Neuropsychopharmacology (2005) 30, 1048–1055

www.neuropsychopharmacology.org

Chronic Reboxetine Desensitizes Terminal but not Somatodendritic α_2-Adrenoceptors Controlling Noradrenaline Release in the Rat Dorsal Hippocampus

Stefania Parini[1], Giuliano Renoldi[1], Angelo Battaglia[2] and Roberto W Invernizzi*[,1]

[1]Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy; [2]Pfizer Italia, Medical Department, Rome, Italy

The slow onset of antidepressant drugs' effects is thought to reflect the time required for the development of adaptive changes such as desensitization of presynaptic autoreceptors controlling the release of neurotransmitters. Using *in vivo* microdialysis in conscious rats, we studied the effect of a continuous infusion of the selective noradrenaline (NA) reuptake inhibitor reboxetine on extracellular concentrations of NA. Doses of 10 mg/kg/day reboxetine through subcutaneous osmotic pumps for 2 days increased extracellular NA by 272% in the dorsal hippocampus (DH) of rats. NA rose significantly more in rats given reboxetine for 7 (469%) and 14 (437%) days. Intraperitoneal injection of 30 μg/kg clonidine, an α_2-adrenoceptor agonist, reduced the release of NA to 49% of basal levels in rats given vehicle or reboxetine for 2 days, but this effect was markedly less in rats given reboxetine for 7 and 14 days. Likewise, the effect of intrahippocampal infusion of clonidine (0.05 and 0.2 μM) on extracellular NA was significantly attenuated in rats given reboxetine for 7 and 14 days, whereas the injection of 0.6 nmol clonidine into the locus coeruleus caused similar reductions of extracellular NA in the DH and prefrontal cortex (PFC) of rats infused with vehicle (DH −64%; PFC −42%) and reboxetine (DH −45%; PFC −28%) for 14 days. The results indicate that chronic treatment markedly enhances the effect of reboxetine on extracellular NA in the DH and suggest that this effect may be due to the desensitization of hippocampal α_2-adrenoceptors.
Neuropsychopharmacology (2005) **30,** 1048–1055, advance online publication, 19 January 2005; doi:10.1038/sj.npp.1300661

Keywords: antidepressants; autoreceptor desensitization; clonidine; locus coeruleus; microdialysis; noradrenaline reuptake inhibitors

INTRODUCTION

Antidepressant drugs take time to develop their effect, typically 2–3 weeks, regardless of the drug class. This delay, which has driven most recent research into the neurobiological effects of antidepressant drugs, suggests that adaptive changes rather than acute pharmacological effects are responsible for the therapeutic effect. Studies with selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRI) have shown that the increase in endogenous 5-HT in the raphe nuclei, by activating somatodendritic 5-HT_{1A} autoinhibitory receptors, attenuated their effect in terminal regions (Adell and Artigas, 1991; Artigas *et al*, 1996; Invernizzi *et al*, 1992). Continuous administration of SSRI desensitizes 5-HT_{1A} autoreceptors. This facilitates their effect on extracellular 5-HT in terminal regions such as the prefrontal cortex (PFC) (Invernizzi *et al*, 1994) and probably contributes to the slow onset of the therapeutic effect (Blier and de Montigny, 1994).

Noradrenergic neurons originating in the locus coeruleus (LC), the main source of noradrenaline (NA) neurons in the brain (Moore and Bloom, 1978), project diffusely to forebrain regions (Tejani-Butt, 1992). NA is taken up by the NA transporter found exclusively on the neuronal membrane of noradrenergic cells (Lorang *et al*, 1994). Activation of terminal α_2-adrenoceptors by the endogenous transmitter or exogenous receptor agonists reduces NA release (L'Heureux *et al*, 1986; Starke, 1977; Westfall, 1977), whereas blockade of these receptors has the opposite effect (Dennis *et al*, 1987; Gobert *et al*, 1997; Mateo *et al*, 1998; Wortley *et al*, 1999). Somatodendritic α_2-adrenoceptors, by controlling the firing activity of NA neurons of the LC (Cedarbaum and Aghajanian, 1976; Svensson *et al*, 1975), contribute to the regulation of action potential-dependent release of NA (Florin-Lechner *et al*, 1996).

Microdialysis studies have shown that systemic injection of NA reuptake inhibitors (NRI) raised extracellular NA in the PFC, hippocampus, and LC (Bymaster *et al*, 2002; Dazzi *et al*, 2001; L'Heureux *et al*, 1986; Mateo *et al*, 1998; Page and Lucki, 2002; Sacchetti *et al*, 1999, 2001). However, their

*Correspondence: Dr RW Invernizzi, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62, Milano 20157, Italy, Tel: +39 02 39014466, Fax: +39 02 3546277,
E-mail: rinvernizzi@marionegri.it
Received 9 September 2004; revised and accepted 29 November 2004
Online publication: 1 December 2004 at http://www.acnp.org/citations/Npp12010404041 6/default.pdf

effect is limited by the concurrent activation of presynaptic α_2-adrenoceptors. Coadministration of α_2-adrenoceptor antagonists strongly enhances the NRI-induced rise of extracellular NA in brain regions containing noradrenergic afferents, such as the PFC and hippocampus (Dennis *et al*, 1987; Mateo *et al*, 1998; Sacchetti *et al*, 1999) and in the LC (Mateo *et al*, 1998).

We found recently that chronic subcutaneous infusion of reboxetine (10 mg/kg/day for 14 days), a selective NRI (Wong *et al*, 2000), through osmotic pumps, enhanced the increase of extracellular NA in the PFC of the rat and reduced the inhibitory effect of intraperitoneal (i.p.) and intracortical clonidine on extracellular NA (Invernizzi *et al*, 2001). Similar findings were recently reported by Meana and co-workers, who showed that the reduction of extracellular NA in the cingulate cortex of rats given clonidine systemically or through a cortical probe was markedly attenuated after repeated injections of desipramine (DMI; 6 mg/kg/day) for 2 weeks (Mateo *et al*, 2001). Although chronic DMI (10 mg/kg/day for 14 days) attenuated the effect of systemic clonidine on NA release in the dorsal hippocampus (DH), the response of hippocampal α_2-adrenoceptors was apparently not affected (Sacchetti *et al*, 2001). Furthermore, reduced sensitivity of α_2-adrenoceptors of the LC during long-term treatment with NRI has been reported in some electrophysiological and biochemical studies (Grandoso *et al*, 2004; Lacroix *et al*, 1991; Svensson and Usdin, 1978) but not confirmed by others (Mateo *et al*, 2001; Szabo and Blier, 2001). Therefore, it is not clear to what extent regional differences in the response to clonidine in rats given chronic NRI reflect intrinsic differences in α_2-adrenoceptor adaptive processes in the cortex and hippocampus or whether differences in drug and schedule of treatment also play a role.

In view of the clear reduction in α_2-adrenoceptor sensitivity found in the PFC of rats given chronic reboxetine through osmotic pumps (Invernizzi *et al*, 2001), in the present study, we used the same schedule to examine whether similar changes occurred in the DH. We examined the effect of the selective α_2-adrenoceptor agonist clonidine (Anden *et al*, 1976) on extracellular NA with the intracerebral microdialysis technique in conscious rats given vehicle or reboxetine for 2, 7 and 14 days. Whenever the response to systemic clonidine differed in rats given chronic vehicle or reboxetine, the drug was infused through the probe or injected into the LC to assess the functional status of terminal and somatodendritic α_2-adrenoceptors.

MATERIALS AND METHODS

Animals and Drug Treatments

Male rats (CD-COBS, Charles River, Italy) weighing 300 ± 30 g (180–200 g at the beginning of chronic experiments), housed under standard laboratory conditions with food and water freely available, were used in all the experiments.

Microdialysis experiments were conducted in conscious, freely moving rats, 24 h after probe implantation. All acute treatments were given once the basal extracellular NA concentrations were stable (two to three consecutive samples not differing by more than 15%).

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. no. 116, G.U., Suppl. 40, 18 Febbraio 1992; Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, December 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

Chronic Reboxetine and Effect of Clonidine

Rats were continuously infused with 10 mg/kg/day reboxetine or vehicle (phosphate-buffered saline, PBS) for 2, 7, or 14 days, by osmotic pumps implanted subcutaneously. This dose of reboxetine for 14 days enhanced the increase of extracellular NA and attenuated the inhibitory effect of clonidine on NA release in the PFC (Invernizzi *et al*, 2001). The daily dose of reboxetine is referred to the rats' mean body weight, which was 250 g after 1 week of treatment.

A group of rats infused with reboxetine or PBS for 2, 7, or 14 days were implanted with dialysis probes in the DH. The next day, once basal extracellular NA was stable, rats were given two consecutive injections of clonidine (10 and 30 μg/kg i.p., the second injection 90 min after the first) and extracellular NA was measured for 90 min after each dose.

The sensitivity of somatodendritic and terminal α_2-autoreceptors in rats given chronic reboxetine was assessed by measuring changes of extracellular NA in response to clonidine injected into the LC or infused through the hippocampal probe.

SURGICAL PROCEDURES

Subcutaneous Osmotic Pumps

Osmotic pumps (Alzet, model 2ML2; Charles River, Calco, Italy) delivering about 5 μl/h for 14 days were used. Rats were anesthetized with 3.5 ml/kg i.p. Equithesin (composition: 1.2 g pentobarbital, 5.3 g chloral hydrate, 2.7 g $MgSO_4$ heptahydrate, 49.5 ml propylene glycol, 12.5 ml ethanol, and 58 ml distilled water) and the skin was shaved and washed with antiseptic solution. An incision was made between the scapulae and a pocket was created by inserting a hemostat in the incision. The pump was filled with reboxetine dissolved in PBS or vehicle under sterile conditions, inserted into the subcutaneous pocket, and the wound was closed with two clips.

Dialysis Probes

Vertical probes were prepared essentially as described by Robinson and Whishaw (1988) with Cuprophan hollow fibers (Sorin Biomedica, Italy; outer diameter 216 μm). The active surface was 2 mm long for the DH and 4 mm for the PFC. Rats were anesthetized with 3.5 ml/kg i.p. Equithesin and placed on a stereotaxic frame (model 900; David Kopf Instruments, Tujunga, CA, USA). Probes were positioned in the DH and PFC at the following stereotaxic coordinates (in mm): DH, AP -3.8, V 4.5, L ± 1.9; PFC, AP $+4.2$, L ± 0.7, V -4.8 from bregma and dura surface (incisor bar -3.3 mm) according to the Paxinos and Watson atlas (1982). In the DH, the active surface of the probe was in contact with the

CA1, CA4, and dentate gyrus. The regions of the cortex accessed by the probe included the rostral division of the Cg1, prelimbic, and medial orbital cortex. Representative dialysis sites are shown in Figure 1. Probes were fixed to the skull with acrylic cement and two stainless-steel screws. Probe perfusion started about 20 h after probe implantation to allow the collection of at least four samples before the drug challenge. Probes were perfused at 1 µl/min with artificial cerebrospinal fluid (aCSF) containing NaCl 145 mM, KCl 3 mM, $CaCl_2$ 1.26 mM, $MgCl_2$ 1 mM in distilled water, pH 7.4 with 2 mM sodium phosphate buffer, and samples collected every 30 min.

Cannulae Aimed at the LC

Rats were anesthetized and mounted on a stereotaxic apparatus as described above. Guide cannulae (23 gauge, 14 mm long) were implanted 2 mm above the LC ipsilaterally to the cortical or hippocampal probe at the following stereotaxic coordinates (incisor bar −8.9 mm): AP +0.9, V −4.1, L ±0.9 from interaural line and dura surface according to the Paxinos and Watson atlas (1982). At the end of surgery, obdurators (30 gauge, 14 mm long) were inserted into the cannulae to prevent clogging. On the next day, once NA release was stable, the obdurators were removed and a needle (30 gauge) protruding 2 mm from the guide was inserted into the LC for the delivery of clonidine or PBS.

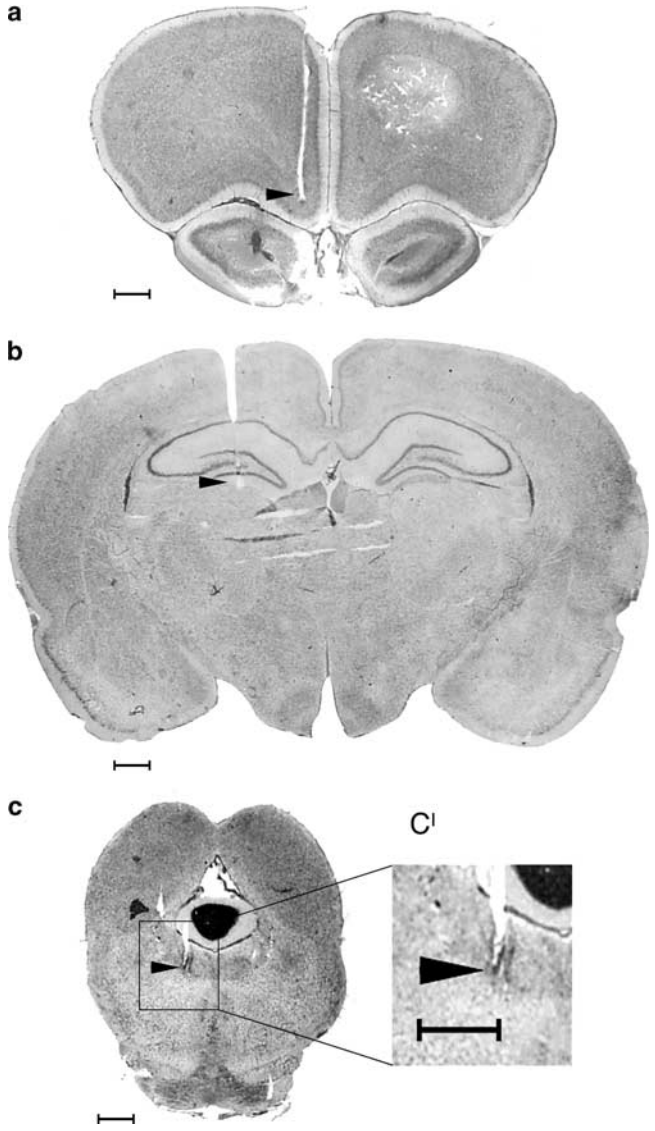

Figure 1 Representative histological sections showing the tract of the microdialysis probes in the PFC (a) and DH (b) and injection needle in the LC (c and c′). Slices (10 µm) were stained with cresyl violet. Arrowheads indicate the position of the tip of the probe or injection needle.

HISTOLOGY

At the end of the experiment, rats were deeply anesthetized (400 mg/kg chloral hydrate) and decapitated. The brain was removed and fixed in 4% paraformaldehyde for 3 days and cryoprotected by immersion in 20% sucrose for 2 days. Coronal slices (10 µm) were cut from frozen brain at the level of the DH, PFC, and LC, mounted on polylysinated slices, and stained with cresyl violet.

The correct placement of the dialysis probes and cannulae was verified by histological examination of the tracts (see Figure 1 for an example of probe and cannula placement). Rats with incorrect probe or cannulae placement were not considered in the results.

ANALYTICAL PROCEDURES

NA in the dialysate was measured by high-performance liquid chromatography with electrochemical detection (HPLC-ED). The detector was a Coulochem II (ESA, Chelmsford, MA) equipped with an analytical cell consisting of two in-series electrodes (model 5011, ESA, Chelmsford, MA). The first electrode was set at +0.2 V and the second at −0.25 V. NA was read as second electrode output signal.

Separation was obtained using a reverse-phase column (Hypersil-ODS 5 µm, 3.1 × 125 mm, Bischoff, Italy). The mobile phase consisted of 5.25 g/l citric acid monohydrate, 3.3 g/l sodium acetate trihydrate, 360 mg/l octyl sodium sulfate, 37 mg/l Na_2EDTA dihydrate, and 80 ml/l methanol pumped at a constant flow of 1 ml/min with an ESA-580 solvent delivery module (ESA, Chelmsford, MA). All chemicals were reagent grade or better from Merck (Bracco, Milan, Italy) or Sigma-Aldrich (Milan, Italy).

DRUGS

Reboxetine methanesulfonate (Pharmacia Corporation, Nerviano, Italy) was dissolved in PBS and infused subcutaneously (10 mg/kg/day) by osmotic pumps. Clonidine HCl (Boehringer Ingelheim, Milano, Italy) was dissolved in PBS and injected i.p. in 2 ml/kg. For intra-LC injections, clonidine HCl (0.2 and 0.6 pmol/0.5 µl) was dissolved in aCSF and injected into the LC through previously implanted guide cannulae. Clonidine or aCSF were delivered at 0.5 µl/min and the needle was left in place for 1 min before withdrawal to allow for diffusion of the drug. All doses are referred to the free base.

DATA CALCULATION AND STATISTICS

The last of three consecutive stable samples was taken as the basal value of extracellular NA, expressed as fmol/30 µl. Basal values of rats infused with PBS or reboxetine for 2, 7, and 14 days through the osmotic pump were compared by one-way ANOVA followed by Tukey–Kramer's test.

The effect of a challenge dose of clonidine in rats given chronic reboxetine or PBS was assessed by ANOVA for repeated measures. When significant effects were found, *post hoc* comparisons between pre- and postinjection values were made by Dunnett's test.

The overall effect of clonidine challenge in rats given chronic reboxetine or vehicle was assessed by the area under the curve (AUC) calculated for each rat on the percentage of basal values and expressed as % × min over the interval indicated in the legends to Figures 1–3. The resulting data were compared by Mann–Whitney's test.

RESULTS

Effect of Chronic Reboxetine on Basal Extracellular NA in the DH

The infusion of 10 mg/kg/day reboxetine for 2 ($n=5$), 7 ($n=12$), and 14 ($n=21$) days through an osmotic pump (still in place during sample collection) significantly increased basal extracellular NA in the DH ($F_{3,49}=28.2$, $p<0.0001$). As shown in Table 1, after 7 and 14 days, basal concentrations of NA were increased by, respectively, 469 and 437%. At both times, reboxetine had more effect on extracellular NA in the DH than in rats receiving PBS ($n=15$) or reboxetine for 2 days (272%). Extracellular NA concentrations in rats infused with reboxetine for 7 and 14 days were not significantly different. As no significant differences in basal levels of NA were found in rats given PBS for 2 ($n=2$), 7 ($n=4$), and 14 ($n=9$) days, the results were pooled.

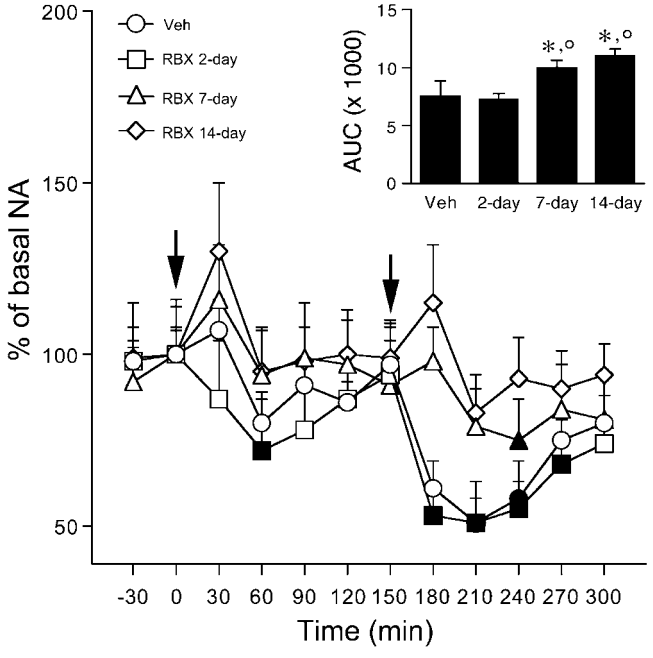

Figure 2 Effect of two consecutive i.p. doses of clonidine (10 and 30 µg/kg) on extracellular NA concentrations in the DH of rats infused with PBS ($n=6$) or 10 mg/kg/day reboxetine for 2 ($n=5$), 7 ($n=6$), and 14 days ($n=11$). Data are mean ± SEM and are expressed as percentages of basal values. The first and second arrows indicate the injection of 10 and 30 µg/kg clonidine. Solid symbols indicate $p<0.05$ *vs* basal values (Dunnett's test). Inset: AUC calculated from 180 to 300 min. *$p<0.05$ *vs* vehicle; °$p<0.05$ *vs* 2-day (Mann–Whitney's test).

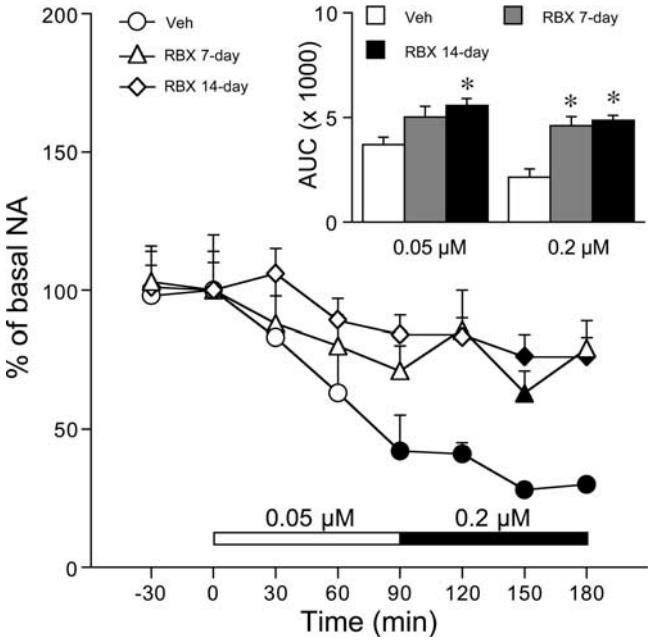

Figure 3 Effect of 0.05 and 0.2 µM clonidine administered through the probe on the extracellular concentrations of NA in the DH of rats infused with PBS ($n=4$) or 10 mg/kg/day reboxetine (RBX) for 7 ($n=6$) and 14 days ($n=6$). Data are mean ± SEM and are expressed as percentages of basal values. Horizontal bars indicate the duration of clonidine infusion. Solid symbols indicate $p<0.05$ *vs* basal values (Dunnett's test). Inset: AUC calculated from 30 to 90 min or 120 to 180 min; *$p<0.05$ *vs* vehicle (Mann–Whitney's test).

Table 1 Extracellular NA in the DH of Rats Infused with PBS or 10 mg/kg/day Reboxetine for 2, 7, and 14 Days

	NA (fmol/30 µl)
PBS	10.2 ± 0.7 (15)
Reboxetine, 2 days	27.7 ± 3.4* (5)
Reboxetine, 7 days	47.8 ± 4.9*,° (12)
Reboxetine, 14 days	44.6 ± 3.2*,° (21)

*$p<0.05$ *vs* PBS; °$p<0.05$ *vs* reboxetine, 2 days (Tukey–Kramer's test). Data are mean ± SEM. Mean basal values were obtained by pooling the data from Figures 2–4 (number of rats in parentheses).

Intraperitoneal Clonidine

As shown in Figure 2, 30 µg/kg clonidine significantly reduced extracellular NA in the DH ($F_{3,23}=15.1$, $p<0.0001$). The effect was significant in rats given vehicle (49% below basal values), 10 mg/kg/day reboxetine for 2

(49%) and 7 days (25%) but not in those infused with the drug for 14 days (17% less than basal values). In all, 10 μg/kg clonidine (Figure 1) or PBS (data not shown) had no significant effect on extracellular NA in any of the experimental groups. The overall effect of 30 μg/kg clonidine on extracellular NA, as estimated by the AUC, was significantly attenuated in rats given reboxetine for 7 and 14 days compared to those receiving PBS or reboxetine for 2 days ($p<0.05$, Mann–Whitney's test).

Intrahippocampal Clonidine

The infusion of clonidine through the probe significantly reduced extracellular NA in the DH of rats given PBS ($F_{3,6}=6.6$, $p=0.0008$) or 10 mg/kg/day reboxetine for 7 ($F_{5,6}=3.0$, $p=0.02$) and 14 days ($F_{5,6}=9.4$, $p<0.0001$) (Figure 3). In rats infused with PBS, extracellular NA reached, respectively, 42 and 28% of basal levels in response to 0.05 and 0.2 μM clonidine. In rats given reboxetine for 7 and 14 days, extracellular NA reached 63 and 76% of basal levels in response to 0.2 μM clonidine and 71 and 83% in those infused with 0.05 μM clonidine. The overall effect of 0.2 μM clonidine on extracellular NA, as evaluated by the AUC, was significantly attenuated by 7 and 14 days at 10 mg/kg/day reboxetine. The effect of 0.05 μM clonidine was also attenuated in the DH of rats given reboxetine for 14 days but not in those given the drug for 7 days. The NA release was stable over the 3-h infusion of normal aCSF (data not shown).

Intra-LC Clonidine

To assess the effect of chronic reboxetine on the sensitivity of somatodendritic α_2-adrenoceptors regulating the NA release in the DH and PFC, rats were injected with clonidine into the LC. The dose of 0.6 nmol/0.5 μl significantly reduced extracellular NA by, respectively, 64 and 42% in the DH ($F_{3,12}=12.4$, $p=0.0006$) and PFC ($F_{3,18}=4.6$, $p=0.01$) of rats given PBS and by 45% ($F_{3,9}=5.0$, $p=0.03$) and 28% ($F_{3,12}=3.9$, $p=0.04$) in those given 10 mg/kg/day reboxetine for 14 days (Figure 4). Comparison of AUCs revealed no significant differences in the effect on intra-LC clonidine on NA release in either the DH or PFC of rats given chronic reboxetine or PBS.

The injection of aCSF or 0.2 nmol/0.5 μl clonidine into the LC had no significant effect on extracellular NA in the DH and PFC (data not shown).

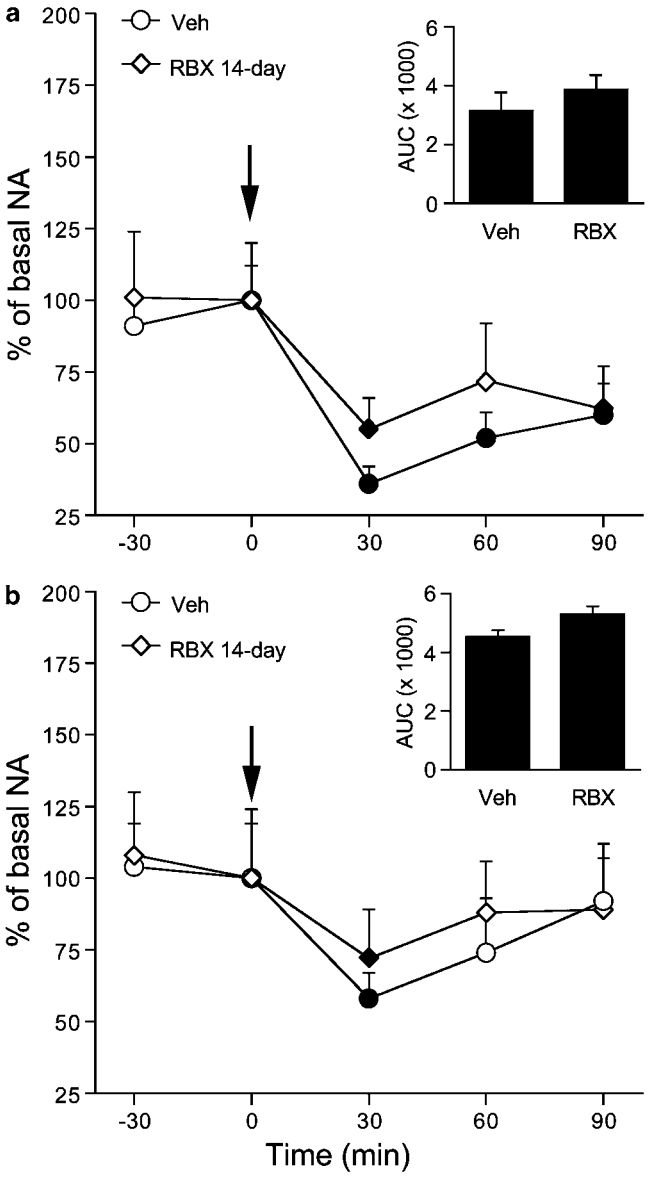

Figure 4 Effect of 0.6 nmol/0.5 μl clonidine injected into the LC on extracellular NA in the DH (a) and PFC (b) of rats infused with PBS ($n=5$ and 7) or 10 mg/kg/day reboxetine for 14 days ($n=4$ and 5). Arrows indicate the injection of clonidine into the LC. Data are mean ± SEM and are expressed as percentages of basal values. Solid symbols indicate $p<0.05$ *vs* basal values (Dunnett's test). Insets: AUC calculated from 0 to 90 min.

DISCUSSION

The present study shows that continuous infusion of 10 mg/kg/day reboxetine through subcutaneous osmotic pumps enhances the drug's ability to raise extracellular NA in the DH and attenuates the inhibitory effect of clonidine on NA release.

Extracellular NA rose more in rats infused with 10 mg/kg/day reboxetine for 7 and 14 days than in those given the drug for 2 days. This suggests that adaptive changes already occurring after 7 days are likely to be responsible for the drug's enhanced effect. The dose of reboxetine used in the present study maximally increased extracellular NA in the PFC (Invernizzi *et al*, 2001) and its effect on extracellular NA is close to that in the DH (240%) and PFC (242%) of rats given a single high dose (15 mg/kg i.p.) (Sacchetti *et al*, 1999). This suggests that under the present experimental conditions, reboxetine maximally inhibits NA reuptake. We have previously shown that brain levels of reboxetine in rats infused with the drug for 2 or 14 days were not significantly different (Invernizzi *et al*, 2001). Thus, it can be excluded that more effective blockade of NA reuptake or changes in drug kinetics accounts for the enhancement of reboxetine's effect.

Previous studies have shown that desensitization of somatodendritic 5-HT_{1A} autoreceptors, developing after chronic blockade of the 5-HT transporter by SSRI, is

responsible for recovery of the normal firing activity of serotonergic neurons of the dorsal raphe nucleus (Blier and de Montigny, 1994) and the enhanced effect of SSRI on extracellular 5-HT in the PFC (Invernizzi *et al*, 1994). We found that the inhibition of hippocampal NA release induced by systemic clonidine was strongly attenuated after 7 and 14 days infusion with reboxetine. Thus, α_2-adrenoceptors are rapidly desensitized in response to reboxetine and this effect takes about the same time to develop as the enhancement of extracellular NA. This suggests a possible link between desensitization of the α_2-adrenoceptors inhibiting NA release and enhancement of extracellular NA.

Since both terminal and somatodendritic α_2-adrenoceptors regulate NA release (Mateo *et al*, 1998), the attenuation of clonidine's effects in rats given chronic reboxetine may be the expression of adaptive changes in the receptors in the cell bodies of the LC, noradrenergic terminals of the DH or both. We attempted to clarify whether the attenuated effect of clonidine depends on desensitization of terminal or somatodendritic α_2-adrenoceptors by infusing clonidine through the hippocampal probe or injecting it into the LC. Intrahippocampal infusion markedly reduced extracellular NA in the DH of rats given PBS and this effect was strongly attenuated in rats given reboxetine for 7 and 14 days.

These results suggest that hippocampal α_2-adrenoceptors become subsensitive during long-term treatment with reboxetine and are consistent with previous findings that hippocampal and cortical α_2-adrenoceptors are desensitized by chronic reboxetine (10 mg/kg/day for 14 days) and DMI (6–10 mg/kg/day for 14 days) (Invernizzi *et al*, 2001; Lacroix *et al*, 1991; Mateo *et al*, 2001). However, other studies found no change in the sensitivity of hippocampal α_2-adrenoceptors after chronic DMI (10–20 mg/kg/day for 21–28 days) (Campbell and McKernan, 1986; Schoffelmeer and Mulder, 1982). We have already seen that chronic DMI (10 mg/kg/day for 14 days) did not affect the suppression of NA release by 30 μg/kg clonidine (Sacchetti *et al*, 2001). The reasons for this difference are not clear but may reflect intrinsic differences between reboxetine and DMI or differences in the experimental conditions. One main difference between the present study and that by Sacchetti *et al* (2001) is the washout period between chronic treatment and clonidine challenge. Whereas in the present study clonidine was injected during reboxetine administration, Sacchetti allowed 48 h washout between the last DMI dose and the injection of clonidine. It could be argued that the activation of autoreceptors by the increased endogenous NA tone might partly prevent clonidine's inhibitory effect on NA release. Thus, the reduced effect of clonidine in rats given chronic reboxetine would not be due to the desensitization of α_2-adrenoceptors but to occupancy of the autoreceptor by the endogenous agonist.

However, several findings argue against this interpretation. First, the reduction of extracellular NA by clonidine was not affected by a 2-day infusion of reboxetine, a condition in which extracellular NA increases two- to three-fold in the DH and five-fold in the LC (see Table 1; unpublished results). Corresponding studies with DMI showed that despite a five-fold increase of extracellular NA, acute blockade of NA reuptake had no effect on clonidine-induced inhibition of NA release in the PFC (Mateo *et al*, 2001). Second, despite the fact that extracellular NA in the LC increased by more than nine times in response to 14 days reboxetine infusion (unpublished results), injection of clonidine into the LC had the same effect in rats given reboxetine or PBS (present study).

Thus, the development of subsensitivity of α_2-adrenoceptors regulating NA release appears to be the more parsimonious interpretation of the reduced effect of clonidine in rats given chronic reboxetine. It remains to be established whether in experimental conditions comparable to the present study chronic treatment with DMI will modify the sensitivity of hippocampal α_2-adrenoceptors.

The present results suggest that desensitization of hippocampal α_2-adrenoceptors plays a major role in the response to systemic clonidine in rats given chronic reboxetine. This interpretation is supported by the finding that the reduction of hippocampal NA release by intra-LC clonidine was not changed by long-term treatment with reboxetine. The latter finding is fully consistent with the results of microdialysis studies showing that the suppressant effect of intra-LC clonidine on NA release was not modified by long-term treatment with DMI (6 mg/kg/day for 14 days) and the monoamine oxidase (MAO) inhibitor clorgyline (Mateo *et al*, 2001). Thus, the firing rate of NA neurons of the LC did not recover and the suppressant effect of clonidine remained unchanged after continuous infusion of reboxetine (2.5 mg/kg/day for 21 days) or repeated doses of MAO inhibitors (Blier and de Montigny, 1985; Blier *et al*, 1986; Szabo and Blier, 2001). This suggests that the sensitivity of somatodendritic α_2-adrenoceptors of the LC does not change in response to treatments increasing the synaptic availability of NA. However, other studies reported that chronic DMI (6–10 mg/kg/day for 14 days) and imipramine (20 mg/kg/day for 11 days) reduced the response to clonidine or brimoxidine (Grandoso *et al*, 2004; Lacroix *et al*, 1991; Svensson and Usdin, 1978).

The reasons for this discrepancy are not clear, but may reflect differences in experimental conditions, mainly because these studies were carried out 12–36 h after the last injection of the chronic schedule, whereas our study and that of Szabo and Blier (2001) were carried out while reboxetine was still being delivered by the osmotic pump. The scarce selectivity of the NRI used in one study (Svensson and Usdin, 1978) and the possible confounding effect of anesthesia, which depresses the activity of LC neurons and may enhance its inhibition (Valentino and Wehby, 1988), could have also contributed.

The present findings and previous work by our group (Invernizzi *et al*, 2001) suggest that desensitization of presynaptic α_2-adrenoceptors in terminal regions but not those in the LC is involved in the enhancement of extracellular NA by reboxetine. The present study did not address the reasons for the different roles of terminal and somatodendritic α_2-adrenoceptors. However, one factor that differentiates presynaptic α_2-adrenoceptors of the DH, PFC and LC might be the large receptor reserve that affects the inhibitory effect of clonidine in the LC (Pineda *et al*, 1997), compared to the smaller reserve for clonidine-induced inhibition of NA release from cortical terminals (Agneter *et al*, 1993). Thus, desensitization of a large proportion of somatodendritic α_2-adrenoceptors may leave the effect of clonidine unaltered.

We previously found that repeated daily injections of 15 mg/kg reboxetine for 14 days did not change the rise of extracellular NA induced by reboxetine and the effect of a challenge dose of clonidine on NA release in the DH (Sacchetti *et al*, 1999). This was very likely due to the rapid clearance of reboxetine in rats (Dostert *et al*, 1997). Stable levels of reboxetine are not achieved with once-daily dosing and this might have prevented the development of adaptive changes in extracellular NA and autoreceptor sensitivity.

Clinical studies using α-methyltyrosine showed that inhibition of catecholamine synthesis rapidly reversed the effect of antidepressant drugs that enhance noradrenergic transmission in depressed patients in remission (Delgado *et al*, 1993, 2002; Miller *et al*, 1996). This highlights the importance of increased brain NA transmission in the therapeutic effect of these antidepressants. Thus, the delayed increase of extracellular NA may be one of the factors in the development of the antidepressant effect of NRI. Blockade of α_2-adrenoceptors is a faster and more efficient way to enhance the effect of NRI on extracellular NA (see Invernizzi and Garattini, 2004 for a recent review). Thus, coadministration of an NRI with an α_2-adrenoceptor antagonist such as idazoxan, which may have antidepressant effects by itself (Osman *et al*, 1989), should accelerate the antidepressant effect of NRI and might possibly enhance their effect in nonresponsive patients. However, clinical studies addressing this issue are scarce and results are still controversial (Charney *et al*, 1986; Lauritzen *et al*, 1992; Schmauss *et al*, 1988), so further trials are necessary to assess the validity of this strategy.

In conclusion, we have shown that reboxetine desensitizes α_2-adrenoceptors controlling NA release. Desensitization of terminal α_2-adrenoceptors plays a major role in enhancing the effect of reboxetine on extracellular NA and may be one factor contributing to the beneficial effects of antidepressant drugs that inhibit NA reuptake.

ACKNOWLEDGEMENTS

This study was supported by Pharmacia Italia (Milan, Italy). We are grateful to J Baggott for language editing.

REFERENCES

Adell A, Artigas F (1991). Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An *in vivo* brain microdialysis study. *Naunyn Schmiedebergs Arch Pharmacol* **343**: 237–244.

Agneter E, Drobny H, Singer EA (1993). Central alpha 2-autoreceptors: agonist dissociation constants and recovery after irreversible inactivation. *Br J Pharmacol* **108**: 370–375.

Anden NE, Grabowska M, Strombom U (1976). Different alpha-adrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. *Naunyn Schmiedebergs Arch Pharmacol* **292**: 43–52.

Artigas F, Romero L, de Montigny C, Blier P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurosci* **19**: 378–383.

Blier P, de Montigny C (1985). Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neuroscience* **16**: 949–955.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* **15**: 220–226.

Blier P, de Montigny C, Azzaro AJ (1986). Effect of repeated amiflamine administration on serotonergic and noradrenergic neurotransmission: electrophysiological studies in the rat CNS. *Naunyn Schmiedebergs Arch Pharmacol* **334**: 253–260.

Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH *et al* (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology* **27**: 699–711.

Campbell IC, McKernan RM (1986). Clorgyline and desipramine alter the sensitivity of [^{3}H]noradrenaline release to calcium but not to clonidine. *Brain Res* **372**: 253–259.

Cedarbaum JM, Aghajanian GK (1976). Noradrenergic neurons of the locus coeruleus: inhibition by epinephrine and activation by the alpha-antagonist piperoxane. *Brain Res* **112**: 413–419.

Charney DS, Price LH, Heninger GR (1986). Desipramine–yohimbine combination treatment of refractory depression. Implications for the beta-adrenergic receptor hypothesis of antidepressant action. *Arch Gen Psychiatry* **43**: 1155–1161.

Dazzi L, Spiga F, Pira L, Ladu S, Vacca G, Rivano A *et al* (2001). Inhibition of stress- or anxiogenic-drug-induced increases in dopamine release in the rat prefrontal cortex by long-term treatment with antidepressant drugs. *J Neurochem* **76**: 1212–1220.

Delgado PL, Miller HL, Salomon RM, Licinio J, Heninger GR, Gelenberg AJ *et al* (1993). Monoamines and the mechanism of antidepressant action: effects of catecholamine depletion on mood of patients treated with antidepressants. *Psychopharmacol Bull* **29**: 389–396.

Delgado PL, Moreno FA, Onate L, Gelenberg AJ (2002). Sequential catecholamine and serotonin depletion in mirtazapine-treated depressed patients. *Int J Neuropsychopharmacol* **5**: 63–66.

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 MS, Poggesi I (1997). Review of the pharmacokinetics and metabolism of reboxetine, a selective noradrenaline reuptake inhibitor. *Eur Neuropsychopharmacol* **7**(Suppl 1): S23–S35 (discussion S71–S73).

Florin-Lechner SM, Druhan JP, Aston-Jones G, Valentino RJ (1996). Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. *Brain Res* **742**: 89–97.

Gobert A, Rivet JM, Cistarelli L, Melon C, Millan MJ (1997). Alpha2-adrenergic receptor blockade markedly potentiates duloxetine- and fluoxetine-induced increases in noradrenaline, dopamine, and serotonin levels in the frontal cortex of freely moving rats. *J Neurochem* **69**: 2616–2629.

Grandoso L, Pineda J, Ugedo L (2004). Comparative study of the effects of desipramine and reboxetine on locus coeruleus neurons in rat brain slices. *Neuropharmacology* **46**: 815–823.

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.

Invernizzi R, Bramante M, Samanin R (1994). Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: role of presynaptic 5-HT$_{1A}$ receptors. *Eur J Pharmacol* **260**: 243–246.

Invernizzi RW, Garattini S (2004). Role of presynaptic α_2-adrenoceptors in antidepressant action: recent findings from microdialysis studies. *Prog Psychopharmacol Biol Psychiatry* **28**: 819–827.

Invernizzi RW, Parini S, Sacchetti G, Fracasso C, Caccia S, Annoni K *et al* (2001). Chronic treatment with reboxetine by osmotic pumps facilitates its effect on extracellular noradrenaline and may desensitize alpha(2)-adrenoceptors in the prefrontal cortex. *Br J Pharmacol* **132**: 183–188.

Lacroix D, Blier P, Curet O, de Montigny C (1991). Effects of long-term desipramine administration on noradrenergic neurotransmission: electrophysiological studies in the rat brain. *J Pharmacol Exp Ther* **257**: 1081–1090.

Lauritzen L, Clemmesen L, Klysner R, Loldrup D, Lunde M, Schaumburg E *et al* (1992). Combined treatment with imipramine and mianserin. A controlled pilot study. *Pharmacopsychiatry* **25**: 182–186.

L'Heureux R, Dennis T, Curet O, Scatton B (1986). Measurement of endogenous noradrenaline release in the rat cerebral cortex *in vivo* by transcortical dialysis: effects of drugs affecting noradrenergic transmission. *J Neurochem* **46**: 1794–1801.

Lorang D, Amara SG, Simerly RB (1994). Cell-type-specific expression of catecholamine transporters in the rat brain. *J Neurosci* **14**: 4903–4914.

Mateo Y, Fernandez-Pastor B, Meana JJ (2001). Acute and chronic effects of desipramine and clorgyline on alpha(2)-adrenoceptors regulating noradrenergic transmission in the rat brain: a dual-probe microdialysis study. *Br J Pharmacol* **133**: 1362–1370.

Mateo Y, Pineda J, Meana JJ (1998). Somatodendritic alpha2-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.

Miller HL, Delgado PL, Salomon RM, Heninger GR, Charney DS (1996). Effects of alpha-methyl-*para*-tyrosine (AMPT) in drug-free depressed patients. *Neuropsychopharmacology* **14**: 151–157.

Moore RY, Bloom FE (1978). Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. *Annu Rev Neurosci* **1**: 129–169.

National Research Council (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press: Washington, DC.

Osman OT, Rudorfer MV, Potter WZ (1989). Chronic alpha-2 antagonism increases norepinephrine, blocks GH release and is antidepressant. *Biol Psychiatry* **25**: 197A.

Page ME, Lucki I (2002). Effects of acute and chronic reboxetine treatment on stress-induced monoamine efflux in the rat frontal cortex. *Neuropsychopharmacology* **27**: 237–247.

Paxinos G, Watson C (1982). *The Rat Brain in Stereotaxic Coordinates*. Academic Press: Sidney.

Pineda J, Ruiz-Ortega JA, Ugedo L (1997). Receptor reserve and turnover of alpha-2 adrenoceptors that mediate the clonidine-induced inhibition of rat locus coeruleus neurons *in vivo*. *J Pharmacol Exp Ther* **281**: 690–698.

Robinson TE, Whishaw IQ (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 RW, 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 *et al* (2001). Chronic treatment with desipramine facilitates its effect on extracellular noradrenaline in the rat hippocampus: studies on the role of presynaptic alpha2-adrenoceptors. *Naunyn Schmiedebergs Arch Pharmacol* **363**: 66–72.

Schmauss M, Laakmann G, Dieterle D (1988). Effects of alpha 2-receptor blockade in addition to tricyclic antidepressants in therapy-resistant depression. *J Clin Psychopharmacol* **8**: 108–111.

Schoffelmeer AN, Mulder AH (1982). ^{3}H-noradrenaline and ^{3}H-5-hydroxytryptamine release from rat brain slices and its presynaptic alpha-adrenergic modulation after long-term desipramine pretreatment. *Naunyn Schmiedebergs Arch Pharmacol* **318**: 173–180.

Starke K (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev Physiol Biochem Pharmacol* **77**: 1–124.

Svensson TH, Bunney BS, Aghajanian GK (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res* **92**: 291–306.

Svensson TH, Usdin T (1978). Feedback inhibition of brain noradrenaline neurons by tricyclic antidepressants: alpha-receptor mediation. *Science* **202**: 1089–1091.

Szabo ST, Blier P (2001). Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. *Eur J Neurosci* **13**: 2077–2087.

Tejani-Butt SM (1992). [^{3}H]nisoxetine: a radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. *J Pharmacol Exp Ther* **260**: 427–436.

Valentino RJ, Wehby RG (1988). Morphine effects on locus coeruleus neurons are dependent on the state of arousal and availability of external stimuli: studies in anesthetized and unanesthetized rats. *J Pharmacol Exp Ther* **244**: 1178–1186.

Westfall TC (1977). Local regulation of adrenergic neurotransmission. *Physiol Rev* **57**: 659–728.

Wong EH, Sonders MS, Amara SG, Tinholt PM, Piercey MF, Hoffmann WP *et al* (2000). Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biol Psychiatry* **47**: 818–829.

Wortley KE, Heal DJ, Stanford SC (1999). Modulation of sibutramine-induced increases in extracellular noradrenaline concentration in rat frontal cortex and hypothalamus by alpha2-adrenoceptors. *Br J Pharmacol* **128**: 659–666.