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Forced Swim Test-Induced Endocrine and Immune Changes in the Rat: Effect of Subacute Desipramine Treatment

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CONNOR, T. J., J. P. KELLY AND B. E. LEONARD. *Forced swim test-induced endocrine and immune changes in the rat: Effect of subacute desipramine treatment.* PHARMACOL BIOCHEM BEHAV **59**(1) 171–177, 1998.—Previously it has been reported that forced swim test (FST) exposure activates the HPA–axis and produces alterations in both cellular and noncellular immunity in rats. Furthermore, there is evidence to suggest that pretreatment with antidepressants has a protective effect against FST-induced immune changes. The purpose of the present study was to examine the effect of subacute treatment with the tricyclic antidepressant desipramine (DMI, 5 and 10 mg/kg; IP) on immobility in the FST, and on FST-induced changes in endocrine and immune parameters in the rat. DMI treatment at a dose of 10 mg/kg produced a significant reduction in immobility time in the FST, while the 5 mg/kg dose was ineffective. FST exposure produced a significant increase in serum corticosterone and a decrease in adrenal ascorbic acid concentrations, neither of which were significantly attenuated by DMI pretreatment. There was a slight but nonsignificant suppression of PHA-induced lymphocyte proliferation 15 min post-FST exposure. However, DMI treatment produced a significant increase in lymphocyte proliferation at this time point. FST exposure caused a reduction in the percentage of lymphocytes and an increase in the percentage of neutrophils in the peripheral blood; DMI treatment failed to significantly alter these stress-induced changes. There was a profound reduction in relative spleen weight observed in DMI-treated animals 120 min post-FST exposure and this was accompanied by an increase in circulating RBC concentrations. In conclusion, although the FST-induced behavioral changes were normalized by DMI treatment the peripheral aberrations induced by FST exposure (with the exception of lymphocyte proliferation) were not. In addition, DMI pretreatment induced stress-like changes in corticosterone, adrenal ascorbic acid and leucocyte subpopulations in the control animals. © 1998 Elsevier Science Inc.

Forced swim test Antidepressants Immune Endocrine Depression Desipramine

THE forced swim test (FST) is a behavioral paradigm that has been considered as a model of the depressive state and is widely used as a predictor of antidepressant activity in rodents (36). When animals are exposed to the FST they typically display an immobile posture that is said to reflect a state of "behavioral despair" on the assumption that the animals have given up hope of escaping (36). Therefore, exposure to this swim stress paradigm produces a change in behavior that is thought to model a symptom of the depressive state, namely that of despair or helplessness. It is well established that subacute antidepressant treatment reduces immobility time in the FST (8).

In addition to being a useful screening test for antidepressant activity, the FST is also a potent psychophysiological stressor that alters physiological (1) endocrine and immune (29,37,39) function. Moreover, it is well established that stress is causally related to, or a concomitant of most major psychiatric illnesses including depression (4,6,25). Recently, we have demonstrated that the FST induces neurochemical, endocrine, and immune alterations that occur in a time dependent manner after exposure (11). These alterations include increased cortical and amygdaloid 5-HT turnover, decreased hypothalamic 5-HT and noradrenaline concentrations, and increased striatal HVA and DOPAC concentrations. We also reported that FST exposure caused raised serum corticosterone concentrations, a reduced percentage of lymphocytes and an increased percentage of neutrophils in the peripheral blood and a transient suppression of mitogen-stimulated lymphocyte proliferation. Other studies have reported reduced neutrophil phagocytosis (39), impaired natural killer (NK) cell cytotoxic responses (29,37), suppression of lymphocyte

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proliferation and interluekin 2 production (37) as a result of exposure to acute swim stress. In addition, many similar alterations in immune function have been reported in depressed patients, such as decreased lymphocyte responsiveness to mitogenic stimulation (23,27), reduced NK-cell activity (21,22), and an impaired neutrophil phagocytic response (33). Depression is also associated with hypercortisolaemia (27), and an increased WBC count that is accompanied by a decreased number of lymphocytes and increased number of neutrophils in the peripheral blood (12,23).

To date, many psychotropic drugs have been screened in the FST, and the majority of clinically active antidepressants are effective in reducing immobility in the FST (8). Although many antidepressants with differing neurochemical specificities attenuate FST-induced immobility, very little is known about the effects of such drugs on FST-induced endocrine and immune alterations. Such an investigation could add to our understanding of the interactions between antidepressants, stress, and the endocrine and immune systems. It would appear that, to date, only one study has examined the effect of antidepressants on swim stress-induced immune changes (29). In this study both paroxetine and nefazodone treatment attenuated the swim stress-induced reduction in splenic NK-cell activity in the rat (29), suggesting that antidepressant treatment had immunoprotective properties against swim stress-induced alterations in cell-mediated immunity.

Desipramine is a well-established tricyclic antidepressant that has been used clinically for many years (24). The main neurochemical action of desipramine is to inhibit reuptake of noradrenaline (and to a lesser extent serotonin) into the presynaptic neuron thereby elevating synaptic concentrations of these transmitters (7). Previous studies have reported that desipramine is active in the FST at a dose of 10 mg/kg upwards (17), but not 5 mg/kg (9), when administered in the subacute regime of 24, 5, and 1 h prior to the 5 min FST session. In the present study 10 mg/kg was chosen as a behaviorally active dose and 5 mg/kg as an inactive dose of desipramine.

The objectives of the present investigation were to examine the effect of subacute desipramine administration of FST-induced endocrine and immune changes in the rat.

METHOD

Subjects and Procedures

Male Sprague–Dawley rats (Harlan Olac, Bichester, UK) weighing approximately 350–400 g were individually housed and maintained on a 12 h:12 h light:dark cycle (lights on at 0800 h) in a temperature-controlled room (22–24°C). Food and water were available ad lib at all times. Desipramine HCl (Sigma Chemical Co., Poole, Dorset, UK) was dissolved in distilled water and was administered in an injection volume of 1 ml/kg (IP); distilled water alone was administered as a vehicle. The rats were randomly assigned to one of nine groups ($n = 9$–10 per group):

Group 1: control + vehicle; *group 2:* control + DMI (5 mg/kg); *group 3:* control + DMI (10 mg/kg); *group 4:* 15 min post-FST + vehicle; *group 5:* 15 min post-FST + DMI (5 mg/kg); *group 6:* 15 min post-FST + DMI (10 mg/kg); *group 7:* 120 min post-FST + vehicle; *group 8:* 120 min post-FST + DMI (5 mg/kg); *group 9:* 120 min post-FST + DMI (10 mg/kg).

Forced Swim Test Procedure

This test was performed using the original method described by Porsolt and co-workers (36). On the first day of the experiment the rats were plunged 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/desipramine injection 15 min after the first FST exposure. The second and third vehicle/desipramine 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 control groups (groups 1, 4, and 7) did not receive either FST exposure (15 min or 5 min). Clean water was used for each behavioral measurement as it has been reported that soiled water decreases immobility time in the FST (2). All other groups received both exposures and were sacrificed prior to, 15, or 120 min after the 5 min FST exposure on day 2.

The study was carried out over a 5-day period and an equal representation from each group was tested on each of the 5 days. In addition, the sequence of testing was randomized throughout the experiment so as to minimize any confounding effects of order of testing.

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 supernatent removed and stored at −20°C until analysis was performed. Serum corticosterone concentrations were measured using a modified method to that described previously (18). A corticosterone stock (Sigma Chemical Co., 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 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.

Adrenal Ascorbic Acid Assay

The method used for adrenal ascorbic acid determination was a spectrophotometric method as previously described (14). The adrenal glands were dissected free of fat, weighed, and placed in 3 ml 7.5% trichloroacetic acid (TCA) (BDH Chemicals Ltd., Poole, Dorset, UK). The tissue was homogenized and stored at −20°C until the assay was performed. On the day prior to assay the samples were thawed and rinsed with 1 ml 7.5% TCA. After centrifugation at 800 × *g* for 20 min using an MSE benchtop centrifuge 3 ml of the supernatant was removed and added to 3 ml 7.5% TCA and 250 mg activated charcoal (Sigma Chemical Co., Poole, Dorset, UK). After vigorous mixing and low-speed centrifugation the supernatent was filtered through 7-cm filters (Whatman No. 2). The resultant protein free filtrate was stored at 4°C overnight and assayed for ascorbic acid the following morning. The ascorbic acid standards were also activated using activated charcoal. A 2,4-Dinitrophenylhydrazine (2,4-DNP) solution was prepared by dissolving 2 g of 2,4-DNP (Sigma Chemical Co., Poole, Dorset, UK) in 100 ml of 9 nH_2SO_4, then adding 4 g of thiourea (Sigma Chemical Co., Poole, Dorset, UK), this light sensitive

solution was filtered through a 7-cm filter. A 250 μg/ml ascorbic acid (BDH Chemicals Ltd., Poole, Dorset, UK) stock was dilluted using 7.5% TCA to produce a range of concentrations for the standard curve (0, 2.5, 5, 10, 20, and 40 μg/ml). To 1 ml of samples and standards was added 250 μl of 2,4-DNP solution, the tubes were mixed and incubated in a water bath at 37°C for 4.5 h. At the end of the incubation period the tubes were cooled in an ice bath for 5 min, and 1.5 ml of 85% H_2SO_4 (BDH Chemicals Ltd., Poole, Dorset, UK) was added to each tube. The tubes were mixed and allowed to stand for 40 min at room temperature, after which time the absorbance of the orange coloured reaction product was read at 515 nm using a spectrophotometer (Shimadzu UV-160). Results were expressed as μg ascorbic acid/mg tissue.

Relative Leucocyte Percentages

Blood smeared slides were stained with Wright's stain using an automated slide stainer (Ames, HEMA-TEK, Japan). The relative leucocyte percentages were counted on each slide using standard hospital procedures, under a microscope.

Mitogen Stimulated Lymphocyte Proliferation

Prior to sacrifice a 5-ml blood sample was removed from each rat into a heparinized syringe via cardiac puncture under ether (BDH Chemicals Ltd., Poole, Dorset, UK) anaesthesia. The blood was mixed with 6% (w/v) dextran (Nycomed AS, Oslo, Norway) (3 ml) and incubated at 37°C for approximately 30 min to sediment the erythrocytes. The leucocyte-rich supernatent was removed and centrifuged at 600 × *g* for 10 min. The pellet was resuspended in 3-ml Hanks balanced salt solution (HBSS) (Gibco Life Technologies, Scotland, UK) and layered onto 4 ml Nycoprep (Nycomed AS, Oslo, Norway) gradient. Following centrifugation at 600 × *g* (Beckman refrigerated benchtop centrifuge) for 25 min at 20°C, two distinct bands of white cells were obtained. The upper layer contained lymphocytes and the lower layer contained neutrophils. For the mitogen-stimulated lymphocyte proliferation, the lymphocyte layer was removed and washed three times in RPMI 1640 medium (Gibco Life Technologies, Scotland, UK). The cells were finally resuspended in complete RPMI 1640 medium [RPMI 1640 +10% (v/v) fetal calf serum (Gibco Life Technologies, Scotland, UK) + 2% (v/v) penicillin/streptomycin (Gibco Life Technologies, Scotland, UK)] and the number of lymphocytes was adjusted to 2×10^6/ml.

Lymphocyte proliferation was preformed as previously described (40). Lymphocyte cultures were performed in triplicate. Briefly, 200 μl aliquots of the lymphocyte preparation were pipetted into wells of a 96-well microtiter plate. To each well was added either no mitogen for background transformation or phytohemagglutinin-P (PHA-P) (100 μg/ml) (Sigma Chemical Co., Poole, Dorset, UK). Cultures were incubated for 62 h at 37°C in a 5% CO_2 atmosphere prior to addition of [^{3}H]thymidine (0.5 μCi/well), the cultures were then incubated for a further 10 h. At the end of the incubation period the plates were removed and stored at −70°C until they were harvested onto GF/C filters using a cell harvestor (Brandel). [^{3}H]thymidine uptake was measured using a liquid scintillation counter (LKB Wallac, 1211 Rackbeta). Mean scintillation counts per minute (CPM) were calculated for the mitogen.

Statistical Analysis of Data

The FST immobility time data was initially analyzed using a two-way analysis of variance. There was no significant difference in immobility time between the groups of animals receiving the same drug/vehicle treatment from the 15 min post-FST and 120 min post-FST time points. Consequently, the data from the same drug/vehicle-treated groups at these two time points was pooled and analyzed by a one-way analysis of variance. The biochemical data was analyzed by a one-way analysis of variance. If any statistically significant change was found, post hoc comparisons were performed using Newman–Keuls multiple range test. Data was deemed significant when $p \leq 0.05$. Results are expressed as group mean ± standard error of the mean.

RESULTS

Behavior

There was no significant difference in immobility time between the groups of animals receiving the same drug/vehicle treatment from the 15 min post-FST and 120 min post-FST time points. Consequently, the behavioural scores presented here are a result of the immobility times of the animals receiving the same drug/vehicle treatment at the 15 min post-FST and 120 min post-FST time points being pooled. One-way ANOVA revealed a significant effect of DMI treatment, $F(2, 55) = 9.67$, $p < 0.0001$. Post hoc analysis revealed that DMI at the 10 mg/kg dose produced a significant ($p < 0.01$) reduction in immobility time in the FST, whereas the 5 mg/kg dose of DMI was ineffective (Fig. 1).

Serum Corticosterone

There was a significant effect of the FST on serum corticosterone concentrations, $F(2, 76) = 11.49$, $p < 0.0001$. There was also a significant effect of drug treatment, $F(2, 76) = 3.23$, $p < 0.05$, and a significant FST × drug treatment interaction, $F(4, 76) = 6.93$, $p < 0.001$. Post hoc analysis revealed a significant ($p < 0.01$) increase in corticosterone in the control + DMI (10 mg/kg) group compared to the control + vehicle group. FST exposure produced a significant ($p < 0.01$) increase in corticosterone 15 min post exposure, and DMI pretreatment did not significantly alter this response. By 120 min after FST exposure, these FST-induced increases had returned to prestress levels (Fig. 2A).

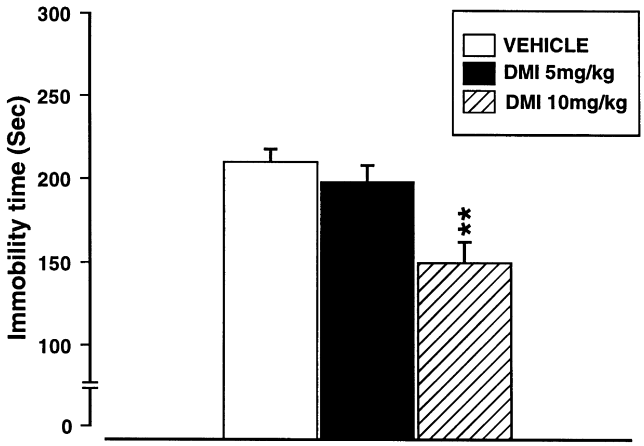


FIG. 1. Effect of subacute desipramine treatment on immobility time in the FST. Data expressed as means ± SEM. **$p < 0.01$ vs. vehicle (Newman–Keuls multiple range test).

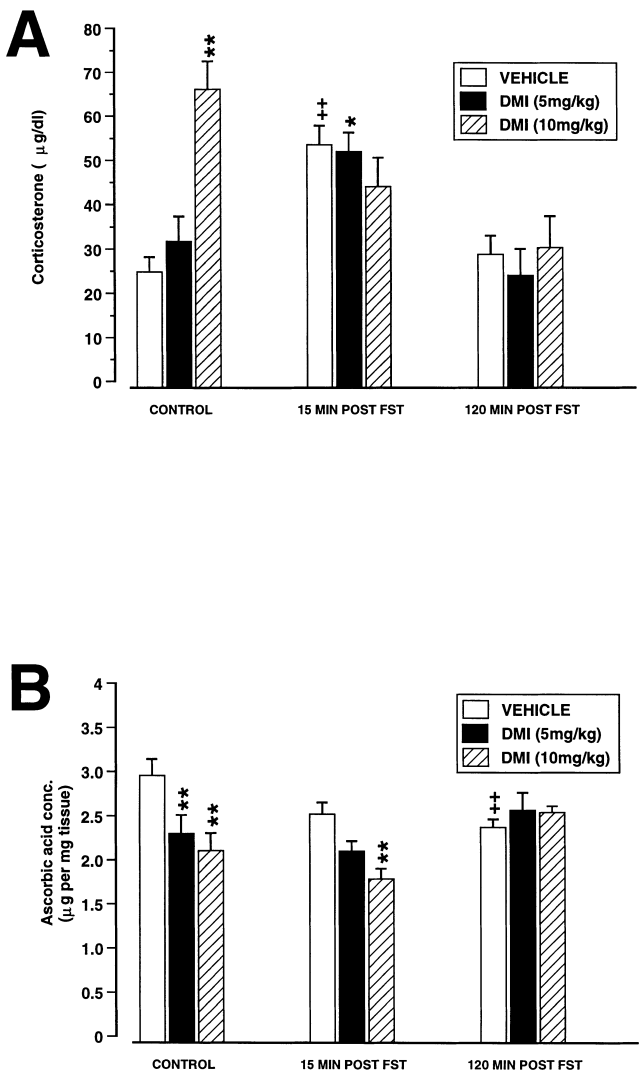


FIG. 2. Effect of FST exposure and subacute desipramine treatment on (A) serum corticosterone concentrations (B) adrenal ascorbic acid concentrations. Data expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle treated counterparts, $^{++}p < 0.01$ vs. control + vehicle (Newman–Keuls multiple range test).

Adrenal Ascorbic Acid

There was a significant effect of the FST on adrenal ascorbic acid concentrations, $F(2, 75) = 7.64$, $p = 0.001$. There was also a significant effect of drug treatment, $F(2, 75) = 5.23$, $p < 0.01$, and a significant FST × drug treatment interaction, $F(4, 75) = 3.93$, $p < 0.01$. Post hoc analysis revealed a significant ($p < 0.01$) reduction in adrenal ascorbic acid in the control + DMI (5 and 10 mg/kg) groups in comparison to the control + vehicle group. There was a similar reduction as a result of desipramine treatment in the animals sacrificed 15 min post-FST exposure. However, this effect was only significant ($p < 0.01$) in the 10 mg/kg DMI group at this time point. In addition, there was a significant ($p < 0.01$) reduction in adrenal ascorbic acid in the vehicle-treated group 120 min post-FST exposure compared to the nonstressed vehicle controls (Fig. 2B).

TABLE 1

THE EFFECT OF FST EXPOSURE AND DESIPRAMINE PRETREATMENT ON RELATIVE LEUCOCYTE PERCENTAGES

Group	% Lymphocytes	% Neutrophils	% Monocytes
Control + vehicle	85.4 ± 1.6	11.1 ± 1.3	3.4 ± 0.9
Control + DMI (5 mg/kg)	80.3 ± 1.9	16.1 ± 1.8	3.4 ± 0.3
Control + DMI (10 mg/kg)	78.8 ± 2.3	17.0 ± 2.1	4.1 ± 0.5
15 min + vehicle	79.1 ± 3.2	15.8 ± 2.8	5.0 ± 0.6
15 min + DMI (5 mg/kg)	85.2 ± 1.8	11.3 ± 1.6	3.3 ± 0.4
15 min + DMI (10 mg/kg)	82.1 ± 2.4	14.5 ± 2.3	3.1 ± 0.3
120 min + vehicle	72.9 ± 2.4*	24.1 ± 2.4*	2.8 ± 0.5
120 min + DMI (5 mg/kg)	65.7 ± 3.1†	31.6 ± 2.9†	2.7 ± 0.5
120 min + DMI (10 mg/kg)	67.2 ± 2.2§	28.5 ± 2.5‡	4.2 ± 0.7

Data expressed as means ± SEM.

$^{*}p < 0.01$ vs. control + vehicle, $\dagger p < 0.01$ vs. control + DMI (5 mg/kg), $\ddagger p < 0.05$, $\S p < 0.01$ vs. control + DMI (10 mg/kg) (Newman–Keuls multiple range test).

Relative Leucocyte Percentages

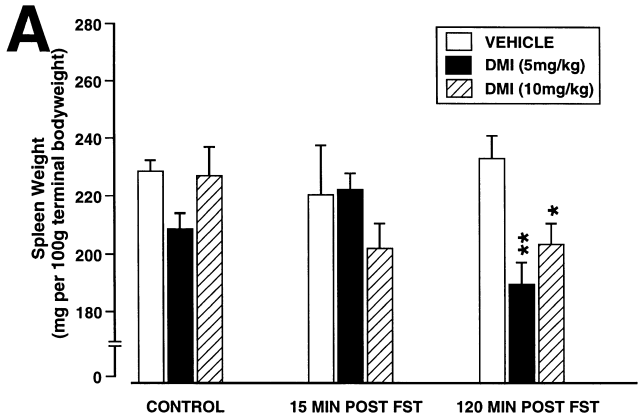
There was a significant effect of the FST on the lymphocyte percentage, $F(2, 75) = 31.28$, $p < 0.0001$, but there was no significant effect of drug treatment. However, there was a significant FST × drug treatment interaction, $F(4, 75) = 2.50$, $p < 0.05$. In addition, there was a significant effect of FST exposure on the neutrophil percentage, $F(2, 75) = 37.23$, $p < 0.0001$, while there was no significant effect of drug treatment, the FST × drug treatment interaction approached significance, $F(4, 75) = 2.10$, $p = 0.08$. There were no significant effects of FST exposure or drug treatment on the monocyte percentage. Post hoc analysis revealed significant ($p < 0.01$) increases in the neutrophil percentage 120 min post-FST exposure in both vehicle and DMI-treated groups. This increase in the neutrophil percentage was accompanied by significant ($p < 0.01$) reductions in the lymphocyte percentage in the same three groups (Table 1).

Relative Spleen Weight

In the case of spleen weights, there was no significant effect of FST exposure, but there was a significant effect of drug treatment, $F(2, 69) = 4.80$, $p = 0.01$, and a significant FST × drug treatment interaction, $F(4, 69) = 2.62$, $p < 0.05$. Post hoc analysis revealed a significant reduction in spleen weight 120 min post-FST exposure in both the 5 mg/kg ($p < 0.01$) and 10 mg/kg ($p < 0.05$) DMI groups compared to the vehicle-treated animals at this time point (Fig. 3A).

Red Blood Cell (RBC) Count

The ANOVA of RBC revealed a significant effect of drug treatment, $F(2, 74) = 6.03$, $p < 0.01$. Post hoc analysis revealed a significant increase ($p < 0.05$) in RBC in the control + DMI (10 mg/kg) group when compared to the control + vehicle



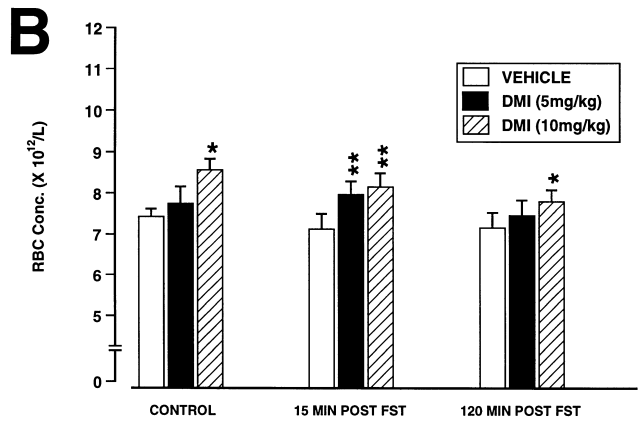


FIG. 3. Effect of FST exposure and subacute desipramine treatment on (A) relative spleen weight (B) RBC count. Data expressed as mean + SEM. $*p < 0.05$, $**p < 0.01$ vs. vehicle treated counterparts (Newman–Keuls multiple range test).

group. There were significant increases ($p < 0.01$) in RBC in both DMI-treated groups 15 min post-FST exposure compared to the vehicle-treated group at time point. In addition, there was a significant increase in RBC in the DMI (10 mg/kg) group 120 min-FST exposure compared to the vehicle-treated group at this time point (Fig. 3B).

Mitogen Stimulated Lymphocyte Proliferation

The ANOVA of PHA-induced lymphocyte proliferation revealed a significant effect of drug treatment, $F(2, 61) = 5.02$, $p < 0.01$, an effect of FST exposure that was approaching statistical significance, $F(2, 61) = 3.04$, $p = 0.054$, and a significant FST × drug treatment interaction, $F(4, 61) = 2.73$, $p < 0.05$. Post hoc analysis revealed a significant increase in lymphocyte proliferation in both the 5 mg//kg DMI ($p < 0.01$) and 10 mg//kg DMI ($p < 0.05$)-treated groups compared to their vehicle-treated counterparts 15-min post-FST exposure (Fig. 4).

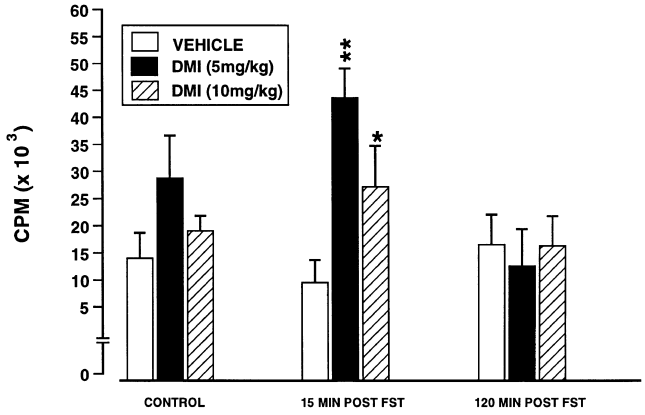


FIG. 4. Effect of FST exposure and subacute desipramine treatment on PHA-induced lymphocyte proliferation. Data expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$ vs. vehicle treated counterparts (Newman–Keuls multiple range test).

DISCUSSION

In the present study, DMI treatment at the 10 mg/kg dose produced a significant reduction in immobility time in the FST, while the 5 mg/kg dose of DMI was ineffective. These behavioral results are consistent with previous findings (9).

DMI pretreatment at either dose had no effect on the magnitude of the corticosterone response 15 min post-FST exposure. This is consistent with previous work from this laboratory, in which chronic treatment with DMI (7.5 mg/kg IP × 28 days) failed to significantly alter the FST-induced increase in serum corticosterone 15 min post exposure (28). In the present study, DMI treatment (10 mg/kg) produced a significant corticosterone response in the control animals; this response was greater in magnitude than the FST-induced increase. The HPA–axis activation elicited by 10 mg/kg DMI in the control animals was possibly due to increased synaptic concentrations of noradrenaline in the PVN of the hypothalamus due to the inhibitory action of DMI on NA uptake. Such an increase in noradrenergic function could cause CRF release at the median eminence thereby activating the HPA–axis (13).

Previously it has been suggested that a reduction in adrenal ascorbic acid concentrations is a physiological indicator of chronic or prolonged stress (16,30,32,35). More recently, acute exposure to restraint stress has been reported to produce a profound reduction in adrenal ascorbic acid concentrations (31). In the present study decreases in adrenal ascorbic acid concentrations were evident as a result of both FST exposure and DMI treatment. The reduction in ascorbic acid concentrations in response to FST exposure was not significant 15 min post exposure. However, by 120 min post exposure there was a highly significant reduction in the adrenal ascorbic acid concentration. Both doses of DMI produced a reduction in ascorbic acid concentrations in both control animals and in animals 15 min post-FST exposure; the effect of DMI treatment had dissipated by 120 min post-FST exposure. Presumably, the reduction in adrenal ascorbic acid concentration is as a result of increased metabolic demand on the adrenal glands as result of a stressful stimulus. Decreased adrenal ascorbic acid concentrations have been previously attributed to both increased synthesis and secretion of corticosterone from the adrenal cortex (31) and increased release of catecholamines from the adrenal medulla (19). In addition, we have recently

demonstrated that adrenal ascorbic acid concentrations remain significantly reduced for up to 8-h post-FST exposure (unpublished data). It is also of interest that ascorbic acid is rapidly released into the blood stream in response to stress (P. Kelliher, personal communication), such a release of ascorbic acid into the peripheral blood may account for the stressor-induced depletion of adrenal ascorbic acid in the present study. Because ascorbic acid has immunoaugmenting properties (26), it is not unreasonable to suggest that stressor-induced release of ascorbic acid into the peripheral blood may play a role in counteracting stress-induced immunosuppression.

In a previous study a reduction in the percentage of lymphocytes and an increase in the percentage of neutrophils in the peripheral blood was reported 120 min after FST exposure (11). In the present study, these findings were replicated. In addition, DMI treatment failed to significantly alter these stress-induced changes. Moreover, there was a tendency of DMI treatment (at both doses) to potentiate the FST induced alterations in WBC subpopulations. This is of interest, as previously it has been reported that both acute and chronic DMI treatment produced an increase in the percentage of neutrophils in the peripheral blood (10,38). The FST-induced alterations in leucocyte subpopulations may be caused by the increased serum corticosterone concentrations or increased sympathetic outflow as a result of stressor exposure, as it is well established that neuroendocrine output can effect leucocyte migration (34). Moreover, a previous study suggests that stress-induced changes in leucocyte numbers are as a result of stress-induced increases in corticosterone concentrations (15). We have previously demonstrated that the alteration in leucocyte subpopulations in response to FST exposure is due to a net reduction in lymphocytes in the peripheral blood without any significant alteration in the absolute number of neutrophils (11).

In addition to the affects of FST exposure on leucocyte subpopulations, it has been demonstrated that swim stress induces alterations in cell mediated immunity (CMI) such as a suppression of natural killer cell (NK-cell) activity (29), mitogen-stimulated lymphocyte proliferation (11,37), and IL-2 production (37) and neutrophil phagocytosis (39). In the present study there was a slight but nonsignificant suppression of PHA-induced lymphocyte proliferation 15 min post-FST exposure. Previously, a significant reduction in PHA-induced lymphocyte proliferation was observed at this time point (11). However, subacute DMI treatment at both doses produced a significant increase in lymphocyte proliferation 15 min post-FST exposure, but this effect had dissipated by 120 min post-exposure. This increase in lymphocyte proliferation in the DMI-treated groups 15 min after exposure to the FST suggests that there was a transient increase in the functional responsiveness of lymphocytes in animals treated with DMI. This is of interest, as recently Menolascino and co-workers reported that treatment with both paroxetine and nefazodone attenuated swim stress-induced suppression of NK-cell activity (29), suggesting that antidepressant treatment had immunoprotective properties against swim stress-induced alterations in cell-mediated immunity. Whether the enhancement of lymphocyte proliferation by DMI, which was noted in the present study, is due to a direct action on the lymphocytes or via a CNS-mediated mechanism remains to be fully elucidated. However, it is likely that a CNS mechanism mediates the observed immunoaugmenting effects of DMI, as previous reports indicate that in vitro exposure to a number of tricyclic antidepressants had a suppressive effect on mitogen-stimulated lymphocyte proliferation (5) and cytokine production (41).

Chronic DMI treatment reduces spleen weight in rats (38). In the present study, a profound reduction in relative spleen weight was observed in DMI (both 5 and 10 mg/kg)-treated animals 120 min post-FST exposure. Such a drastic reduction in spleen weight within such a short time is probably as a result of a mobilization of erythrocytes within the spleen, which then leave the spleen and enter the peripheral blood. This effect is most likely an interaction between FST exposure and DMI treatment, as previously it was demonstrated that 10 mg/kg DMI alone did not reduce spleen weight 3 h post administration (10). In the present study an increase in RBC concentrations was evident as a result of DMI treatment in both control, 15 min post-FST and 120 min post-FST groups. These increases occurred in a dose-dependent manner and were most evident 15 min post-FST exposure. This is in contrast to the reductions in spleen weight, which were most evident 120 min post-FST exposure. Nevertheless, the increase in RBC count may be due to an efflux of RBC from the spleen, and this may be responsible for the reduction in spleen weight observed in the present study. It has been previously reported that the FST causes an increase in O_2 demand, which is highly correlated with the degree of mobility in the test (3). In addition, DMI increases mobility in the FST; therefore, it is possible that DMI could synergistically increase O_2 demand during the FST. It is well established that increased O_2 demand causes an increase in circulating RBC count (as a natural compensatory mechanism) (20). In the present study FST exposure alone was not sufficient to decrease spleen weight and to increase circulating RBC but the FST + DMI combination was. However, as in the present study the time of maximal increases in the RBC count (15 min post-FST) did not coincide with the time of maximal reduction in spleen weight (120 min post-FST); the mechanisms involved in this phenomenon require further clarification in future studies. In addition, it is not clear how DMI (10 mg/kg) increased RBC in the control animals.

In conclusion, subacute DMI pretreatment (3×10 mg/kg) attenuated immobility in the FST. In contrast, pretreatment with DMI (at both doses) potentiated FST-induced changes in adrenal ascorbic acid concentrations and leucocyte subpopulations. In addition, DMI induced stress-like changes in corticosterone, adrenal ascorbic acid, and leucocyte subpopulations in the control animals. Therefore, even though the FST-induced behavioral changes were normalized by DMI treatment, the peripheral abberations induced by FST exposure (with the exception of lymphocyte proliferation) were not. Furthermore, this model could be used to examine the effects of antidepressants with different neurochemical specificities (SSRIs, MAOIs, agonists of specific 5-HT receptor subtypes, etc.) on FST-induced neurochemical, endocrine, and immune alterations. Such investigations may give some insight into which central neurochemical changes are responsible for the FST-induced alterations in endocrine and immune function.

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