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European Journal of Pharmacology 476 (2003) 55–61
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Antagonism of the stress-induced increase in cortical norepinephrine output by the selective norepinephrine reuptake inhibitor reboxetine

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Received 24 April 2003; received in revised form 14 July 2003; accepted 22 July 2003

Abstract

We have previously shown that long-term treatment of rats with antidepressant drugs that affect the activity of noradrenergic and serotonergic neurons by different mechanisms, inhibits the increase in cortical norepinephrine output induced by stress. With the use of microdialysis, we have now evaluated the effects of reboxetine, an antidepressant drug that selectively inhibits norepinephrine reuptake, on the increase in cortical norepinephrine output elicited in rats by exposure to foot-shock stress or by the acute administration of *N*-methyl-β-carboline-3-carboxamide (FG 7142) (20 mg/kg, i.p.). Foot-shock stress and FG 7142 each induced a marked increase in the cortical extracellular concentration of norepinephrine (+200 and +90%, respectively) in control rats. Long-term treatment with reboxetine (10 mg/kg, i.p., once a day for 21 days) reduced the effect of foot-shock stress and completely antagonized the effect of FG 7142 on cortical norepinephrine output. Our results suggest that changes in the activity of noradrenergic neurons in the cortex might be relevant to the anxiolytic and antidepressant effects of reboxetine.
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Keywords: Anxiety; Microdialysis; Norepinephrine; Cortex prefrontal; Reboxetine; Rat

1. Introduction

Norepinephrine is thought to be an important mediator of the stress-induced development of anxiety and depression (Ninan, 1999). Various experimental evidence has suggested that the locus coeruleus noradrenergic system plays a major role in modulation of behavioral arousal in response to sudden environmental stimuli, a phenomenon that is crucial in the response to stress and anxiogenic stimuli (Cole and Robbins, 1992; Robbins and Everitt, 1995; McQuade et al., 1999). Noradrenergic function has been proposed to be altered in individuals with depression or anxiety disorders, possibly explaining the altered behavioral responses associated with these conditions.

Clinical evidence suggests that stress can precipitate episodes of anxiety or depression, both of which disorders are characterized by poor concentration, reduced attention, altered memory, reduced socialization and an altered state of arousal. Indeed, episodes of depression are often preceded by stressful conditions (Hammen et al., 1986; Post, 1992; Mizoguchi et al., 2000; Persaud, 2000). Experimental data have also shown that long-term exposure of rats to various stressful protocols induces many of the motivational, neuroendocrine and behavioral changes apparent in human depression (Brady, 1994). Such protocols also increase the sensitivity of cortical and hippocampal noradrenergic neurons to an acute stress (Nisenbaum et al., 1991; Gresch et al., 1994, 1995) as well as down-regulate the expression of specific neurotrophic factors (Duman et al., 1999, 2001).

Long-term treatment with antidepressants induces effects on the firing rate of locus coeruleus and other noradrenergic neurons that are opposite to those elicited by stress (Huang et al., 1980; Nestler et al., 1990; Valentino et al., 1990; Melia et al., 1992; Smith et al., 1995). Such chronic antidepressant treatment antagonizes the actions of stress both by promoting neurogenesis in the hippocampus and other brain regions (Duman et al., 1999, 2001) as well as by modulating the expression of genes for specific neurotrophic factors (Mallei et al., 2002). Long-term administration of antidepressant drugs that potentiate both noradrenergic and serotonergic

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0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)02130-7

transmission (Stimmel et al., 1997) also reduces behaviors linked to anxiety both in rats (Commissaris and Hill, 1994; Teixeira et al., 2000) and in humans with either generalized anxiety disorder or mixed depression-anxiety syndrome (Falkai, 1999; Goodnick et al., 1999; Sheehan, 1999; Thompson, 1999). Consistent with these observations, we recently showed that long-term administration of antidepressant drugs that alter, by two different mechanisms of action, the activity of both norepinephrine and serotonin systems resulted in partial or complete inhibition of the effect of acute stress on rat cortical noradrenergic neurons (Dazzi et al., 2002a,b). The effects of chronic treatment with such antidepressants thus mimicked those of an acute injection of benzodiazepine. We suggested that this reduced sensitivity of noradrenergic neurons to stressful stimuli might be functionally associated with the plastic adaptive response of these neurons to long-term treatment with antidepressants. These functional changes might contribute to amelioration of the symptoms associated with mood and anxiety disorders.

We have now investigated the effect of long-term administration of reboxetine, a selective inhibitor of norepinephrine reuptake, on the increase in the extracellular concentration of norepinephrine in the rat prefrontal cortex induced by acute exposure to foot-shock stress. In addition, we also determined the effect of reboxetine treatment on cortical norepinephrine output induced by *N*-methyl-β-carboline-3-carboxamide (FG 7142), a benzodiazepine receptor inverse agonist that triggers, in both humans and animals, biochemical and behavioral changes similar to those either elicited by stressful stimuli or often associated with anxiety disorders (Ninan et al., 1982; Corda et al., 1983; Dorow et al., 1983). The experiments were performed 48 h after the last chronic administration of the drug, as we were interested in evaluating the adaptation or changes in the sensitivity of noradrenergic neurons to stressful stimuli following chronic treatment with antidepressant drugs.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley CD rats (Charles River, Como, Italy), with initial body masses of 200–220 g, were maintained under an artificial 12-h light, 12-h dark cycle (light on 0800–2000 h) at a temperature of 22 ± 2 °C and 65% humidity. Food and water were freely available, and the animals were acclimatized for at least 8 days before use. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocols were approved by the Animal Ethics Committee of the University of Cagliari.

2.2. Drugs and long-term treatment protocol

Rats were treated with reboxetine (10 mg/kg, i.p.), which was kindly provided by Pharmacia-Upjohn and dissolved in distilled water, or with vehicle once a day for 21 days. FG 7142 (Schering, Berlin, Germany) was dissolved in distilled water with one drop of Tween 80 per 5 ml. Drugs or vehicle were injected in a volume of 3 ml/kg body mass.

2.3. Microdialysis probe implantation and experimental procedures

Twenty-four hours after the last injection of the long-term treatment protocol, rats were subjected to implantation under chloral hydrate anesthesia (0.4 g/kg, i.p.) of a concentric dialysis probe with a 2-mm zone of active membrane (AN69 HF; Hospal Dasco, Bologna, Italy). The probe was directed unilaterally into the ventromedial frontal (infralimbic) cortex (A: +3.2, ML: +0.8, V: −5.3 relative to the bregma) according to the atlas of Paxinos and Watson (1982). Experiments were performed after animals were allowed to recover for ~24 h, between 0900 and 1800 h. Ringer's solution containing 3 mM KCl, 125 mM NaCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 23 mM $NaHCO_3$ and 1.5 mM potassium phosphate buffer (pH 7.3) was pumped through the dialysis probe at a constant rate of 2 μl/min. Samples were collected every 20 min and immediately analyzed by high-performance liquid chromatography with electrochemical detection as described (Dazzi et al., 2002a). Absolute norepinephrine concentrations were determined with the use of external standards. The average neurotransmitter concentration in the last three samples before treatment was taken as 100%, and all subsequent values were expressed as a percentage of basal values. All microdialysis probes were tested for in vitro recovery before implantation, and only those with an average recovery of $20 \pm 3\%$ were used. The absolute concentrations of norepinephrine were not corrected for these recovery values. The position of the probe was verified histologically at the end of each experiment. Rats in which the probe was detected outside of the prefrontal cortex were excluded from data analysis.

2.4. Foot shock

The foot-shock apparatus (Lafayette Instruments, Lafayette, IN) consisted of a Plexiglas box with two opaque sides, measuring $28 \times 22 \times 27$ cm. The box was connected to a scrambler controller that delivered intermittent shocks. The experimental animals received an 8-min foot-shock stress (0.2 mA, for 500 ms every second) 48 h after the last injection of the long-term drug treatment protocol. During stress exposure, a 10-min sample was taken; all subsequent samples were taken again every 20 min.

2.5. Statistical analysis

All data are presented as means ± S.E.M. Comparisons among groups were performed by two-way analysis of variance (ANOVA) for repeated measures. Post hoc comparisons were performed by Newman–Keuls test. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Effect of acute reboxetine administration on cortical norepinephrine output

The basal extracellular concentration of norepinephrine in the prefrontal cortex of naïve rats, not corrected for in vitro recovery, was 27.48 ± 3.29 fmol/40 μl sample (Fig. 1; $n = 35$). The administration of a single injection of reboxetine (2.5–10 mg/kg, i.p.) induced a dose-dependent increase in norepinephrine output in the medial prefrontal cortex of freely moving rats (Fig. 1). The increase in the extracellular concentration of norepinephrine induced at the dose of 10 mg/kg was maximal (+100%) about 60 min after drug injection and remained significant for ~ 120 min, before returning to basal levels by 140 min (data not shown). The increase in the cortical norepinephrine concentration induced by reboxetine at a dose of 5 mg/kg was also maximal (+70%) about 60 min after injection and persisted for ~ 100 min. The lowest dose of reboxetine (2.5 mg/kg) had no significant effect on norepinephrine output. Overall, ANOVA revealed a significant main effect of treatment [$F(3,111) = 59.867$, $P < 0.001$], a significant main effect of repeated measures [$F(6,111) = 2.065$, $P < 0.001$] and a significant interaction between factors [$F(12,111) = 1.958$, $P < 0.001$].

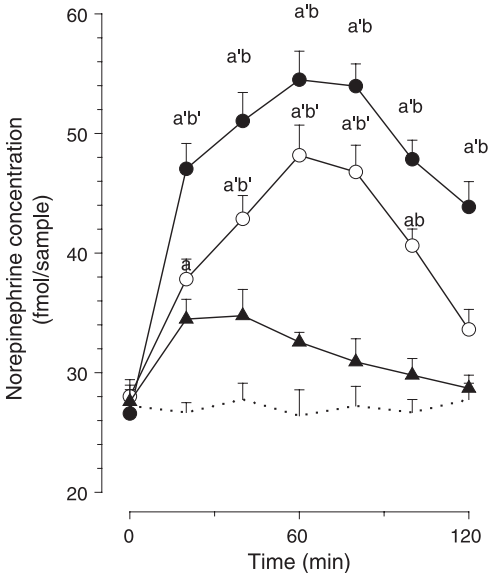

Fig. 1. Time course and dose-dependence of the increase in norepinephrine output in the rat prefrontal cortex induced by acute administration of reboxetine. Animals were injected intraperitoneally either with reboxetine at doses of 2.5 (triangles), 5 (open circles) or 10 (closed circles) mg/kg or with vehicle (no symbols, dotted line). Data are means ± S.E.M. and are expressed as fmols/40 μl sample. $^{a}P < 0.05$, $^{a'}P < 0.01$ vs. basal values; $^{b}P < 0.05$, $^{b'}P < 0.01$ vs. vehicle-treated rats ($n = 5$ per dose).

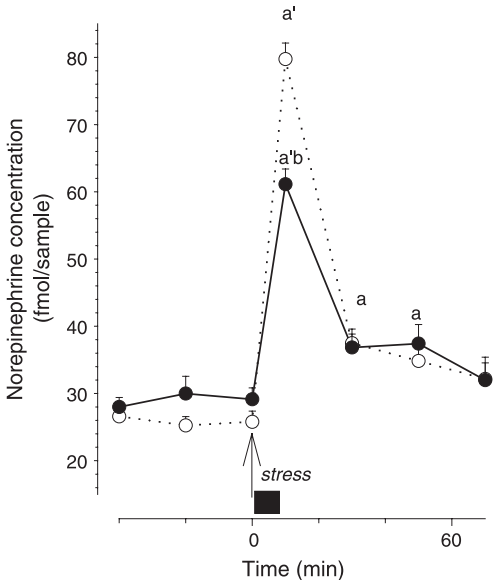

Fig. 2. Inhibition of the foot shock-induced increase in norepinephrine output in the prefrontal cortex of rats by chronic treatment with reboxetine. Animals were treated with reboxetine (10 mg/kg, i.p., once a day for 21 days; closed circles) or with vehicle (open circles), and 48 h after the last injection (indicated as time 0), they were subjected to foot shock for 8 min. Data are means ± S.E.M. of values and are expressed as fmols/40 μl sample. $^{a}P < 0.05$, $^{a'}P < 0.01$ vs. basal values; $^{b}P < 0.01$ vs. vehicle-treated rats ($n = 5$ per group).

3.2. Effect of chronic treatment with reboxetine on basal norepinephrine extracellular concentration

In agreement with previous data (Sacchetti et al., 1999), long-term administration of reboxetine (10 mg/kg, i.p., once a day for 21 days) did not significantly affect the basal extracellular concentration of norepinephrine in the prefrontal cortex measured 48 h after the last injection, compared to the value for rats chronically treated with vehicle [26.58 ± 3.21 and 28.56 ± 4.05 fmol/40 μl sample ($n = 25$), respectively; $P = 0.62$; Fig. 2].

3.3. Effect of chronic administration of reboxetine on the sensitivity of cortical noradrenergic neurons to foot-shock stress

As expected (Dazzi et al., 2002a,b), exposure of the vehicle-treated rats 48 h after the last injection to foot-shock stress (0.2 mA for 500 ms, every second for 8 min) resulted in a marked increase (+200%) in the extracellular concentration of norepinephrine in the prefrontal cortex (Fig. 2). In contrast, such stress increased norepi-

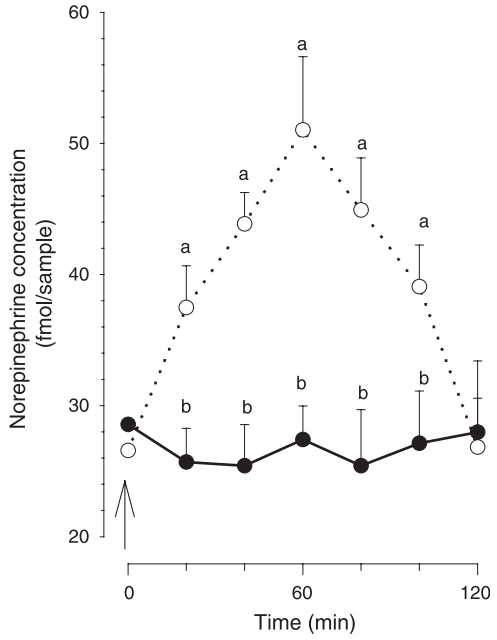

Fig. 3. Prevention of the FG 7142-induced increase in norepinephrine ouput in the prefrontal cortex of rats by long-term administration of reboxetine. Animals were subjected to a single injection of FG 7142 (20 mg/kg, i.p.) 48 h after (indicated as time 0) the last injection of long-term treatment with reboxetine (10 mg/kg, i.p., once a day for 21 days; closed circles) or vehicle (open circles). Data are means ± S.E.M. and are expressed as fmols/40 μl sample. [a]$P<0.01$ vs. basal values; [b]$P<0.01$ vs. vehicle-treated rats ($n=5$ per group).

nephrine output by only 115% in rats chronically treated with reboxetine. ANOVA revealed a significant main effect of treatment [$F(1,69)=8.368$, $P<0.05$] and a significant main effect of repeated measures [$F(6,69)=2.987$, $P<0.05$].

3.4. Effect of chronic administration of reboxetine on the FG 7142-induced increase in cortical norepinephrine output

Administration of a single dose of the anxiogenic drug FG 7142 to rats chronically treated with vehicle, 48 h after the last vehicle injection, induced an increase in cortical norepinephrine efflux that was maximal (+90%) at 60 min and persisted for ~ 100 min (Fig. 3). Long-term administration of reboxetine completely inhibited the effect of FG 7142 on cortical norepinephrine output. ANOVA revealed a significant main effect of treatment [$F(1,69)=42.358$, $P<0.0001$].

3.5. Effects of acute administration of reboxetine on the response of cortical noradrenergic neurons to foot shock or FG 7142

Acute administration of reboxetine (10 mg/kg, i.p.) to naïve rats 40 min before foot shock or FG 7142 injection potentiated the stimulatory effects of these latter treatments on cortical norepinephrine output. Thus, in rats injected with reboxetine, the increase in the extracellular norepinephrine concentration induced by foot shock (Fig. 4A) or by acute administration of FG 7142 (Fig. 4B) was significantly greater than that in control rats (the net increase being +120 vs. +70% for foot shock, and +110 vs. +90% for FG 7142) [$F(1,69)=8.752$, $P<0.001$].

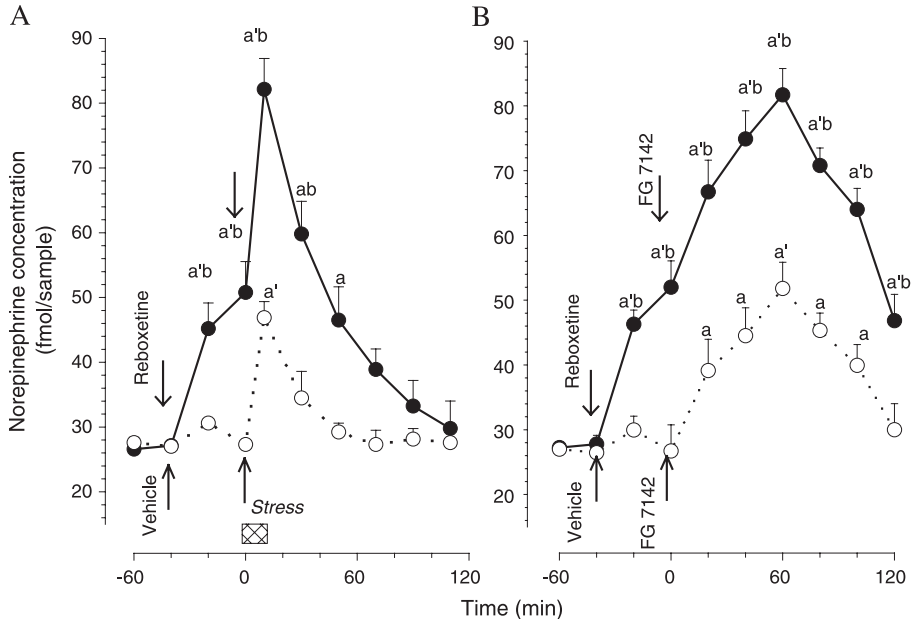

Fig. 4. Potentiation of the stimulatory effects of foot-shock stress (A) or of acute administration of FG 7142 (B) on norepinephrine output in the prefrontal cortex of rats by a single injection of reboxetine. Animals were injected with a single dose of reboxetine (10 mg/kg, i.p.) or vehicle 40 min before exposure to foot shock or administration of FG 7142 (20 mg/kg, i.p.). Data are means ± S.E.M. and are expressed as fmols/40 μl sample. [a]$P<0.05$, [a']$P<0.01$ vs. basal values; [b]$P<0.01$ vs. vehicle-treated rats ($n=5$ per group).

4. Discussion

Locus coeruleus noradrenergic neurons play an important role in modulating the behavioral response to stressful stimuli (Aston-Jones et al., 1991; Cole and Robbins, 1992; Robbins and Everitt, 1995; McQuade et al., 1999; Usher et al., 1999). Increases in norepinephrine output elicited by stress are required both for the focusing of attention on the stimulus and for an oriented response (Aston-Jones et al., 1991). Moreover, whereas acute exposure to stress increases the cortical output of noradrenergic neurons (Abercrombie et al., 1988; Rossetti et al., 1990; Gresch et al., 1994), experimental models have shown that chronic exposure to stressful stimuli reduces basal norepinephrine output and increases the sensitivity of noradrenergic neurons projecting to the cortex to an acute stress (Irwin et al., 1986; Adell et al., 1988; Nisenbaum et al., 1991; Gresch et al., 1994, 1995).

The increased sensitivity of noradrenergic neurons to stressful stimuli that is apparent after chronic exposure to stress might be responsible for the unrealistic or excessive anxiety as well as for the inappropriate behavioral reactions to stressful situations that are characteristic of anxiety disorders. Moreover, depression is thought to result from an inability to make the appropriate adaptive responses to stress or other aversive stimuli. Chronic stress has thus been proposed to play a causal role in several psychological conditions including depression, anxiety disorders and schizophrenia. Aston-Jones et al. (1999) suggested that the responsiveness of locus coeruleus neurons to environmental stimuli might be increased in individuals with posttraumatic stress disorder or with generalized anxiety disorder, and that the symptoms of these disorders might be ameliorated by drugs that reduce the discharge rate of these neurons.

Our present data show that long-term administration of reboxetine, an antidepressant drug that selectively inhibits the reuptake of norepinephrine, results in a reduction in the sensitivity of cortical noradrenergic neurons to stress. These results extend our previous findings that chronic treatment with antidepressant drugs that target both norepinephrine and serotonin systems (imipramine, venlafaxine, mirtazapine) inhibits the stress-induced increase in the extracellular concentration of norepinephrine in the rat prefrontal cortex (Dazzi et al., 2002a,b). Moreover, treatment with these drugs, like that with reboxetine, prevented the increase in cortical norepinephrine output elicited by the acute administration of FG 7142. Chronic treatment with reboxetine was also recently shown to induce a marked decrease (-88%) in the firing rate of locus coeruleus noradrenergic neurons, without significantly affecting the electrical activity of dorsal raphe serotonergic neurons, in anesthetized rats (Szabo and Blier, 2001). Both this previous study and our present data thus suggest that the anxiolytic and antidepressant efficacies of reboxetine might be associated with normalization of the sensitivity of norepinephrine neurons to stressful stimuli.

In contrast to our present results, Page and Lucki (2002) showed that the response to stress in rats chronically exposed to reboxetine (10 mg/kg/day, delivered by an osmotic minipump) was characterized by a net greater increase in norepinephrine output over a shorter time compared with that apparent in control animals. However, the percentage increase in norepinephrine ouput induced by stress was similar in rats chronically treated with reboxetine and in those treated with vehicle ($+33\%$ and $+45\%$, respectively). Differences in the basal norepinephrine concentration (almost sixfold higher in the reboxetine group), due to the presence of a high plasma concentration of the drug, complicate comparisons of the effect of stress between the two groups of rats in this previous study. In our experimental protocol, rats were exposed to stress 48 h after the last injection of the chronic drug treatment regimen, at which time the basal extracellular concentration of norepinephrine did not differ significantly between animals treated with reboxetine and those that received vehicle. Under these conditions, both the net increase and the percentage increase in the extracellular concentration of norepinephrine induced by stress or FG 7142 administration were markedly reduced after chronic reboxetine treatment. These effects of reboxetine are consistent with our previous observations that chronic treatment with imipramine, mirtazapine or venlafaxine reduces the response of noradrenergic neurons to stressful stimuli (Dazzi et al., 2002a,b).

Long-term exposure of rats to stressful stimuli results in extensive atrophy of apical dendrites of hippocampal pyramidal neurons (Woolley et al., 1990; Watanabe et al., 1992). Furthermore, chronic treatment of rats with corticosterone induces reorganization of apical dendrites in pyramidal neurons in the prefrontal cortex (Wellman, 2001). These plastic changes are also consistent with an increased sensitivity of locus coeruleus monoaminergic neurons projecting to the cortex and hippocampus to the effects of an acute stressful stimulus (Irwin et al., 1986; Adell et al., 1988; Nisenbaum et al., 1991; Gresch et al., 1994, 1995). Long-term administration of reboxetine reduces the basal electrical activity of noradrenergic neurons (Szabo and Blier, 2001), consistent with the reduced sensitivity of such neurons to stressful stimuli observed in rats chronically treated with this drug in the present study. Chronic treatment with other antidepressants has also been shown to affect the expression of genes for neurotrophic factors (Duman et al., 1999, 2001; Mallei et al., 2002). The reduction in the sensitivity of cortical noradrenergic neurons to the effects of stress or FG 7142 induced by long-term treatment with reboxetine or other antidepressants (Dazzi et al., 2002a,b) may thus result in an enhanced ability of the stressed individual to cope with environmental challenges that would otherwise provoke anxiety-related responses. Such an action would be consistent with clinical evidence that antidepressant drugs are effective anxiolytics (Davidson et al., 1999; Falkai, 1999; Goodnick et al., 1999; Silverstone and Ravindran, 1999; Thompson, 1999; Rickels et al., 2000). Moreover, the fact

that the antistress effect of antidepressant drugs is evident only after long-term treatment is also consistent with the notion that plastic adaptive changes in the properties of noradrenergic neurons (or of other neurons that might modulate noradrenergic function) elicited by such treatment are important for the anxiolytic action of these drugs.

Previous experimental evidence (Page and Abercrombie, 1997) and our preliminary data (Dazzi et al., 1999) suggest that chronic treatment with selective serotonin reuptake inhibitors, which in clinical practice exhibit anxiolytic efficacy (Nutt, 2000; Seedat et al., 2000; Kasper and Resinger, 2001; Rausch et al., 2001; Stein et al., 2001; Varia and Rauscher, 2002), does not modify the effect of stress on the cortical output of norepinephrine. Consistent with these data, we have recently shown that fluoxetine does not antagonize the activation of the hypothalamic–pituitary–adrenal axis induced by stress (Serra et al., 2002). In contrast, antidepressant drugs, such as mirtazapine, that affect both norepinephrine and serotonin systems manifest greater antistress efficacy and completely antagonize the stress-induced increase in both the plasma and brain concentrations of neuroactive steroids (Dazzi et al., 2001; Serra et al., 2002). These observations, together with the present data showing that reboxetine, a selective norepinephrine reuptake inhibitor, significantly reduced the sensitivity of cortical noradrenergic neurons to stressful stimuli, suggest that a selective action on cortical noradrenergic neurons may be crucial for the anxiolytic effect of this antidepressant drug. However, evidence indicating that antidepressant drugs that potentiate both norepinephrine- and serotonin-mediated transmission are more efficacious in antagonizing the effects of stress than are those that target only noradrenergic neurons suggests that serotonergic transmission might also play a role in this action. This conclusion is consistent with the clinical efficacy of selective serotonin reuptake inhibitors in various anxiety disorders (Nutt, 2000; Seedat et al., 2000; Kasper and Resinger, 2001; Rausch et al., 2001; Stein et al., 2001; Varia and Rauscher, 2002).

In conclusion, our data suggest that normalization of the activity of norepinephrine neurons projecting to the cortex, which contribute to the regulation of emotion and affective processes, appears to be important for the antistress action of reboxetine. Given that reboxetine shares this action with other antidepressants that affect both noradrenergic and serotonergic transmission, a common mechanism might underlie the time-dependent amelioration by these drugs of the symptoms of anxiety often associated with mood disorders (Falkai, 1999; Goodnick et al., 1999; Thompson, 1999).

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