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Reboxetine attenuates forced swim test-induced behavioural and neurochemical alterations in the rat

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Abstract

The forced swim test is a behavioural paradigm that is predicative of antidepressant activity in rodents. Until recently, research has focused on the ability of antidepressant drugs to decrease immobility in the forced swim test paradigm, but the neurochemical sequelae induced by swim stress, or the neurochemical basis of antidepressant-induced behavioural changes have received little attention. In this regard, we have recently demonstrated that forced swim test exposure increases serotonergic activity in the amygdala, frontal cortex and hippocampus and dopamine turnover in the striatum. In addition, forced swim test-exposure activates the hypothalamic pituitary adrenal axis. The purpose of the present study was to examine the effect of treatment with the selective noradrenaline reuptake inhibitor reboxetine (3, 10 and 30 mg/kg; i.p.) on immobility and defaecation scores in the forced swim test, and on forced swim test-induced neurochemical and hypothalamic pituitary adrenal axis changes in the rat. Reboxetine treatment (10 and 30 mg/kg) significantly decreased immobility and defaecation in the forced swim test in dose dependent manner. Furthermore, reboxetine produced a dose dependent attenuation of forced swim test-induced increases in serotonin turnover in the amygdala and frontal cortex and dopamine turnover in the striatum. Reboxetine (30 mg/kg) produced a modest, but non-significant, attenuation of forced swim test-induced increases in serum corticosterone concentrations. These data demonstrate that, in addition to the behavioural activity of reboxetine in the rat forced swim test paradigm, a dose-dependent attenuation of swim stress-induced increases in serotonergic and dopaminergic activity occurred in a region specific manner. These are the first data to demonstrate that treatment with the selective noradrenaline reuptake inhibitor, reboxetine can impact on the activity of other neurotransmitter systems in response to stress. Moreover, these data further demonstrate that this paradigm is a valuable tool in studying the effect of antidepressants, on both behaviour and swim stress-related alterations in central neurotransmitter function and hypothalamic pituitary adrenal axis activity in the rat.

Keywords: Antidepressant; Depression; Forced swim test; Noradrenaline; Reboxetine; Serotonin; Stress

1. Introduction

Given the clinical evidence associating stress with depression (Anisman and Zacharko, 1982; Bidzinska, 1984; Dinan, 1994; Holsboer and Barden, 1996), many of the preclinical models for assessing antidepressant activity have been based on abnormal behaviours precipitated by stress (Willner, 1990). One such paradigm is the forced swim test which was developed 20 years ago as a screening test for antidepressants in rodents (Porsolt et al., 1978). When rodents are exposed to the forced swim test, they typically display an immobile posture which is said to reflect a state of ''behavioural despair'' (Porsolt et al., 1978). Therefore, exposure to swim stress produces a change in behaviour which is thought to model a key symptom of the depressive state, namely that of despair or helplessness.

Until recently, research has focused on the ability of antidepressant drugs to increase escape motivated behaviour in the forced swim test paradigm, with little examination of the neurochemical consequences of swim stress or the neurochemical basis of antidepressant-induced behavioural changes that occur in this test. However, we have recently demonstrated that forced swim test exposure produces a number of region specific and time dependent neurochemical changes in the rat (Connor et al., 1997). The most consistent neurochemical changes observed following forced swim test-exposure are increased dopamin-

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ergic activity in the striatum and increased serotonergic activity in the frontal cortex and amygdala (Connor et al., 1997; Kelliher et al., 1998a,b). Such stress-related changes in central dopaminergic and serotonergic activity are consistent with previous findings following swim stress exposure (Jordan et al., 1994; Petty et al., 1997) and exposure to other stressors (Dunn, 1988; Inoue et al., 1993; Kawahara et al., 1993; Davis et al., 1994; Inoue et al., 1994; Ge et al., 1997).

There is substantial evidence to suggest that noradrenaline is involved in the aetiology of depression and in the mechanism of action of antidepressant drugs (Potter, 1996; Leonard, 1997; Redmond and Leonard, 1997). It has been shown that increasing synaptic noradrenaline concentrations by means of reuptake inhibitors such as desipramine, or by antagonism of presynaptic α_2-adrenoceptors using mianserin or mirtazapine, is effective in the treatment of depression (Potter, 1996; Nutt and Pinder, 1996). Reboxetine, (RS)-2-[(RS)-α-(2-ethoxyphenoxy) benzyl] morpholine sulphonate [FCE 20124], is a new selective noradrenaline reuptake inhibitor (Riva et al., 1989; Wong et al., 1997) shown to be active in pharmacological and biochemical models predicative of antidepressant activity (Melloni et al., 1984; Riva et al., 1989; Harkin et al., 1999). However, in contrast to tricyclic antidepressants such as desipramine, it lacks affinity for α-adrenergic, histaminergic and muscarinic cholinergic receptors (Riva et al., 1989; Wong et al., 1997), and therefore represents an effective and safe option for antidepressant therapy. In addition to its antidepressant activity, the selectivity of reboxetine for noradrenaline reuptake inhibition makes it a particularly useful psychopharmacological tool for investigating the role of noradrenaline in modulating other neurotransmitter systems.

The purpose of the present study was to examine the dose related effect of treatment with reboxetine (3, 10 and 30 mg/kg; i.p.) on immobility and defaecation in the forced swim test, and also on forced swim test-induced serotonergic alterations in the amygdala, frontal cortex and hippocampus and dopaminergic changes in the striatum. In addition, the effect of reboxetine treatment on forced swim test-induced hypothalamic pituitary adrenal axis activation was evaluated.

2. Materials and methods

2.1. Subjects and drug treatment

Male Sprague–Dawley rats (Harlan Olac, Bicester, UK) weighing approximately 250–300 g were used in this experiment. Rats were housed in groups of four and maintained on a 12 h:12 h light:dark cycle (lights on at 8 A.M.) in a temperature controlled room (22–24°C). Food and water were available ad libitum. Reboxetine methanesulphonate (Pharmacia and Upjohn, Kalamazoo, USA) was dissolved in 0.9% saline and was administered in an injection volume of 1 ml/kg (i.p.); 0.9% saline alone was administered as a vehicle. The doses of reboxetine used in the present study were based on previous studies conducted in our laboratory (Harkin et al., 1999).

At the beginning of the study, rats were assigned to one of eight groups.

Group 1: Control + Vehicle; Group 2: Control + Reboxetine (3 mg/kg); Group 3: Control + Reboxetine (10 mg/kg); Group 4: Control + Reboxetine (30 mg/kg); Group 5: FST + Vehicle; Group 6: FST + Reboxetine (3 mg/kg); Group 7: FST + Reboxetine (10 mg/kg); Group 8: FST + Reboxetine (30 mg/kg).

The experimental protocol was carried out under the guidelines of the Animal Welfare Committee, National University of Ireland, Galway, Ireland, and were in compliance with the European Communities council directive of 24th November 1986 (86/609/EEC).

2.2. Forced swim test procedure

This test was performed using the method described by Porsolt et al. (1978). On the first day of the experiment, the rats were placed individually into a container 40 cm high and 18 cm in diameter containing 20 cm of water at 25°C. The animals were left to swim in the water for 15 min before being removed, allowed to dry beside a heater and returned to their home cage. The animals received their first vehicle/antidepressant injection 15 min after the first forced swim test exposure. Control animals remained in their home cages and received their antidepressant/vehicle injections at an equivalent timepoint. The second and third vehicle/antidepressant injections were administered 5 h and 1 h prior to the second forced swim test exposure 24 h later. In the second forced swim test exposure rats were allowed to swim for a duration of 5 min and immobility times were recorded by two trained raters that were blind to the drug treatments. The defaecation rate was quantified as the number of faecal boli excreted during the 5 min testing session. The sequence of testing was randomized throughout the experiment so as to minimize any confounding effects of order of testing. The control groups (groups 1–4) did not receive either forced swim test exposure (15 min or 5 min). Animals which were exposed to the swim stress procedure were sacrificed 45 min following exit from the apparatus on day 2 of the test, whereas control animals were sacrificed at the same timepoint following antidepressant administration as the animals which were exposed to swim stress (1 h 50 min following the final injection). This timepoint was chosen as we have previously demonstrated that serotonin turnover in the frontal cortex and amygdala reaches a maximal increase 45 min following forced swim test exposure (Connor et al., 1997, 1998). In addition, stress-induced

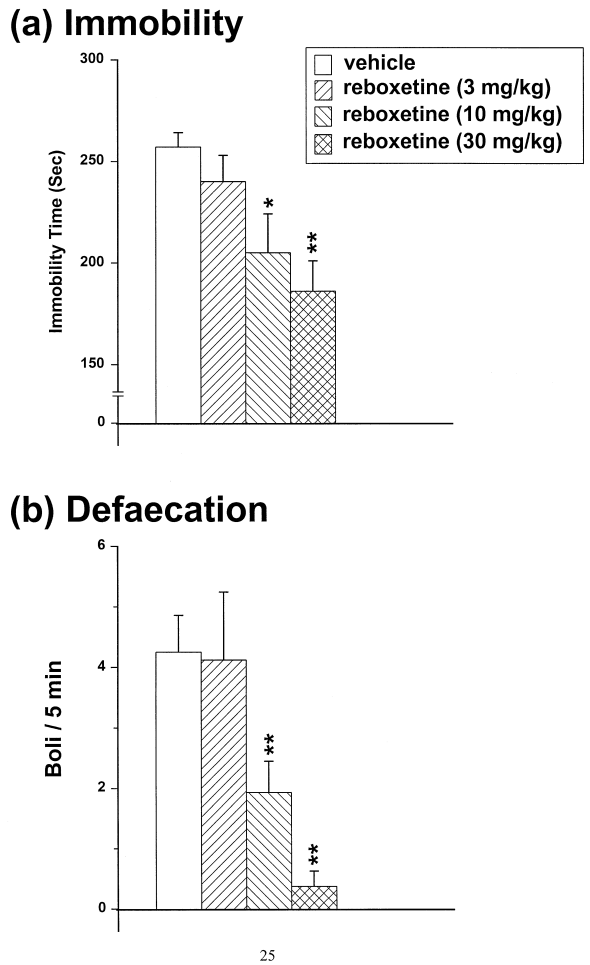

Fig. 1. Effect of subacute reboxetine treatment on (a) immobility time and (b) defaecation in the rat forced swim test. Data expressed as means with standard errors ($n = 7$–8). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle (Fisher's LSD multiple range test).

increases in circulating corticosterone concentrations could be measured at this timepoint in order to examine the possibility that reboxetine treatment could alter swim stress-induced activation of the hypothalamic pituitary adrenal axis.

2.3. Determination of neurotransmitter concentrations

The rats were sacrificed by decapitation. After sacrifice, the brain was rapidly removed and the left frontal cortex, amygdala, hippocampus and striatum were dissected on an ice-cold plate (Popov et al., 1967). Concentrations of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were measured by high performance liquid chromatography (HPLC) coupled with electrochemical detection (Seyfried et al., 1986). All brain regions were homogenized by sonication in 1 ml of mobile phase (pH 2.8) that was spiked with 2 ng/50 μl of *N*-methyl dopamine (Sigma, Poole, Dorset, UK) as an internal standard. The mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 0.1 mM EDTA (BDH Chemicals, Poole, Dorset, UK), 1.4 mM octane-1-sulphonic acid (Sigma, Poole, Dorset, UK) and 10% (v/v) methanol (Lab-Scan, Dublin, Ireland) and was adjusted to pH 2.8 using 4 N NaOH (BDH Chemicals, Poole, Dorset, UK). Homogenates were centrifuged at 12,000 rpm in a Hettich Mikro/K refrigerated centrifuge for 15 min. A 20 μl sample of the supernatant was injected directly onto a reverse phase column (LI Chrosorb RP-18, 25 cm × 4 mm internal diameter, particle size 5 μm) for separation of indoles and catecholamines (flow rate 1 ml/min). An electrochemical detector (Shimadzu) was coupled to the HPLC system and was set at a potential of +0.8 V for the detection of monoamine neurotransmitters and metabolites. Neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator and expressed as ng of neurotransmitter per g fresh weight of brain tissue. The ratio of 5-HIAA/5-HT was used as an index of serotonin turnover and the ratio of HVA/dopamine and DOPAC/dopamine were

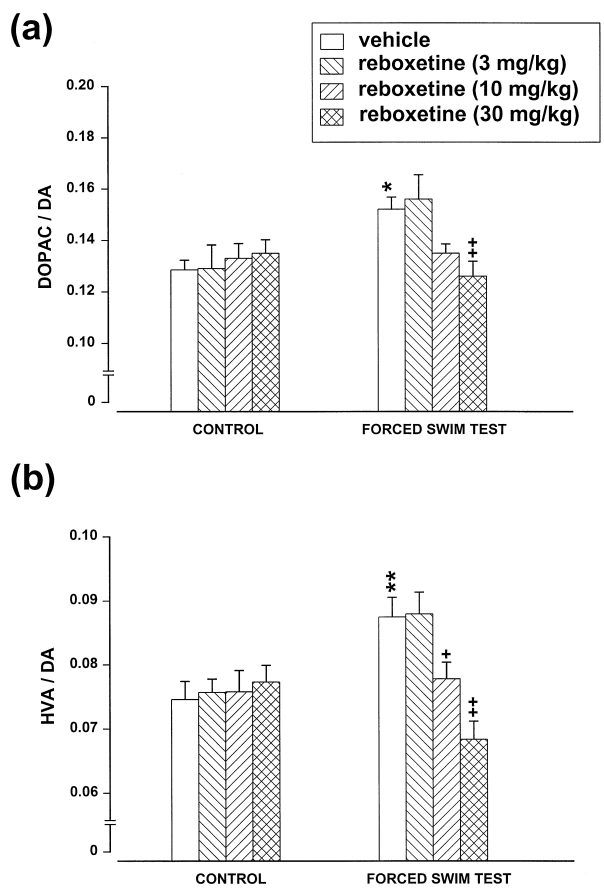

Fig. 2. Effect of subacute reboxetine treatment on forced swim test-induced increases in the (a) DOPAC/dopamine ratio and (b) HVA/dopamine ratio in the striatum. Data expressed as means with standard errors ($n = 7$–8). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control + vehicle, $+P < 0.05$, $++P < 0.01$ vs. forced swim test + vehicle (Fisher's LSD multiple range test).

Table 1
Effect of forced swim test exposure and subacute reboxetine treatment on dopamine, DOPAC and HVA concentrations in the striatum
Rats were sacrificed 45 min following forced swim test exposure. Dopamine, DOPAC and HVA concentrations are expressed as ng per g fresh weight of brain tissue.

Group	Dopamine	DOPAC	HVA
Control			
Vehicle	8951 ± 940	1147 ± 115	663 ± 68
Reboxetine (3)	9004 ± 494	1149 ± 85	684 ± 47
Reboxetine (10)	7757 ± 359	1037 ± 71	586 ± 37
Reboxetine (30)	8255 ± 596	1109 ± 67	632 ± 35
Forced swim test			
Vehicle	9164 ± 617	1395 ± 97	780 ± 55
Reboxetine (3)	8749 ± 303	1387 ± 127	772 ± 43
Reboxetine (10)	7950 ± 431	1079 ± 71[a]	618 ± 39[b]
Reboxetine (30)	9029 ± 405	1136 ± 66[a]	614 ± 29[b]
Swim test effect			
f value	0.37	4.81	3.50
p value	0.543	0.032	0.066
df	1, 55	1, 55	1, 55
Drug effect			
f value	1.96	2.85	4.63
p value	0.130	0.045	0.058
df	3, 55	3, 55	3, 55
Interaction			
f value	0.31	0.90	1.10
p value	0.817	0.442	0.351
df	3, 55	3, 55	3, 55

Data expressed as means ± S.E.M. ($n = 7–8$).
[a] $P < 0.05$; [b] $P < 0.01$ vs. forced swim test + vehicle (Fisher's LSD multiple range test).

used as indices of dopamine turnover (Dunn, 1988; Davis et al., 1994; Connor et al., 1997).

2.4. Serum corticosterone concentrations

After sacrifice a trunk blood sample was collected and allowed to clot at room temperature. The blood was centrifuged at $800 \times g$ for 10 min, the supernatant removed and stored at −20°C until analysis was performed. Serum corticosterone concentrations were measured using a fluorometric assay as described previously (Grealy and O'Donnell, 1991). A corticosterone stock (Sigma, Poole, Dorset, UK) solution (100 μg/dl) was prepared and diluted to produce a range of concentrations (10–80 μg/dl). Serum samples and corticosterone standards were then mixed in 600 μl of dichloromethane for 15 s. 500 μl of the resulting dichloromethane (Lab Scan, Dublin, Ireland) extract phase was then transferred into a tube containing 400 μl of concentrated sulphuric acid: absolute ethanol (65:35) and the tubes were thoroughly mixed for 15 s. Samples were then placed in the dark for 45 min, a 300-μl aliquot of the lower phase was removed and the fluorescence measured at excitation 474 nm and emission 518 nm with a Perkin Elmer LS-5 spectrophotofluorimeter. The results were expressed as μg corticosterone per dl of serum.

2.5. Statistical analysis of data

The FST immobility time and defaecation data was analysed using a one-way analysis of variance and the biochemical data was analysed by a two-way analysis of

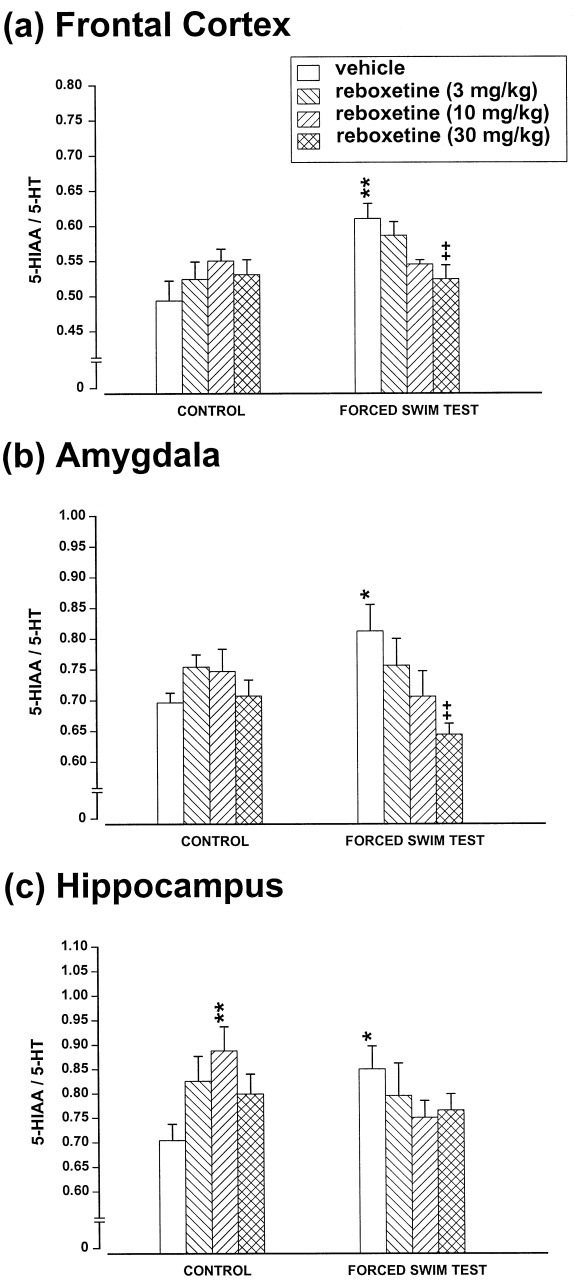

Fig. 3. Effect of subacute reboxetine treatment on forced swim test-induced increases in the 5-HIAA/5-HT ratio in the (a) frontal cortex, (b) amygdala and (c) hippocampus. Data expressed as means with standard errors ($n = 7–8$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control + vehicle, $++ P < 0.01$ vs. forced swim test + vehicle (Fisher's LSD multiple range test).

variance. If any statistically significant change was found, post-hoc comparisons were performed using the Fishers LSD multiple range test. Data was deemed significant when $P < 0.05$. Results are expressed as group mean ± S.E.M.

3. Results

3.1. Behaviour in the forced swim test

There was a significant effect of reboxetine treatment on immobility time in the forced swim test [$F(3,27) = 4.93$, $P < 0.01$]. Post hoc analysis revealed that reboxetine produced a dose dependent decrease in immobility time in the forced swim test. Both 10 mg/kg ($P < 0.05$) and 30 mg/kg ($P < 0.01$) significantly decreased forced swim test-related immobility, while 3 mg/kg was without effect on this parameter (Fig. 1a). In addition, there was a significant effect of reboxetine treatment on defaecation during the swim test session [$F(3,27) = 6.72$, $P < 0.01$]. Both 10 mg/kg and 30 mg/kg ($P < 0.01$) significantly decreased forced swim test-related defaecation, while 3 mg/kg was without effect on this parameter (Fig. 1b).

3.2. Dopamine turnover in the striatum

There was a significant forced swim test x drug interaction on the striatal DOPAC/DA ratio; [$F(3,55) = 3.85$, $P < 0.05$] and HVA/DA ratio; [$F(3,55) = 6.31$, $P < 0.001$]. Post-hoc analysis revealed that forced swim test exposure produced a significant increase in both the DOPAC/DA ($P < 0.05$) and HVA/DA ($P < 0.01$) ratios in the striatum. Reboxetine treatment (10 and 30 mg/kg) attenuated these forced swim test-induced increases in the striatal HVA/DA ratio ($P < 0.05$-0.01), while 30 mg/kg alone significantly reduced the swim stress-induced increase in the DOPAC/DA ratio ($P < 0.01$) (Fig. 2). These changes observed in striatal dopamine turnover were predominantly due to alterations in the concentrations of the dopamine metabolites, DOPAC and HVA as opposed to any net change in the concentrations of the parent amine (Table 1).

Table 2
Effect of forced swim test exposure and subacute reboxetine treatment on 5-HT and 5-HIAA concentrations in the frontal cortex, amygdala and hippocampus
Rats were sacrificed 45 min following forced swim test exposure. 5-HT and 5-HIAA concentrations are expressed as ng per g fresh weight of brain tissue. Data expressed as means ± S.E.M. ($n = 7$–8).

Group	Frontal cortex		Amygdala		Hippocampus	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Control						
Vehicle	545 ± 21	267 ± 15	926 ± 35	644 ± 24	598 ± 34	418 ± 22
Reboxetine (3)	540 ± 14	282 ± 12	857 ± 26	637 ± 19	552 ± 21	457 ± 37
Reboxetine (10)	532 ± 13	291 ± 6	830 ± 30	613 ± 7	565 ± 29	502 ± 43
Reboxetine (30)	539 ± 19	284 ± 8	817 ± 30[a]	573 ± 11	563 ± 24	447 ± 22
Forced swim test						
Vehicle	544 ± 19	330 ± 13[b]	788 ± 60[b]	639 ± 53	568 ± 25	479 ± 24
Reboxetine (3)	614 ± 18[d]	359 ± 13	943 ± 22[d]	709 ± 33	678 ± 34[c]	528 ± 33
Reboxetine (10)	603 ± 15[c]	329 ± 5	913 ± 35[c]	644 ± 40	697 ± 37[d]	526 ± 43
Reboxetine (30)	602 ± 20[c]	316 ± 16	901 ± 24[c]	580 ± 20	667 ± 27[c]	506 ± 20
Swim test effect						
f value	17.13	39.62	1.42	1.63	15.81	5.77
p value	0.0001	0.0001	0.2377	0.2067	0.0002	0.0197
df	1, 55	1, 55	1, 52	1, 52	1, 54	1, 54
Drug effect						
f value	1.25	1.49	0.69	3.92	0.92	1.50
p value	0.2999	0.2251	0.5614	0.0135	0.4342	0.2245
df	3, 55	3, 55	3, 52	3, 52	3, 54	3, 54
Interaction						
f value	1.98	1.64	5.40	0.70	3.38	0.21
p value	0.1274	0.1904	0.0026	0.5521	0.0245	0.8873
df	3, 55	3, 55	3, 52	3, 52	3, 54	3, 54

[a] $P < 0.05$; [b] $P < 0.01$ vs. control + vehicle.
[c] $P < 0.05$; [d] $P < 0.01$ vs. forced swim test + vehicle (Fisher's LSD multiple range test).

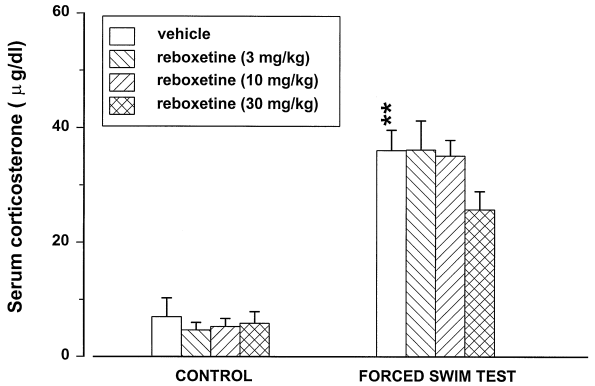

Fig. 4. Effect of subacute reboxetine treatment on forced swim test-induced increases in corticosterone. Data expressed as means with standard errors ($n = 7–8$). $^{**}P < 0.01$ vs. control + vehicle (Fisher's LSD multiple range test).

3.3. Serotonin turnover in the frontal cortex, amydala and hippocampus

There was a significant forced swim test × drug interaction on the 5-HIAA/5-HT ratio in the frontal cortex [$F(3,55) = 3.58$, $P < 0.05$], amygdala [$F(3,52) = 2.87$, $P < 0.05$] and hippocampus [$F(3,54) = 3.06$, $P < 0.05$]. Post-hoc analysis revealed that forced swim test exposure produced a significant increase in the 5-HIAA/5-HT ratio in the frontal cortex ($P < 0.01$), amygdala and hippocampus ($P < 0.05$). Reboxetine treatment (30 mg/kg) attenuated these forced swim test-induced increases in the 5-HIAA/5-HT ratio in the frontal cortex and amygdala ($P < 0.01$), but failed to significantly alter swim stress-related increases in the 5-HIAA/5-HT ratio in the hippocampus (Fig. 3). These swim stress related changes in serotonin turnover were due to a combination of changes in both 5-HT and 5-HIAA (Table 2) as previously observed (Connor et al., 1997).

3.4. Serum corticosterone concentrations

There was a significant effect of forced swim test exposure on serum corticosterone concentrations; [$F(1,52) = 148.86$, $P < 0.0001$]. However, the effect of drug treatment did not reach statistical significance; [$F(3,52) = 1.25$, $P = 0.29$]. Post-hoc analysis revealed that FST exposure produced a significant ($P < 0.01$) increase in corticosterone 45 min post exposure and treatment with reboxetine 30 mg/kg produced a modest, but non-significant, attenuation of this response (Fig. 4).

4. Discussion

In the present study, we observed that reboxetine produced a dose-dependent attenuation of immobility and defaecation in the rat forced swim test paradigm. These data are in accordance with previous reports from our laboratory where it was observed that reboxetine displayed anti-immobility effects in the rat forced swim test (Harkin et al., 1999). The dose-dependent attenuation of defaecation produced by reboxetine pretreatment in the forced swim test may indicate that reboxetine reduces emotional reactivity in response to stressor exposure. It is noteworthy that a similar inhibition of defaecation during swim stress exposure is seen in rats pretreated with other noradrenaline reuptake inhibitors such as desipramine (Unpublished data) or talsupram (Kelliher et al., 1998b). However, it is also possible that the ability of noradrenaline reuptake inhibitors to antagonise stress-induced defaecation in the forced swim test may be simply due to increased noradrenaline availability in sympathetic nerve terminals in the gastrointestinal tract.

The data presented in this study also demonstrate that reboxetine treatment produced a dose-dependent attenuation of swim stress-induced increases in 5-HT turnover in the amygdala and frontal cortex, two brain regions which we previously found to be particularly sensitive to swim stress exposure (Connor et al., 1997, 1998; Kelliher et al., 1998a). Reboxetine treatment also attenuated swim stress-related increases in 5-HT turnover in the hippocampus, but this effect did not reach statistical significance. Subacute treatment with other noradrenaline reuptake inhibitors such as desipramine (Unpublished data) and talsupram (Kelliher et al., 1998b) display behavioural activity in the forced swim test and also block forced swim test-induced increases in serotonin turnover in the amygdala and frontal cortex. In addition, chronic treatment with desipramine attenuates forced swim test-induced increases in 5-HT turnover in both the amygdala and frontal cortex (Connor et al., 1998). Thus, the ability to attenuate forced swim test-induced increases in cortical and amygdaloid serotonergic activity may be a property shared by a number of noradrenaline reuptake inhibitors.

In the present study, reboxetine provoked a robust ''stress like'' increase in basal 5-HT turnover in the hippocampus. This increase in hippocampal 5-HT turnover may account for the inability of reboxetine to significantly attenuate the forced swim test-induced increase in 5-HT turnover in this brain region.

The amygdala has been implicated as a key site of antidepressant action in the forced swim test, inasmuch as direct injection of imipramine and pargyline into particular amygdaloid nuclei, but not other brain structures produces behavioural responses similar to i.p. injections of these drugs (Duncan et al., 1986). Although reboxetine produces a dose-dependent decrease in immobility in the forced swim test and also dose-dependently attenuates the forced swim test-induced increases in amygdaloid 5-HT turnover, it is unlikely that this effect of reboxetine on the serotonergic system mediates its behavioural effects in the forced swim test paradigm. This view is supported by the observation that a serotonergic amygdaloid lesion produced using 5,7-dihydroxytryptamine failed to alter the anti-im-

mobility effect of desipramine in the forced swim test, whereas a 6-hydroxydopamine lesion in this region impaired the ability of desipramine to reduce immobility (Araki et al., 1985). In addition, serotonin reuptake inhibitors are largely inactive in the traditional forced swim test paradigm, at least at therapeutically relevant doses (Araki et al., 1985; see Borsini, 1995; Connor et al., 1998; Kelliher et al., 1998b). However, recent studies using a modified forced swim test paradigm with a novel behavioural scoring system have indicated that selective serotonin reuptake inhibitors display behavioural activity in the test (Detke et al., 1995, 1997). The anti-immobility effect of reboxetine may be more closely related to its ability to antagonise the stressor-induced increase in dopamine turnover in the striatum, in that it was previously suggested that mesocorticolimbic dopaminergic activity may play a role in the behaviour of rats in the forced swim test (Willner, 1995).

With regard to the ability of noradrenaline reuptake inhibition to influence serotonergic responses to stress, or indeed under basal conditions, it has been reported that noradrenaline can modulate serotonergic activity by different mechanisms (see Stahl, 1997). For instance, when 5-HT neurons receive excitatory noradrenergic input at the raphe nuclei cell bodies, activation of α_1-adrenoceptors on 5-HT neurons leads to 5-HT release from the nerve terminal (Baraban and Aghajanian, 1980a,b; Hertel et al., 1998). Thus, it is reasonable to suggest that the reduced locus coeruleus firing which occurs following acute reboxetine treatment (Erik Wong, personal communication) would result in reduced noradrenaline release at serotonergic cell bodies, thus having an inhibitory effect on 5-HT release. Such a mechanism may have contributed to the attenuation of stress-related increases in serotonin turnover following reboxetine treatment observed in the present study. Conversely, 5-HT neurons also receive inhibitory noradrenergic inputs at the axon terminal, mediated by presynaptic α_2-heteroceptors on 5-HT neurons (Galzin et al., 1984; Trendelenburg et al., 1994). Thus, it is possible that the reduced locus coeruleus firing which occurs following acute reboxetine treatment (Erik Wong, personal communication) would relieve the inhibitory effect of nerve terminal α_2-heteroreceptor activation by noradrenaline on 5-HT release. Therefore, such a mechanism may have contributed to the basal increase in 5-HT turnover in the hippocampus which was provoked by reboxetine. However, further studies are required in order to elucidate the definite mechanisms by which reboxetine alters serotonergic activity in different brain regions. The selectivity of reboxetine for noradrenaline reuptake inhibition makes it a particularly useful tool for investigating the role of noradrenaline in modulating other neurotransmitters systems.

The realisation that the noradrenaline reuptake inhibitor reboxetine, alters 5-HT function is of interest, as both noradrenaline and 5-HT are implicated in the pathophysiology of depressive disorders. In fact several reports indicate that a treatment combination of a noradrenergic tricyclic antidepressant and a selective serotonin reuptake inhibitor can lead to both a more rapid onset of antidepressant action, and also can have increased efficacy compared to treatment with either agent alone (see Nelson, 1998). Thus, it has been suggested that antidepressants which act concurrently on the noradrenergic and serotonergic systems (e.g., milnacipran and venlafaxine) may be more efficacious, or display a more rapid onset of antidepressant activity, than agents which act on either system alone (Schweizer et al., 1991; Guelfi et al., 1995; Benkert et al., 1996; Lopez-Ibor et al., 1996). Although reboxetine has no direct effect on the serotonergic uptake mechanism (Wong et al., 1997), these data demonstrate that by an indirect mechanism(s) reboxetine can influence serotonergic activity within the CNS both under basal conditions and in response to stress.

In addition to the ability of reboxetine to modulate the serotonergic response to stress, an interaction was observed between reboxetine treatment and stress-related increases in striatal dopaminergic activity, in that noradrenaline reuptake inhibition by reboxetine antagonised stressor-induced increases in dopaminergic activity in the striatum. This is a property which is shared with the tricyclic antidepressant desipramine, which is also a noradrenaline reuptake inhibitor (Unpublished data). Regarding the mechanism by which noradrenaline reuptake inhibitors can affect striatal dopaminergic activity, it is well established that ventral tegmental area dopamine neurons which project to the striatum receive noradrenergic inputs from the locus coeruleus (see Roth et al., 1987). Also, it was previously shown that α_1-adrenoceptor antagonism attenuates the burst firing in ventral tegmental area dopamine neurons without affecting their basal firing rate (Grenhoff and Svensson, 1993). Moreover, α_1-adrenoceptor antagonism markedly attenuates MK-801-induced dopamine release at the nucleus accumbens without altering basal dopamine release in this brain region (Mathe et al., 1996). Thus, blockade of α_1-adrenoceptors which are located on the cell bodies of ventral tegmental area dopamine neurons (see Grenhoff and Svensson, 1993) has a suppressive effect on stimulated dopamine release. It is well established that acute administration of tricyclic antidepressants such as desipramine inhibit locus coeruleus firing by increasing the local concentration of noradrenaline in the vicinity of the somatodendritic α_2-autoreceptors (Valentino et al., 1991; Curet et al., 1992; see Svensson et al., 1998). Similarly, reboxetine is a potent inhibitor of locus coeruleus firing (Erik Wong, personal communication) and would therefore decrease noradrenaline release in the vicinity of α_1-adrenoceptors located on ventral tegmental area dopamine cell bodies. Such a mechanism could account for the ability of an noradrenaline reuptake inhibitor such as reboxetine to attenuate the stressor-induced increase in striatal dopaminergic activity which was observed in the present study.

The noradrenergic input from the locus coeruleus to the hypothalamic paraventricular nucleus stimulates the release of corticotropin releasing factor, thereby activating the hypothalamic pituitary adrenal axis (Alonso et al., 1986; see Delbende et al., 1992). As previously outlined, reboxetine inhibits locus coeruleus firing, thus it is not unreasonable to suggest that reboxetine would attenuate the hypothalamic pituitary adrenal axis activation in response to an acute stressor. The results of the present study demonstrate that at the higher dose of reboxetine (30 mg/kg) there was a modest impairment of forced swim test-induced corticosterone secretion. This may indicate that hypothalamic pituitary adrenal axis activation in response to swim stress was blunted by prior administration of reboxetine. However, as many other neurotransmitters and neuropeptides play a role in the activation of the hypothalamic pituitary adrenal axis (see Delbende et al., 1992), it is unlikely that an agent that selectively modulates the noradrenergic system would totally block stressor-induced corticosterone secretion.

In conclusion, these data demonstrate that in addition to the behavioural activity of reboxetine in the rat forced swim test, pretreatment with this noradrenaline reuptake inhibitor attenuates swim stress-induced increases in serotonergic and dopaminergic activity in a region specific manner. These are the first data to demonstrate that treatment with the selective noradrenaline reuptake inhibitor reboxetine, can reduce the activity of the serotonergic and dopaminergic systems in response to stress. Moreover, these data further demonstrate that this paradigm is a useful way to study the effect of antidepressants on both behaviour, and swim stress-related alterations in central neurotransmitter function and hypothalamic pituitary adrenal axis activity in the rat.

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