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Effect of Subchronic Antidepressant Treatments on Behavioral, Neurochemical, and Endocrine Changes in the Forced-Swim Test

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CONNOR, T. J., P. KELLIHER, Y. SHEN, A. HARKIN, J. P. KELLY AND B. E. LEONARD. *Effect of subchronic antidepressant treatments on behavioral, neurochemical, and endocrine changes in the forced-swim test.* PHARMACOL BIOCHEM BEHAV **65**(4) 591–597, 2000.—The purpose of the present study was to examine the effect of subchronic treatment (24 days) with antidepressants displaying differential effects on noradrenaline and serotonin reuptake, on behavior, neurochemistry, and hypothalamic–pituitary–adrenal (HPA) axis activity following FST exposure in the rat. Desipramine (7.5 mg/kg, IP) significantly decreased immobility in the FST, whilst paroxetine (7.5 mg/kg IP) and venlafaxine (10 mg/kg, IP) were without effect. Nonetheless, treatment with all three antidepressants significantly attenuated stress-related increases in amygdaloid and cortical serotonin turnover. Of the three antidepressants examined, only desipramine attenuated the stress-associated elevation in serum corticosterone. In conclusion, although FST-induced increases in serotonin turnover in the frontal cortex and amygdala were attenuated following treatment with all three antidepressants, FST-induced behavioral changes and increased HPA axis activity were normalized only following desipramine treatment. In addition, these results suggest that neurochemical mechanisms independent of increased serotonergic activity subserve the normalization of behavior and HPA axis responses in the FST. These data also add to our understanding of the interactions between antidepressants and stress-induced behavioral, neurochemical, and endocrine alterations, and illustrates important differences between classes of antidepressants. © 2000 Elsevier Science Inc.

Amygdala Antidepressant Depression Forced-swim test HPA axis Noradrenaline Serotonin Stress

GIVEN the clinical evidence associating stress with depression, many of the preclinical models for assessing antidepressant activity have been based on abnormal behaviors precipitated by stress (56). One such paradigm is the forced-swim test (FST), which was developed 20 years ago as a screening test for antidepressants in rodents (46). When rodents are exposed to the FST, they typically display an immobile posture, which is said to reflect a state of "behavioral despair" on the assumption that the animals have given up hope of escaping (46). Therefore, exposure to swim stress produces a change in behavior that is though to model a key symptom of the depressive state, namely that of despair or helplessness. It is well established that either subacute (three doses within the 24-h period prior to behavioral scoring) or subchronic antidepressant treatment reduces immobility time in the FST (7). While the ability of antidepressants that possess noradrenaline reuptake inhibiting activity to decrease immobility in the rat FST following subacute treatment is well established (12,15, 23,27), data concerning the effectiveness of selective serotonin reuptake inhibitors (SSRIs) in this test has proved more

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controversial (6). However, it has been suggested that SSRIs may be behaviorally active in the rat FST following subchronic treatment (18,40).

Until recently, research has focused on the ability of antidepressant drugs to decrease immobility in the FST paradigm, with little examination of the neurochemical consequences of swim stress or the neurochemical basis of antidepressant-induced behavioral changes in this test. However, we have recently demonstrated that FST exposure produces a number of region specific and time-dependent neurochemical changes in the rat (14). One of the most consistent neurochemical changes observed following FST exposure is an increase in cortical and amygdaloid 5-HT turnover (14,33,34). Such stress-related increases in cortical and amygdaloid serotonergic activity are consistent with previous findings following swim-stress exposure (31) and exposure to other stressors (25,29,30,32,44). Although many antidepressants with differing neurochemical specificities attenuate FST-induced immobility, very little is known about the effects of such drugs on FST-induced neurochemical alterations.

It would appear that, to date, only one study has examined the effect of antidepressant treatment on swim stress-induced changes in central serotonergic activity in the rat (36).

As the FST is a potent activator of the HPA axis (14,19), the paradigm provides an ideal opportunity to evaluate the effect of antidepressant treatments on stressor-induced HPA axis activation. While it has been reported that both tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) attenuate stress-related increases in HPA axis activity (51,52), less attention has been given to the effect of SSRIs on stressor-induced HPA axis activation.

Desipramine is a well-established TCA that has been used clinically for many years (38). The main neurochemical action of desipramine is to inhibit reuptake of noradrenaline into the presynaptic neuron, thereby elevating synaptic concentrations of this neurotransmitter (4). Previous studies have reported that desipramine is active in the FST after both subacute (12,15,23) and subchronic (20) administration. Paroxetine is an SSRI, and selectively inhibits the reuptake of serotonin (38). While SSRIs fail to alter immobility in the rat FST following subacute treatment, it has been suggested that they may be more effective in the FST following subchronic administration (18,40). Venlafaxine is a novel compound that has recently been developed as an antidepressant. Both in vitro (5) and in vivo (3), venlafaxine blocks the reuptake of serotonin and noradrenaline (serotonin noradrenaline reuptake inhibitor; SNRI) and unlike TCAs, has low affinity for muscarinic, histaminergic, and α-adrenergic receptors. Consequently, it does not display any of the adverse effects seen with TCAs (1), and possesses a similar side-effect profile to that of SSRIs (43). In addition, it has been reported that venlafaxine displays antidepressant activity in a number of preclinical tests, including both the mouse (39,49) and rat (50) FST, the olfactory bulbectomized rat model of depression (41,48), and the resident–intruder social interaction paradigm (42).

The objectives of the present investigation were to examine the effect of subchronic treatment (24 days) with the antidepressants desipramine, paroxetine, and venlafaxine on both immobility and stress-induced increases in amygdaloid and cortical serotonin turnover in the rat FST paradigm. In addition, serum corticosterone concentrations were measured following FST exposure to evaluate the effect of the different antidepressant treatments on stress-related increases in HPA axis activity.

METHOD

Subjects and Drug Treatment

Male Sprague–Dawley rats (obtained from an in house breeding program), weighing 300–350 g, were used in this experiment. Rats were individually housed and maintained on a 12:12-h light:dark cycle (lights on at 0800 h) in a temperature-controlled room (20–22°C). Food and water were available ad lib. Animals were treated once daily for 24 days with either desipramine HCl (7.5 mg/kg, IP; Sigma Chemical Co., Poole, Dorset, UK), paroxetine HCl (7.5 mg/kg, IP; SmithKline Beecham, Harlow, UK), or venlafaxine HCl (10 mg/kg, IP; Wyeth-Ayerst laboratories, Taplow, UK). All drugs were dissolved in a vehicle that consisted of 0.9% (w/v) NaCl containing 0.25% (v/v) Tween 80, and were administered in an injection volume of 1 ml/kg. Controls received vehicle alone. The doses selected in the present study previously demonstrated activity following repeated administration in various animal models of depression (15,16,41,55). Rats were assigned to one of eight groups (n = 6–7 per group): group 1: control + vehicle; group 2: control + desipramine; group 3: control + paroxetine; group 4: control + venlafaxine; group 5: FST + vehicle; group 6: FST + desipramine; group 7: FST + paroxetine; group 8: FST + venlafaxine.

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 24 November 1986 (86/609/EEC).

Forced-Swim Test Procedure

This test was performed using the original method described by Porsolt et al. (46). On the first day of the swim stress procedure (day 23 of drug treatment) 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 a vehicle/antidepressant injection 15 min after the first FST exposure. Control animals remained in their home cages at all times, and received their antidepressant/vehicle injections at an equivalent timepoint. The final two vehicle/antidepressant injections were administered 5 h and 1 h prior to the second FST exposure 24 h later. In the second FST exposure rats were allowed to swim for a duration of 5 min, and immobility times were recorded by observers that were blind to the drug treatments. The sequence of testing was randomized throughout the experiment so as to minimize any confounding effects of the order of testing. The control groups (groups 1–4) did not receive FST exposure (15 min or 5 min). Animals that were exposed to the swim-stress procedure were sacrificed 45 min after exposure on day 2 of the test, whereas control animals were sacrificed at the equivalent time point following antidepressant administration 1 h 50 min following the final injection). This time point was chosen, as we have previously demonstrated that serotonin turnover in the frontal cortex and amygdala are maximally increased 30–60 min following FST exposure (14). In addition, this time point is ideal for measuring stress-induced increases in circulating corticosterone concentrations to examine the effect of antidepressant treatment on FST-induced activation of the HPA axis.

Determination of Brain Biogenic Amine Concentrations

The rats were sacrificed by decapitation. After sacrifice, the brain was rapidly removed, and the left frontal cortex and amygdaloid cortex were dissected on an ice-cold plate (45). Concentrations of serotonin and its metabolite 5-HIAA were measured by high-performance liquid chromatography (HPLC) coupled with electrochemical detection (54), and the ratio of 5-HIAA/5-HT was used as an index of serotonin turnover (14,22). Both brain regions were homogenized by sonication in 1.0 ml of the mobile phase (pH 2.8) that was spiked with 20 ng/50 μl of *N*-methyl dopamine (Sigma Chemical Co., 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, Ltd., Poole, Dorset, UK), 1.4 mM octane-1-sulphonic acid (Sigma Chemical Co.), and 10% (v/v) methanol (Lab-Scan, Dublin, Ireland). The mobile phase was adjusted to pH 2.8 using 4 N NaOH (BDH Chemicals Ltd.). 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 into a reverse-phase column (LI Chrosorb RP-18, 25 cm × 4 mm internal diameter, particle size 5 μm) for separation of 5-HIAA and 5-HT (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 serotonin and 5-HIAA. The neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator, and concentrations were expressed as ng of neurotransmitter per g fresh weight of brain tissue.

Serum Corticosterone Concentrations

After sacrifice, a trunk blood sample was collected and allowed to clot at room temperature. The blood was centrifuged at 800 × *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 (26). A corticosterone stock (Sigma Chemical Co.,) 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 (Lab Scan, Dublin, Ireland) for 15 s. Five hundred microliters of the resulting dichloromethane extract phase was then transferred into a tube containing 400 μl of concentrated sulphuric acid:absolute ethanol (65:35) and the tubes vortexed for 15 s. Samples were then placed in the dark for 45 min and a 300-μl aliquot of the lower phase was removed and the fluoresence measured at excitation 474 nm and emission 518 nm (Perkin-Elmer LS-5 spectrophotofluorimeter). The results were expressed as μg corticosterone per dl of serum.

Statistical Analysis of Data

FST immobility time data was initially analyzed using a one-way analysis of variance. The biochemical data was analyzed by a two-way analysis of variance. If any statistically significant change was found, post hoc comparisons were performed using Fishers LSD multiple-range test. Data was deemed significant when $p < 0.05$. Results are expressed as group mean with standard error of the mean.

RESULTS

Behavior in the Forced-Swim Test

There was a significant effect of antidepressant treatment on immobility time in the FST, $F(3, 24) = 4.64$, $p = 0.01$]. Post hoc analysis revealed that desipramine produced a significant ($p < 0.01$) reduction in immobility time in the FST, whereas paroxetine and venlafaxine were without effect (Fig. 1).

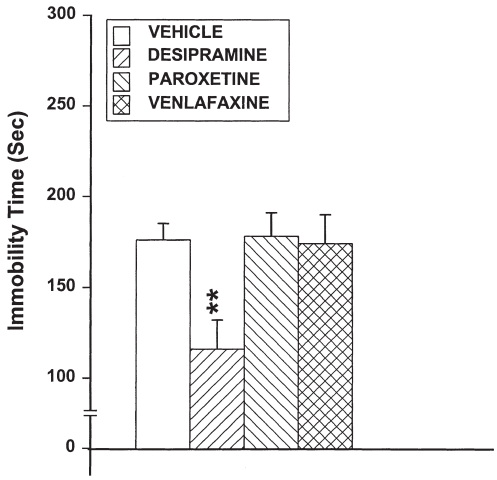

FIG. 1. Effect of subchronic treatment with desipramine, paroxetine, and venlafaxine on immobility in the rat forced-swim test. Data expressed as means with standard errors ($n = 7$). $^{**}p < 0.01$ vs. vehicle (Fishers LSD multiple range test).

Serotonin Turnover in the Frontal Cortex and Amygdaloid Cortex

There was a significant effect of FST on the 5-HIAA/5-HT ratio in the frontal cortex, $F(1, 45) = 8.90$, $p < 0.01$, and amygdaloid cortex, $F(1, 45) = 5.13$, $p < 0.05$. There was also a significant effect of drug treatment [frontal cortex: $F(3, 45) = 39.50$, $p < 0.0001$; amygdaloid cortex: $F(3, 45) = 51.76$, $p < 0.0001$] and a significant FST × drug treatment interaction [frontal cortex: $F(3, 45) = 6.18$, $p < 0.01$; amygdaloid cortex: $F(3, 45) = 5.05$, $p < 0.01$]. Post hoc analysis revealed that FST exposure produced a significant ($p < 0.01$) increase in the 5-HIAA/5-HT ratio in both the frontal cortex and amygdala. Subchronic treatment with all three antidepressants attenuated ($p < 0.01$) this FST-induced increase in the 5-HIAA/5-HT ratio. In addition, treatment with both paroxetine and venlafaxine produced a significant ($p < 0.01$) decrease in the 5-HIAA/5-HT ratio in control animals, whereas desipramine treatment did not significantly alter this ratio in control animals (Fig. 2a and b). In all cases, the changes in serotonin turnover were predominantly due to changes in 5-HIAA, without any significant alteration in serotonin concentrations (Table 1).

Serum Corticosterone Concentrations

There was a significant effect of FST on serum corticosterone concentrations, $F(1, 43) = 112.61$, $p < 0.0001$. There was also a significant effect of drug treatment, $F(3, 43) = 4.45$, $p < 0.01$, and a significant FST × drug treatment interaction, $F(3, 43) = 3.13$, $p < 0.05$. Post hoc analysis revealed that FST exposure produced a significant increase in corticosterone 45-min post exposure ($p < 0.01$) and subchronic treatment with desipramine significantly attenuated this response ($p < 0.01$). Subchronic treatment with paroxetine or venlafaxine failed to alter the swim stress-induced increase in serum corticosterone (Fig. 3).

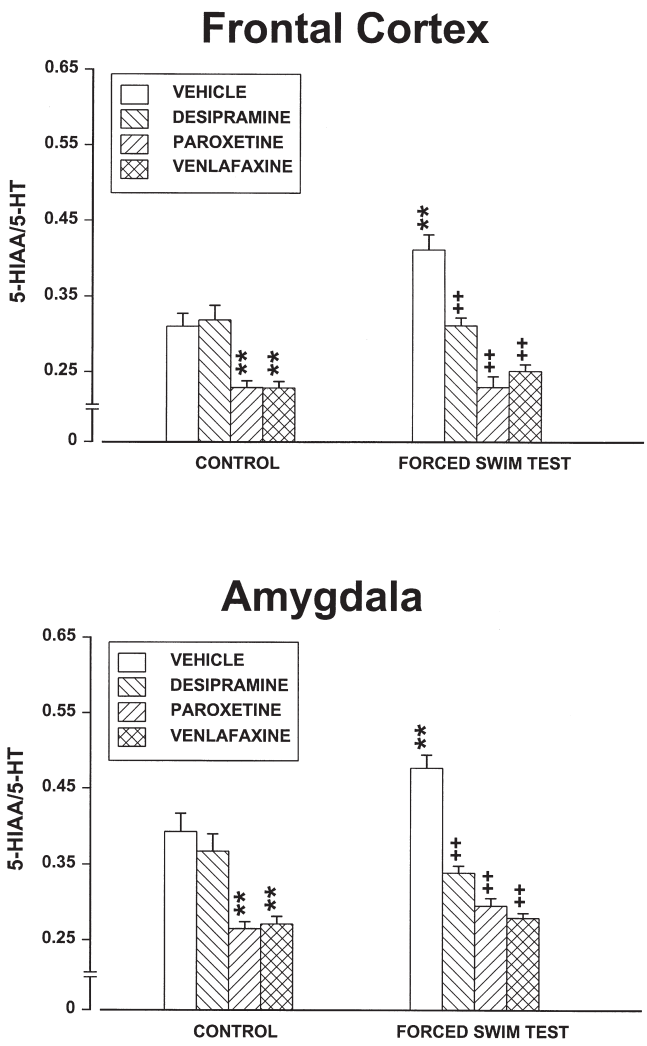

FIG. 2. Effect of subchronic treatment with desipramine, paroxetine, and venlafaxine on forced-swim test-induced increases in the 5-HIAA/5-HT ratio in the (a) frontal cortex and (b) amygdaloid cortex. Data expressed as means with standard errors (n = 6–7). **$p <$ 0.01 vs. control + vehicle ++$p < 0.01$ vs. FST + vehicle (Fishers LSD multiple range test).

TABLE 1

EFFECT OF FORCED-SWIM TEST EXPOSURE AND SUBCHRONIC ANTIDEPRESSANT TREATMENT ON 5-HIAA AND 5-HT CONCENTRATIONS IN THE FRONTAL CORTEX AND AMYGDALOID CORTEX

	Frontal Cortex		Amygdaloid Cortex	
Group	5-HT	5-HIAA	5-HT	5-HIAA
Control groups				
Vehicle	735 ± 23	227 ± 10	1075 ± 38	420 ± 23
Desipramine	676 ± 21	214 ± 8	958 ± 98	341 ± 22
Paroxetine	684 ± 52	155 ± 9*	972 ± 62	256 ± 16*
Venlafaxine	772 ± 30	177 ± 11*	998 ± 54	271 ± 18*
FST groups				
Vehicle	796 ± 23	326 ± 13*	1077 ± 45	513 ± 24*
Desipramine	803 ± 85	249 ± 16‡	1212 ± 91	411 ± 31‡
Paroxetine	741 ± 24	172 ± 14‡	958 ± 60	281 ± 15‡
Venlafaxine	788 ± 33	195 ± 7‡	1056 ± 74	294 ± 17‡
FST effect				
f-value	6.10	27.06	2.38	11.82
p-value	0.0173	0.0001	0.1293	0.0013
df	1,45	1,45	1,45	1,45
Drug effect				
f-value	1.213	38.06	1.28	36.49
p-value	0.3156	0.0001	0.2926	0.0001
df	3,45	3,45	3,45	3,45
FST × Drug				
f-value	0.934	5.77	1.61	1.287
p-value	0.4319	0.002	0.2001	0.2903
df	3,45	3,45	3,45	3,45

Data expressed as means with standard errors (n = 6–7).
**$p < 0.01$ vs control + vehicle.
‡$p < 0.01$ vs. FST + vehicle (Fishers LSD multiple range test).
5-HT and 5-HIAA concentrations are expressed as ng per gram fresh weight of tissue.

DISCUSSION

In this study desipramine treatment significantly decreased immobility in the FST, while paroxetine and venlafaxine were without effect. Nonetheless, subchronic treatment with all three antidepressants significantly attenuated FST-induced increases in serotonin turnover in the amygdaloid and frontal cortices. Although previous reports suggest that SSRIs may be behaviorally active in the rat FST following subchronic treatment (18,40), these data demonstrate that subchronic treatment for 24 days with the SSRI paroxetine, and the SNRI venlafaxine, at doses that provoke profound changes in central serotonin turnover fail to decrease immobility in the rat FST. The reason for the discrepancy between these studies is largely unclear, although Detke et al. (18) used a novel behavioral scoring system that measures a variety of behavioral parameters in the rat FST. Such a scoring method may confer greater sensitivity to this test in detecting SSRIs. Similarly, in the case of venlafaxine Reneric and Lucki (50) reported that venlafaxine displayed a dose-dependent decrease in immobility in the rat FST using this modified scoring method. In that study the behavioral profile exhibited by venlafaxine was similar to that of an SSRI (17,18). These data suggest that the Porsolt FST is predominantly a test that identifies catacholaminergic agents, and is not sensitive to detecting serotonin reuptake inhibitors. Thus, the advent of the modified FST by the Lucki group is a welcome development in psychopharmacology that has enhanced the sensitivity of the FST, thus making it more useful for the detection of antidepressants.

While all three antidepressants attenuated the FST-induced increase in serotonin turnover in both the amygdala and frontal cortex, the effect of paroxetine and venlafaxine provoked a significant reduction in serotonin turnover in nonstressed rats, whereas desipramine did not. These data are consistent with many previous reports concerning the ability of SSRIs to reduce serotonin turnover in the rodent brain (11,24,34,35). In addition, the similarity between the effect of paroxetine and venlafaxine on serotonin turnover is consistent with data demonstrating that venlafaxine is a potent serotonin reuptake inhibitor (5,9). As only desipramine displayed antidepressant-like activity in the FST paradigm, whereas all three antidepressants attenuated the stress-related increase in serotonin turnover, it would appear that antidepressant-induced attenuation of FST-related increases in serotonin turnover is not indicative of behavioral activity in this test. However, it must be pointed out that in the present study we cannot equate the at-

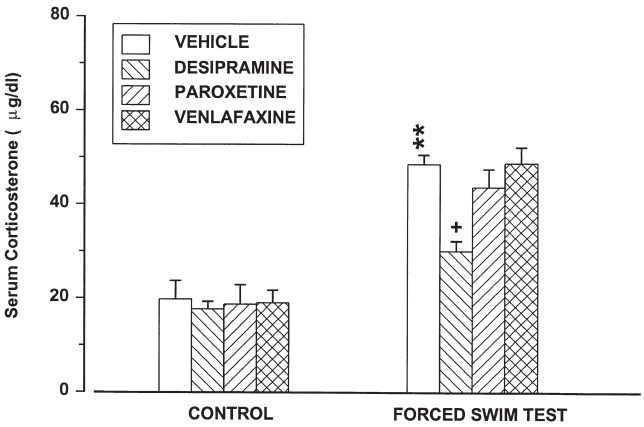

FIG. 3. Effect of subchronic treatment with desipramine, paroxetine, and venlafaxine on forced-swim test-induced increases in serum corticosterone concentrations. Data expressed as means with standard errors (n = 6–7). $^{**}p < 0.01$ vs. vehicle-treated counterparts. $+p < 0.01$ vs. FST + vehicle (Fishers LSD multiple range test).

tenuation of stress-induced increases in serotonin turnover in the paroxetine- and venlafaxine-treated animals to decreased stressor-induced serotonin release. A reduction in serotonin turnover in stressed rats (when compared to control rats) may be simply related to an acute pharmacological action of these drugs in inhibiting serotonin reuptake and metabolism. However, further studies employing washout periods following chronic antidepressant treatment regimens will address this issue.

Desipramine is both behaviorally active in the FST and also attenuates FST-induced increases in serotonin turnover in the amygdala. The amygdala has been implicated as a key site of antidepressant action in the FST, inasmuch as direct injection of imipramine and pargyline into this brain region, but not into other brain structures, produces antiimmobility responses similar to systemic administration of these drugs (21). Although desipramine produces a dose-dependent decrease in immobility in the FST and also dose dependently attenuates the FST induced increases in amygdaloid 5-HT turnover, it is unlikely that this effect of desipramine on the serotonergic system mediates its behavioral effects in the FST paradigm. This view is supported by the observation that a serotonergic amygdaloid lesion produced using 5,7-DHT failed to alter the antiimmobility effect of desipramine in the FST, whereas a 6-OHDA lesion in this region impaired the ability of desipramine to reduce immobility (2). In addition, as observed in the present study and elsewhere, serotonin reuptake inhibitors are largely inactive in the FST paradigm, at least at therapeutically relevant doses (2,6,34).

In addition to the behavioral and neurochemical consequences of exposure to stress there is an abundance of literature dealing with the attenuating effect of antidepressant treatments on stress-induced HPA axis activation (28,51,52). Of the three antidepressants examined in the present study only desipramine attenuated the stress associated elevation in serum corticosterone. This is of interest, and is concordant with reports that subchronic desipramine treatment upregulates hippocampal glucocorticoid receptor (GR) mRNA expression (53), and thus enhances the feedback inhibitory effect of corticosterone on the HPA axis. Interestingly, Duncan et al. (20) reported that subchronic treatment with desipramine and other noradrenaline reuptake inhibitors, but not SSRIs, attenuated swim stress-induced increases in Fos-LI in the hypothalamic PVN, which is the major site of corticotropin releasing factor (CRF) synthesis. Moreover subchronic desipramine treatment decreases CRF mRNA expression in the hypothalamic PVN (8) and attenuates foot shock-induced corticosterone secretion in rats (13). Thus, in addition to the ability of subchronic desipramine treatment to cause GR upregulation, desipramine treatment may also dampen HPA axis responsiveness by reducing hypothalamic CRF release in response to swim stress. While TCAs such as imipramine, desipramine, and amitriptyline upregulate central GR mRNA expression (53) and GR receptor number (10,37,47) following subchronic treatment, reports indicate that chronic treatment with SSRIs such as citalopram (10,53) or atypical antidepressants such as mianserin (10) fail to alter hippocampal GR mRNA expression or receptor number. Similarly, the present study also demonstrates that subchronic treatment with either paroxetine or venlafaxine failed to alter the HPA axis response to swim stress, suggesting that feedback inhibition on the HPA axis was not facilitated by subchronic treatment with these two antidepressants, which act predominantly on the serotonergic system. As only a single dose of each antidepressant was employed, it may be argued that these agents would have altered behavioral and HPA axis activity in the FST had higher doses been examined. However, the doses of paroxetine and venlafaxine used provoked a large reduction in 5-HIAA in both the amygdala and frontal cortex, indicating that they profoundly alter central serotonergic activity. Furthermore, the doses of paroxetine and venlafaxine used in the present study are either equivalent to, or in excess of, those that produce antidepressant activity in other rodent models predictive of antidepressant activity (16,41,42). Nonetheless, higher doses of venlafaxine that would have a greater effect on noradrenaline reuptake (3) may display a positive antidepressant profile in this test as observed in a recent studies in a modified rat FST (50) and mouse FST (49). In this regard it was previously demonstrated that the dual-acting SNRI, milnacipran, which displays more equal effects on both noradrenaline and serotonin reuptake in comparison to venlafaxine (9), displayed an noradrenaline reuptake inhibitor-like antidepressant effect in the modified rat FST paradigm (50).

In conclusion, although FST-induced increases in serotonin turnover in the frontal cortex and amygdala were attenuated following subchronic treatment with all three antidepressants, FST-induced immobility and increased serum corticosterone concentrations were attenuated only following treatment with the TCA desipramine. Thus, these data clearly demonstrate that subchronic treatment with paroxetine and venlafaxine at doses that provoke robust changes in central serotonergic activity fail to display an antidepressant profile in the FST paradigm. In addition, these results suggest that neurochemical mechanisms independent of increased serotonergic activity subserve the behavioral and HPA axis responses in the FST paradigm. Furthermore, the present study adds to our understanding of the interactions between antidepressants and stress-induced behavioral, neurochemical, and endocrine alterations, and illustrates important differences between different classes of antidepressants.

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