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# Forced Swim Test-Induced Neurochemical, Endocrine, and Immune Changes in the Rat

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CONNOR, T. J., J. P. KELLY AND B. E. LEONARD. *Forced swim test-induced neurochemical, endocrine and immune changes in the rat*. PHARMACOL BIOCHEM BEHAV **58**(4) 961–967, 1997—The forced swim test (FST) is a behavioral paradigm that is widely used as a screening test for antidepressant activity in rodents. The objectives of the present study were to characterize the corticosterone and immune responses and in addition to examine neurotransmitter levels, in five brain regions at intervals (15, 30, 60, 90, and 120 min) following the second exposure to the FST. There was a significant but transient reduction in noradrenaline and 5-HT concentrations, in the hypothalamus 15 min post-FST exposure. 5-HT turnover in the frontal cortex and amygdala was significantly increased between 20–120 min post-FST exposure. The FST elicited a robust corticosterone response that peaked significantly at 30 min and had almost returned to baseline 120 min after exposure. There was a significant reduction in total white blood cell count 120 min after the FST, which was accompanied by a significantly reduced percentage of lymphocytes 90 and 120 min post-FST exposure. In addition, there was a significant but transient suppression of both PHA and Con A-induced lymphocyte proliferation 15 min following FST exposure. This study demonstrates that there are neurochemical changes that are coincident with the endocrine and immune changes associated with FST exposure in rats. Furthermore, this model could be used to examine the effects of manipulation of this stress response by antidepressant drugs. Such an investigation could add to our understanding of the interactions between antidepressants, stress and the neuroendocrine and immune systems. © 1997 Elsevier Science Inc.

Forced swim test Behavioral despair Stress Depression Animal model Monoamines Corticosterone Immune

THE forced swim test (FST) is a behavioral paradigm that is widely used as a predictor of antidepressant activity in rodents (34). In this paradigm rats are forced to swim in water at 25°C for 15 min on the first day of testing, and for 5 min on the second day of testing. On the second day of testing behavioral monitoring reveals that the animals quickly adopt an immobile posture, and this is said to reflect a state of "behavioral despair." Subchronic and chronic antidepressant treatment largely attenuates FST induced immobility; both typical and atypical antidepressants are active in the FST (6).

In addition to being a useful screening test for antidepressant activity, the FST is also a potent psychophysiological stressor that is accompanied by a variety of physiological, endocrine, and immune changes (1,27,41,42). Furthermore, neurochemical changes have been demonstrated in response to a variety of stressors including swim stress (21,32), conditioned fear (15,24), foot shock (11,16,37), and immobilization (32,40). These stressor-induced neurochemical alterations include increases in dopamine, noradrenaline, and serotonin release and turnover in a variety of brain regions. Such alterations have been detected using both ex vivo postmortem tissue analysis (11,16) and in vivo microdialysis studies (21,40). In addition to stressor-induced monoamine alterations, there have been reports that stressors cause the release of amino acid neurotransmitters such as glutamate and aspartate (28) and neuropeptides such as corticotropin releasing factor (CRF) (15,26). Therefore, a multitude of central neurochemical alterations may by responsible for the stressor-induced behavioral, endocrine, and immune alterations seen following exposure to stressful stimuli in laboratory animals.

It is now well established that there is a dynamic equilibrium between the neuroendocrine and immune systems. Altered CNS function can affect peripheral immunocompetence via the neuroendocrine and sympathetic nervous systems (4,7). In addition, products of the immune system such as cytokines can modulate CNS function (5). Recently, it has been

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demonstrated that cytokines (Interleukin-1 and -6) are secreted in response to stress (23,39,44), and it has been reported that pretreatment with an interleukin-1 receptor antagonist (IL-1ra) attenuates stress-induced monoamine release in the hypothalamus (40) suggesting that IL-1 plays a role in mediating stress-induced central monoamine changes (39,40).

The appropriate distribution of immune cells between different body tissues is paramount to the performance of surveillance and effector functions of the immune system (9). In addition, it is well established that glucocorticoids can effect leucocyte migration (30). Furthermore, stressor-induced alterations in leucocyte subpopulations have been recently reported in rats (9,10). In addition to the changes in leucocyte numbers after stressor exposure, there is evidence that demonstrates stress-induced alterations in cellular immune function. For instance, suppression of natural killer cell (NK-cell) activity, neutrophil phagocytosis, and mitogen-stimulated lymphocyte proliferation has been demonstrated following acute stressors such as swim stress, footshock, and conditioned fear (18,24,41,42).

Until recently the FST was used mainly for the screening of antidepressant compounds. However, little or no research has been devoted to examining the effects of the FST on changes in neurotransmitter, endocrine, and immune system homeostasis. A knowledge of such changes may give greater insight into the mechanisms in which this stressor induces behavioral despair, and in addition, may shed light on the mechanisms by which antidepressants attenuate FST-induced immobility.

Stressor-induced neurochemical, endocrine, and immunological alterations may be related in a time-dependent manner. Many earlier studies that examined stressor-induced monoamine, endocrine and immune changes have been limited by the fact that only a single time point after stressor application was examined (16,41). As stressor-induced neurochemical, endocrine, and immune changes may demonstrate differential times of onset, and differential response duration, the objective of the present study was to monitor these parameters at different intervals following exposure to the FST so as to elucidate the kinetics/dynamics of this stress response on both central and peripheral systems.

## METHOD

### *Subjects and Procedures*

Male Sprague–Dawley rats (Harlan Olac, Bicester, 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.

### *Forced Swim Test Procedure*

This test was performed using the original method described by Porsolt and co-workers (34). On the first day of the experiment the rats were plunged individually into a container 30 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, and returned to their home cage. Twenty-four hours later the procedure was repeated except the rats were allowed to swim for a duration of 5 min. The control group were not exposed to the FST on either day. All other groups received both exposures and were sacrificed 15, 30, 60, 90, or 120 min after the 5-min FST exposure on day 2.

### *Determination of Brain Biogenic Amine Concentrations*

The rats were sacrificed by decapitation while under ether anesthesia. After sacrifice, the brain was rapidly removed and the left frontal cortex, striatum, amygdala, hippocampus, and hypothalamus were dissected on an ice-cold plate (33). Concentrations of noradrenaline, dopamine, 5-HT, 5-HIAA, HVA, and DOPAC were measured by high-performance liquid chromatography (HPLC) coupled with electrochemical detection (36). The brain regions were homogenized by sonication in 1.0 ml of 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 (BDH Chemicals Ltd., Poole, Dorset, UK), 1.4 mM octane-1-sulphonic acid (Sigma Chemical Co., Poole, Dorset, UK), 0.1 mM EDTA (BDH Chemicals Ltd., Poole, Dorset, UK) 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., Poole, Dorset, UK).

Homogenates were centrifuged at 12,000 rpm in a Hettich Mikro/K refrigerated centrifuge for 15 min. A 50 μl sample of the supernatent 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 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. The neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator. Neurotransmitter concentrations were expressed as ng of neurotransmitter per gram fresh weight of brain tissue. In addition, the ratio of 5-HIAA/5-HT was used as an index of serotonin turnover.

### *Serum Corticosterone Concentrations*

After sacrifice a trunk blood sample was collected and was allowed to clot at room temperature. Serum corticosterone concentrations were measured using a modified method to that described previously (13). A corticosterone (Sigma Chemical Co., Poole, Dorset, UK) stock 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 (BDH Chemicals Ltd., Poole, Dorset, UK): absolute ethanol (Lab Scan, Dublin, Ireland) [65:35] and the tubes were mixed for 15 s using a vortex mixer. 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.

### *Total and Differential Leucocyte Counts*

Prior to sacrifice the animals were anesthetized with diethyl ether (Lab Scan, Dublin, Ireland) and a blood sample for the immune assays was obtained by cardiac puncture into a heparinized syringe. The total leucocyte count was performed on heparinized whole-blood samples using a haematology counter (Serono 9000). Blood smeared slides were stained with Wright's stain using an automated slide stainer (Ames, HEMA-TEK,

Japan) and the lymphocytes, neutrophils, and monocytes were counted under a microscope.

*Mitogen Stimulated Lymphocyte Proliferation*

Heparinized 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 of Nycoprep (Nycomed AS, Oslo, Norway) gradient. Following centrifugation (Beckman refrigerated benchtop centrifuge) at 600 × *g* 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 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) heat inactivated 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 $2 \times 10^6$/ml.

Lymphocyte proliferation was performed in triplicate as previously described (43). 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, concanavalin A (ConA) (Sigma Chemical Co., Poole, Dorset, UK) (5 and 25 μg/ml) or phytohemagglutinin-P (PHA-P) (Sigma Chemical Co., Poole, Dorset, UK) (50 and 100 μg/ml). 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) (Amersham International Ltd., Bucks, UK), 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 harvester (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 each mitogen.

*Statistical Analysis of Data*

All data with the exception of the lymphocyte proliferation was analyzed by a one-way analysis of variance. Lymphocyte proliferation data was analyzed using a two-way analysis of variance. If any statistically significant change was found, post hoc comparisons were performed using Fischer's LSD multiple range test. Data were deemed significant when $p < 0.05$. Results are expressed as group mean ± standard error of the mean.

## RESULTS

*Neurochemistry*

FST exposure elicited numerous neurochemical changes in the brain regions examined.

*Noradrenaline.* There was a significant effect of FST exposure on hypothalamic noradrenaline, $F(5, 50) = 2.97$, $p < 0.05$. Post hoc analysis revealed significant ($p < 0.05$) but transient reduction in a hypothalamic noradrenaline concentration 15-min post exposure (Table 1).

*Dopamine.* There was a significant effect of FST exposure on dopamine in the frontal cortex, $F(5, 50) = 2.35$, $p = 0.05$. Post hoc analysis revealed a significant ($p < 0.05$) but transient increase in dopamine concentrations 30 min after FST exposure. There was a significant effect of FST exposure on the dopamine concentration in the amygdaloid cortex $F(5, 49) = 2.55$, $p < 0.05$. Post hoc analysis revealed a significant ($p < 0.05$) but transient reduction in amygdaloid dopamine concentrations 15 min post-FST exposure. There were significant effects of FST exposure on striatal DOPAC and HVA concentrations; $F(5, 47) = 2.78$, $p < 0.05$, and, $F(5, 47) = 2.34$, $p = 0.05$, respectively. Post hoc analysis revealed a significant ($p < 0.05$) increase in striatal DOPAC concentrations 15 min and from 60–90 min post-FST exposure. In addition, there was a significant increase in HVA 60 and 90 min post exposure (Table 2).

*Serotonin.* There was a significant effect on the 5-HIAA/5-HT ratio in the frontal cortex, $F(5, 52) = 2.42$, $p < 0.05$. Post hoc analysis revealed a significant increase in the 5-HIAA/5-HT ratio 30 ($p < 0.01$), 60 and 120 min ($p < 0.05$) post-FST exposure. In addition, there was a significant effect of FST exposure on 5-HIAA/5-HT ratio in the amygdala, $F(5, 53) = 2.62$, $p < 0.05$. Post hoc analysis revealed a significant ($p < 0.05$) increase in the amygdaloid 5-HIAA/5-HT ratio 60 and 120 min post exposure. There was also a significant effect of FST exposure on hypothalamic 5-HT concentrations, $F(5, 49) = 2.83$, $p < 0.05$; a significant ($p < 0.05$), but transient, reduction in the hypothalamic 5-HT concentration occurred 15 min post-FST exposure (Table 3).

There were no significant effects of FST exposure on any of the neurotransmitters examined in the hippocampus (data not shown).

*Corticosterone*

There was a significant effect of FST exposure on serum corticosterone concentration $F(5, 51) = 5.81$, $p < 0.0001$. Post hoc analysis revealed an increase in corticosterone 15 min post-FST exposure ($p < 0.01$), which peaked significantly (510% increase) ($p < 0.01$) 30 min post exposure; this increase was still significant at 60 min post exposure ($p < 0.05$). However, by 90 and 120 min the concentrations was no longer significantly different from the control value (Fig. 1).

*Total and Differential Leucocyte Counts*

FST exposure produced a significant effect on the total leucocyte count, $F(5, 50) = 3.21$, $p = 0.05$. Post hoc analysis revealed a significant ($p < 0.05$) reduction in leucocytes 120 min post-FST exposure (Table 4). There was a significant effect of FST exposure on absolute lymphocyte number, $F(5, 49) = 2.68$, $p < 0.05$, with post hoc analysis revealing a signifi-

TABLE 1

EFFECT OF FST EXPOSURE ON NORADRENALINE CONCENTRATIONS IN THE HYPOTHALAMUS

| Group | Noradrenaline |
|---|---|
| Control | 3939 ± 291 |
| 15 min | 2573 ± 320* |
| 30 min | 3079 ± 302 |
| 60 min | 3494 ± 297 |
| 90 min | 3054 ± 324 |
| 120 min | 3022 ± 202 |

Data expressed as means ± SEM. *$p < 0.05$ vs. control (Fisher's LSD multiple range test) ($n$ = 9–10). Neurotransmitter concentrations are expressed as ng/g fresh weight of tissue. Control noradrenaline in brain regions which had no significant changes; Frontal cortex: 445 ± 60; amygdala: 662 ± 67; striatum: 1619 ± 214.

TABLE 2

EFFECT OF FST EXPOSURE ON DOPAMINE, DOPAC, AND HVA CONCENTRATIONS

| Group | Dopamine | DOPAC | HVA |
|---|---|---|---|
| Striatum | | | |
| Control | 35527 ± 6284 | 3624 ± 498 | 2847 ± 402 |
| 15 min | 48210 ± 4451 | 5462 ± 515* | 3976 ± 244 |
| 30 min | 38872 ± 1977 | 4242 ± 200 | 3471 ± 198 |
| 60 min | 45942 ± 2830 | 5491 ± 385* | 4103 ± 274* |
| 90 min | 46006 ± 3898 | 5975 ± 755* | 4201 ± 109* |
| 120 min | 45432 ± 5476 | 5476 ± 711 | 3996 ± 444 |
| Frontal Cortex | | | |
| Control | 248 ± 76 | 52 ± 7 | 76 ± 5 |
| 15 min | 320 ± 90 | 72 ± 13 | 103 ± 17 |
| 30 min | 584 ± 164* | 80 ± 13 | 100 ± 9 |
| 60 min | 271 ± 107 | 51 ± 6 | 97 ± 18 |
| 90 min | 253 ± 64 | 68 ± 11 | 93 ± 9 |
| 120 min | 263 ± 160 | 89 ± 11 | 112 ± 12 |
| Amygdala | | | |
| Control | 390 ± 56 | 87 ± 11 | ND |
| 15 min | 196 ± 21* | 54 ± 6 | ND |
| 30 min | 307 ± 49 | 61 ± 8 | ND |
| 60 min | 238 ± 30 | 61 ± 4 | ND |
| 90 min | 253 ± 52 | 62 ± 10 | ND |
| 120 min | 275 ± 32 | 56 ± 5 | ND |

Data expressed as means ± SEM. *$p < 0.05$ vs. control (Fisher's LSD multiple range test) ($n$ = 9–10). Neurotransmitter concentrations are expressed as ng/g fresh weight of tissue. DA: dopamine; DOPAC: 3,4-dihydroxyphenylacetic acid; HVA: homovanillic acid. Control amine concentrations in brain regions which had no significant changes; hypothalamus: DA 553 ± 60, DOPAC ND, HVA ND. ND = not detected.

TABLE 3

EFFECT OF FST EXPOSURE ON 5-HT AND 5-HIAA CONCENTRATIONS AND THE 5-HIAA/5-HT RATIO

| Group | 5-HT | 5-HIAA | 5-HIAA/5-HT |
|---|---|---|---|
| Frontal cortex | | | |
| Control | 433 ± 45 | 295 ± 28 | 0.69 ± 0.02 |
| 15 min | 462 ± 69 | 347 ± 32 | 0.75 ± 0.03 |
| 30 min | 440 ± 31 | 369 ± 37 | 0.83 ± 0.03** |
| 60 min | 434 ± 50 | 370 ± 49 | 0.85 ± 0.05* |
| 90 min | 485 ± 41 | 436 ± 67 | 0.88 ± 0.07 |
| 120 min | 436 ± 32 | 349 ± 18 | 0.82 ± 0.05* |
| Amygdala | | | |
| Control | 927 ± 76 | 328 ± 11 | 0.37 ± 0.02 |
| 15 min | 834 ± 80 | 309 ± 27 | 0.38 ± 0.02 |
| 30 min | 771 ± 40 | 323 ± 19 | 0.42 ± 0.02 |
| 60 min | 829 ± 41 | 354 ± 17 | 0.43 ± 0.02* |
| 90 min | 809 ± 47 | 352 ± 18 | 0.44 ± 0.02* |
| 120 min | 819 ± 35 | 330 ± 10 | 0.41 ± 0.01 |
| Hypothalamus | | | |
| Control | 990 ± 28 | 918 ± 111 | 2.6 ± 0.6 |
| 15 min | 733 ± 81* | 667 ± 79 | 2.1 ± 0.3 |
| 30 min | 799 ± 36 | 735 ± 56 | 2.4 ± 0.4 |
| 60 min | 929 ± 62 | 873 ± 111 | 2.8 ± 0.6 |
| 90 min | 831 ± 52 | 706 ± 75 | 2.6 ± 0.2 |
| 120 min | 842 ± 52 | 706 ± 80 | 2.8 ± 0.3 |

Data expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$ vs. Control (Fisher's LSD multiple range test) ($n$ = 9–10). Neurotransmitter concentrations are expressed as ng/g fresh weight of tissue. 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid. Control amine concentrations in brain regions which had no significant changes; striatum: 5-HT 3007 ± 353, 5-HIAA 2059 ± 200, 5-HIAA/5-HT 0.70 ± 0.05.

cant ($p < 0.01$) reduction in the absolute number of lymphocytes both 90 and 120 min post-FST exposure (Table 4).

*Mitogen Stimulated Lymphocyte Proliferation*

There was a significant but transient suppression of both PHA and Con A-induced lymphocyte proliferation at both concentrations of each mitogen examined as a result of FST exposure. The ANOVA of Con A-induced proliferation revealed a significant effect of FST exposure, $F(5, 82) = 2.45$, $p < 0.05$. There was also a significant effect of Con A concentration, $F(1, 82) = 16.73$, $p = 0.0001$, but there was no significant FST × Con A concentration interaction. Post hoc analysis revealed a significant ($p < 0.05$) suppression of Con A-induced proliferation 15 min post-FST exposure in cells treated with Con A (5 μg/ml) and both 15 and 30 min post-FST exposure in the cells treated with Con A (25 μg/ml) (Fig. 2a). The ANOVA of PHA-induced proliferation revealed a significant effect of FST exposure, $F(5, 82) = 4.43$, $p < 0.01$. Post hoc analysis showed a significant ($p < 0.05$) reduction in PHA-induced lymphocyte proliferation 15 min post-FST exposure at both PHA concentrations (50 and 100 μg/ml) (Fig. 2b).

## DISCUSSION

In the present study a variety of neurochemical alterations elicited by FST exposure were observed. These alterations were region specific, and also occurred in a time-dependent manner. The ratio 5-HIAA/5-HT was used as an index of 5-HT turnover. The increased 5-HT turnover, which was observed in the amygdala and frontal cortex as a result of FST exposure, is indicative of increased release and/or metabolism of 5-HT. These findings are consistent with previous reports that examined the effects of other stressors on 5-HT turnover in the brain (11,16,25). Thus, Inoue and co-workers reported significantly increased 5-HT turnover in the prefrontal cortex, nucleus accumbens, and lateral hypothalamus in response to intermittent footshock stress (16). Other studies also report increased 5-HT turnover and tryptophan concentrations in a number of brain regions in response to foot shock stress (11,25). A limitation of these studies was that the animals were sacrificed at a single timepoint at the termination of stressor exposure, whereas in the present study the FST-induced changes were monitored in a time course fashion for up to 2 h after exposure, thereby giving a more comprehensive profile.

The transient reductions in hypothalamic 5-HT and noradrenaline concentrations observed in the present study are indicative of increased release of these neurotransmitters in response to FST exposure. The findings are in agreement with a previous study where 15 min after exposure of rats to ether stress or footshock there was a significant reduction in hypothalamic 5-HT (46). Other investigators have reported an increase in hypothalamic noradrenaline turnover as a result of stressor exposure (11,14,45). The stress-induced decreases in hypothalamic noradrenaline and 5-HT coincided with the activation of the HPA axis observed in the present study. This is of interest, as it is well established that both hypothalamic no-

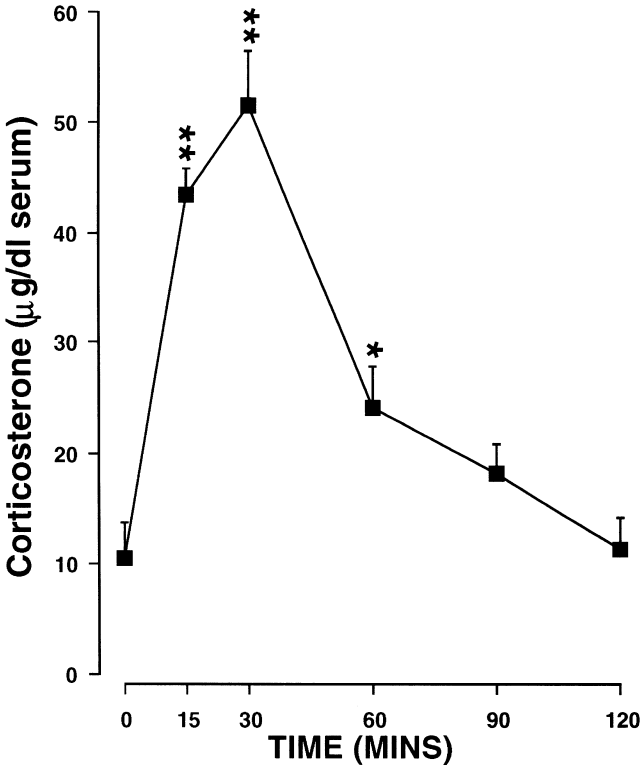


FIG. 1. Effect of FST exposure on serum corticosterone concentrations. Data expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$ vs. control (Fisher's LSD multiple range test) ($n$ = 9–10).

radrenaline and 5-HT release are involved in HPA-axis activation (8).

Significant increases in striatal DOPAC and HVA concentrations occurred in response to FST exposure; these changes are indicative of increased mesocortcolimbic dopaminergic activity. In addition to the changes observed in striatal DOPAC and HVA concentrations, nonsignificant increases in HVA and DOPAC concentrations were also observed in the frontal cortex. Previous studies have reported increased DOPAC and HVA concentrations in several brain regions as a result of footshock stress with the prefrontal cortex displaying the largest increases (11,16). However, in the present study FST exposure had a more profound effect on striatal than on cortical dopaminergic function, which may be due to the fact that the FST is a stressor that induces increased motor output. In vivo microdialysis studies have reported increased DA, NA, and 5-HT release in response to stress in a number of brain regions including the prefrontal cortex, paraventricular nucleus of the hypothalamus, hippocampus, nucleus accumbens, and striatum (21,22,28,31,35).

FST exposure elicited a robust corticosterone response that peaked at 30 min post exposure and returned to baseline values by 120 min. This result is consistent with previous findings that reported HPA axis activation in response to swim stress (1,41,42) and other stressors such as acute footshock or ether stress (46).

It is well established that immune cell trafficking is central to the performance of the surveillance as well as effector functions of the immune system (10). In a previous study from our laboratory a single exposure to 5 min of swim stress produced a significant reduction in the percentage of lymphocytes and a concomitant increase in the percentage of neutrophils in the peripheral blood (42). In the present study, a similar reduction in the percentage of lymphocytes and an increase in the percentage of neutrophils was observed in response to expo-

TABLE 4

EFFECT OF FST EXPOSURE ON ABSOLUTE NUMBERS OF LEUCOCYTES

| Group | Total leucocytes ($\times 10^9$/l) | Lymphocytes ($\times 10^9$/l) | Neutrophils ($\times 10^9$/l) | Monocytes ($\times 10^9$/l) |
|---|---|---|---|---|
| Control | 18.0 ± 1.4 | 13.4 ± 1.4 | 2.6 ± 0.6 | 0.3 ± 0.04 |
| 15 min | 15.1 ± 1.5 | 12.6 ± 1.3 | 2.1 ± 0.3 | 0.5 ± 0.08 |
| 30 min | 16.6 ± 1.4 | 13.3 ± 1.8 | 2.4 ± 0.4 | 0.4 ± 0.08 |
| 60 min | 13.2 ± 1.3 | 10.6 ± 1.0 | 2.8 ± 0.6 | 0.4 ± 0.09 |
| 90 min | 12.9 ± 0.9 | 9.9 ± 0.8* | 2.6 ± 0.2 | 0.4 ± 0.05 |
| 120 min | 12.8 ± 0.5* | 9.6 ± 0.3* | 2.8 ± 0.3 | 0.4 ± 0.04 |

Data expressed as means ± SEM. *$p < 0.05$ vs. control (Fisher's LSD multiple range test) ($n$ = 9–10).

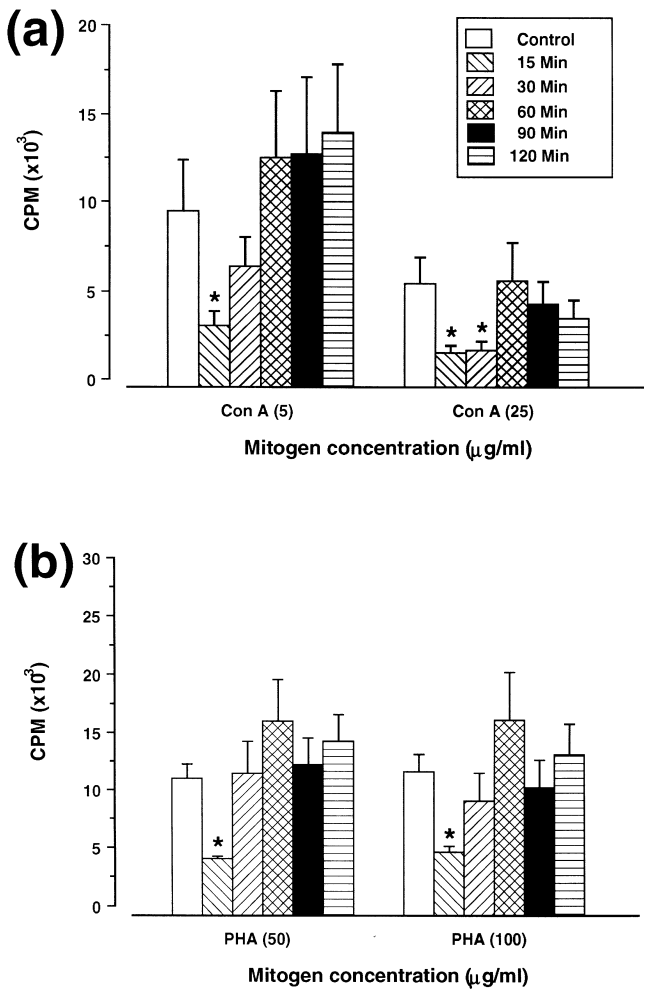


FIG. 2. Effect of FST exposure on (a) Con A and (b) PHA induced lymphocyte proliferation. Data expressed as means ± SEM. *$p < 0.05$ vs. control (Fisher's LSD multiple range test) ($n$ = 9–10).

sure to the classical FST (data not shown). In addition, a reduction in the total leucocyte count occurred that reached significance 120 min after FST exposure. When the differential and total leucocyte counts were multiplied, absolute lymphocyte, neutrophil and monocyte counts were obtained. These results demonstrated that the changes in the differential leucocyte count as a result of FST exposure were predominantly due to a reduction in the absolute number of lymphocytes as opposed to an increase in the absolute numbers of neutrophils. Previous studies have reported a similar decrease in total leucocyte count during restraint stress (9,10). Furthermore, the study by Dhabhar and co-workers also reported a decreased percentage of lymphocytes and increased percentage of neutrophils as a result of stressor application (10). In addition, they demonstrated that these stress-induced alterations in leucocyte subpopulations were transient and reversible upon cessation of the stressor (10), suggesting that the reduction in total leucocytes and lymphocytes in response to stress were due to immune cell redistribution as opposed to cell death or apoptosis.

It is generally accepted that neuroendocrine output can effect leucocyte migration (30). The stress-induced changes in leucocyte numbers in the present study occur 60–90 min after peak serum corticosterone concentrations. Therefore, the stress-induced increase in serum corticosterone may be at least partially responsible for the changes in peripheral leucocyte concentrations. In support of this hypothesis, it has been reported that adrenalectomy significantly attenuated restraint stress-induced reduction in lymphocytes (10). In addition, administration of exogenous corticosterone to nonadrenalectomized, nonstressed animals produced changes in leucocyte numbers that were similar in magnitude and duration to the changes induced by stress (10). This suggests that it is the release of corticosterone from the adrenal cortex and not adrenal catecholamines from the adrenal medulla that mediate stressor-induced alterations in leucocyte subpopulations. Furthermore, corticosteroids can induce the release of granulocytes from the bone marrow (38). However, in the present study there was no change observed in absolute neutrophil numbers, so it appears that lymphocytes were signaled to leave the peripheral blood and enter other immune compartments (lymph nodes, spleen, bone marrow, Peyers patches, or many other tissues) in response to FST exposure. It is not clear how long it would take for the FST-induced reduction in leucocytes to return to control values. To ascertain this information further time points post FST exposure would need to be examined. It is likely that these changes in leucocyte distribution are mediated by stress-induced changes in the expression or affinity of adhesion molecules on the leucocyte and/or endothelial cell membranes (30).

In addition to the changes observed in leucocyte numbers in the present study, altered functional responsiveness of lymphocytes as a result of FST exposure was also observed. There was a significant, but transient, suppression of mitogen stimulated lymphocyte proliferation 15 min after FST exposure. Both Con A- and PHA-induced proliferation were suppressed, indicating that both T and B lymphocytes were affected. This is in agreement with the results of a previous study in which rats were subjected to three swim stress sessions over 12 h (41). Recently, it has been reported that exposure to a single 15-min swim stress in rats produced a suppression of NK cell activity immediately after cessation of the stressor (27).

It is well established that glucocorticoids, including corticosterone, have immunosuppressive properties (3), and many previous studies have implicated corticosterone to be a key mediator in stress-induced suppression of cell mediated immunity (CMI) (41,42). However, others have demonstrated that stress-induced suppression of CMI occurs even in the absence of corticosterone secretion, suggesting that corticosterone secretion is not responsible for the impaired CMI after stress (19,20). In addition to stress-induced CRF release causing activation of the HPA axis, CRF also causes sympathetic nervous system (SNS) activation, and recently it has been suggested that SNS activation in response to a stressor is an important contributary factor in stress-induced suppression of CMI (17). Moreover, adrenoceptors, which are present on lymphocyte membranes, provide a means by which both noradrenaline and adrenaline can affect lymphocyte function (2). It has been previously suggested that stressor-induced alterations in CMI, such as reductions in mitogen-stimulated lymphocyte proliferation, occur as a result of an alteration in leucocyte subpopulations in response to stressor exposure. However, in a recent study, increased macrophage activity as a result of stress has been suggested to be responsible for stress-induced suppression of lymphocyte proliferation as opposed to stress-induced alterations in leucocyte subpopulations (12). The rapidity of the suppressive effect of swim stress on mitogen-stimulated lymphocyte proliferation in the present study, and NK-cell activity in the study by Menolascino and co-workers (27), implicates a SNS-mediated effect as opposed to an effect mediated by corticosterone. However, further studies are necessary to elucidate the exact mechanism of the FST-induced reduction in lymphocyte proliferation observed in the present study.

In conclusion, FST exposure produced a variety of time-dependent neurochemical, endocrine, and immune alterations in the rat. This study demonstrates the importance of examining such stressor-induced changes in a time course fashion so to elucidate the kinetics/dynamics of the stress response on both central and peripheral systems. Furthermore, this model could be used to examine the effects of pharmacological manipulation of the stress response with antidepressant drugs on different systems. Such an investigation could add to our understanding of the interactions between antidepressants, stress, and the neuroendocrine and immune systems.

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