronically.

To the best of our knowledge, this is the first demonstration of an apparent desensitization of terminal α_2-adrenoceptors controlling NA release in the prefrontal cortex of rats treated chronically with a selective inhibitor of NA uptake.

As mentioned in the Introduction, with the exception of one electrophysiological study (Lacroix *et al*., 1991), changes in terminal autoreceptors controlling NA release have generally not been seen in the dorsal hippocampus of rats treated chronically with desipramine. It is not clear to what extent this reflects intrinsic differences in α_2-adrenoceptor mechanisms between the dorsal hippocampus and prefrontal cortex or whether differences in drugs and treatment schedules play a role. One study on how the reboxetine chronic schedule used in the present study affects terminal α_2-adrenoceptors in the dorsal hippocampus may help clarify this.

Continuous infusion of reboxetine for 2 and 14 days did not modify extracellular concentrations of 5-HT in the prefrontal cortex, in line with *in vitro* findings that the selectivity ratio for inhibiting 5-HT and NA uptake is about 130 (Wong *et al*., 2000). Two days' infusion of reboxetine significantly raised extracellular DA in the prefrontal cortex. A direct effect on dopaminergic neurons can be excluded since reboxetine has no affinity for the DA transporter (Wong *et al*., 2000). Moreover, unlike DA reuptake inhibitors, single and repeated injections of reboxetine had no effect on extracellular DA in the striatum (Sacchetti *et al*., 1999). As previously suggested for desipramine (Carboni *et al*., 1990; Pozzi *et al*., 1994), a likely explanation is that reboxetine raised extracellular DA in the prefrontal cortex by blocking DA reuptake into cortical noradrenergic neurons. The effect of 14 days' infusion of reboxetine on extracellular DA was not significantly different from that after two days suggesting that no adaptive changes had occurred in the mechanisms involved in this effect.

In conclusion, the present study has shown that chronic treatment with reboxetine facilitates its effect on extracellular NA in the prefrontal cortex and that this effect is associated with a reduced responsiveness of α_2-adrenoceptors in this region, perhaps as a result of their desensitization in the face of prolonged exposure to elevated NA levels. Infusion of reboxetine for 2 and 14 days had no effect on extracellular 5-HT but increased extracellular DA in the prefrontal cortex. This is probably secondary to the drug's effect on NA uptake and may contribute to beneficial effects of reboxetine in depression.

This work was supported by a grant from Pharmacia & Upjohn, Milano, Italy.

References

BLIER, P. & DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.,* **15,** 220–226.

CACCIA, S. (1998). Metabolism of newer antidepressants. An overview of the pharmacological and pharmacokinetics implications. *Clin. Pharmacokinet.,* **34,** 281–302.

CAMPBELL, I.C. & MCKERNAN, R.M. (1986). Clorgyline and desipramine alter the sensitivity of [^{3}H]-noradrenaline release to calcium but not to clonidine. *Brain Res.,* **372,** 253–259.

CARBONI, E., TANDA, G., FRAU, R. & DI CHIARA, G. (1990). Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. *J. Neurochem.,* **55,** 1067–1070.

DENNIS, T., L'HEUREUX, R., CARTER, C. & SCATTON, B. (1987). Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by in vivo dialysis) in the rat. *J. Pharmacol. Exp. Ther.,* **241,** 642–649.

DOSTERT, P., BENEDETTI, M.S. & POGGESI, I. (1997). Review of the pharmacokinetics and metabolism of reboxetine, a selective noradrenaline reuptake inhibitor. *Eur. Neuropsychopharmacol.,* **7** (Suppl. 1)**,** S23–S35.

EDWARDS, D.M.F., PELLIZZONI, C., BREUEL, H.P., BERARDI, A., CASTELLI, M.G., FRIGERIO, E., POGGESI, I., ROCCHETTI, M., DUBINI, A. & STROLIN BENEDETTI, M. (1995). Pharmacokinetics of reboxetine in healthy volunteers. Single oral doses, linearity and plasma protein binding. *Biopharm. Drug Disp.,* **16,** 443–460.

FRIGERIO, E., PIANEZZOLA, E. & STROLIN BENEDETTI, M. (1994). Sensitive procedure for the determination of reboxetine enantiomers in human plasma by reversed-phase high-performance liquid chromatography with fluorimetric detection after chiral derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate. *J. Chromatog. A,* **660,** 351–358.

HEAL, D.J., PROW, M.R. & BUCKETT, W.R. (1991). Effects of antidepressant drugs and electroconvulsive shock on pre- and postsynaptic α_2-adrenoceptor function in the brain: rapid down-regulation by sibutramine hydrochloride. *Psychopharmacology,* **103,** 251–257.

INVERNIZZI, R., BELLI, S. & SAMANIN, R. (1992). Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. *Brain Res.,* **584,** 322–324.

KAPUR, S. & MANN, J.J. (1992). Role of the dopaminergic system in depression. *Biol. Psychiatry,* **32,** 1–17.

LACROIX, D., BLIER, P., CURET, O. & DE MONTIGNY, C. (1991). Effect of long-term desipramine administration on noradrenergic neurotransmission: electro-physiological studies in the rat brain. *J. Pharmacol. Exp. Ther.,* **257,** 1081–1090.

MATEO, Y., PINEDA, J. & MEANA, J.J. (1998). Somatodendritic α_2-adrenoceptors in the locus coeruleus are involved in the in vivo modulation of cortical noradrenaline release by the antidepressant desipramine. *J. Neurochem.,* **71,** 790–798.

PALIJ, P. & STAMFORD, J.A. (1996). Rauwolscine potentiates the effect of desipramine on limbic noradrenaline efflux. *Neuroreport,* **7,** 1121–1124.

PAXINOS, G. & WATSON, C. (1982) The rat brain in stereotaxic coordinates, Academic Press: Sydney.

POZZI, L., INVERNIZZI, R., CERVO, L., VALLEBUONA, F. & SAMANIN, R. (1994). Evidence that extracellular concentrations of dopamine are regulated by noradrenergic neurons in the frontal cortex of rats. *J. Neurochem.,* **63,** 195–200.

ROBINSON, T.E. & WHISHAW, I.Q. (1988). Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. *Brain Res.,* **450,** 209–224.

SACCHETTI, G., BERNINI, M., BIANCHETTI, A., PARINI, S., INVERNIZZI, R.W. & SAMANIN, R. (1999). Studies on the acute and chronic effects of reboxetine on extracellular noradrenaline and other monoamines in the rat brain. *Br.J.Pharmacol.,* **128,** 1332–1338.

SACCHETTI, G., BERNINI, M., GOBBI, M., PARINI, S., PIRONA, L., MENNINI, T. & SAMANIN, R. (2000). Chronic treatment with desipramine facilitates its effect on extracellular noradrenaline in the rat hippocampus: studies on the role of presynaptic α_2-adrenoceptors. *N.S. Arch. Pharmacol.,* **363,** 66–72.

SCHOFFELMEER, A.N.M. & MULDER, A.H. (1982). ^{3}HNoradrenaline and ^{3}H-5-hydroxytryptamine release from rat brain slices and its presynaptic α-adrenergic modulation after long-term desipramine pretreatment. *N.S. Arch. Pharmacol.,* **318,** 173–180.

WONG, E.H.F., SONDERS, M.S., AMARA, S.G., TINHOLT, P.M., PIERCEY, M.F.P., HOFFMANN, W.P., HYSLOP, D.K., FRANKLIN, S., PORSOLT, R.D., BONSIGNORI, A., CARFAGNA, N. & MCARTHUR, R.A. (2000). Reboxetine: A pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biol. Psychiatry,* **47,** 818–829.

(Received July 21, 2000
Revised October 9, 2000
Accepted October 31, 2000)