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Effect of Tryptophan on the Behavior of Nonstressed and Stressed Mice in Porsolt's Swim Test

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HILAKIVI-CLARKE, L. A., M. J. DURCAN, R. G. LISTER AND M. LINNOILA. *Effect of tryptophan on the behavior of nonstressed and stressed mice in Porsolt's swim test.* PHARMACOL BIOCHEM BEHAV **37**(2) 273–276, 1990.—The effect of tryptophan on immobility in Porsolt's swim test was studied in male NIH Swiss mice. Preexposure to a swim or fight-stressor was included in the design. Doses of tryptophan (0, 12.5, 50, 75, 100, 125 and 200 mg/kg) were administered intraperitoneally 60 min prior to the swim test. In the nonstressed mice tryptophan had an U-shaped dose-response relationship: immobility in the water was dose-dependently shortened after doses from 0 to 100 mg/kg, whereas after 125 and 200 mg/kg tryptophan the immobility times did not differ from the values obtained after a saline injection. Preexposure to a swim- or fight-stressor did not make mice more sensitive to the effects of tryptophan. Tryptophan (0–300 mg/kg) had no effect on exploratory behavior or locomotor activity in the holeboard, suggesting that sedation was not a factor in the swim test results. The findings suggest that tryptophan has antidepressant -like properties in Porsolt's swim test.

Tryptophan Porsolt's swim test Stress Holeboard Mice

TRYPTOPHAN, the precursor of serotonin (5-HT), has been suggested to be as effective as tricyclic antidepressants in the treatment of mild to moderate depression in humans (12). On the other hand, a tryptophan-free diet has been shown to produce depressive symptoms in healthy humans (13). In the present study we investigated the effects of tryptophan in an animal model used to predict antidepressant efficacy, the Porsolt's swim test (2, 8, 10). In this test an animal is put into a cylinder containing water, and the time it spends immobile is used as an index of "depressive" behavior. Antidepressant drugs generally shorten the period of immobility in the water (8).

Porsolt's swim test has been previously reported to be insensitive to antidepressant drugs which inhibit the reuptake of 5-HT from the synaptic cleft [see (2)]. However, it has recently been found that when animals were exposed to chronic electrical tail shock prior to the swim test, the relatively selective 5-HT uptake inhibitor, clomipramine, completely prevented the increase in immobility caused by previous exposure to that stressor (5).

In the present study, the effect of tryptophan was examined with a slightly modified version of Porsolt's swim test [see (6,7)]. Exposure of mice to a swim- or fight-stressor was also investigated to assess if this influences the response to tryptophan in the swim test [see (1)]. The fight-stress was due to some animals having been housed in cages with a dominant mouse which frequently attacked its cage mates (6). The swim-stressed mice were put in the water twice before the actual test was carried out.

The swim test may falsely indicate antidepressant potency of a compound which alters locomotor activity (2). Therefore, we investigated effects of tryptophan on locomotor activity using the holeboard test of exploratory behavior and locomotor activity (4).

METHOD

Male NIH Swiss mice, weighing approximately 23–26 g, served as subjects. The mice, maintained on a 12-hr light:12-hr dark cycle (lights on 06:00) and allowed free access to food and water, were housed in groups of 10. The test room was dimly lit and maintained at 25°C. All experiments were performed between 09:00 and 15:00 hr.

The mice were divided into three groups: 1) control mice which were not exposed to a stressor prior to testing; 2) mice which on day 1 and 3 were put into a plastic pail containing water (for details see below) for 5 min (called swim-stressed mice), and actually tested in the swim test on day 6; and 3) mice which were

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exposed to frequent attacks in the home cage by a dominant cage mate, and had lesions on their tail and body (called fight-stressed mice). The duration of the fight-stress was 1–3 weeks.

Porsolt's Swim Test

Each mouse was placed in a plastic container (height 17 cm, inside diameter 21 cm), with 8 cm deep water (temperature 25–26°C) for 10 min. During the last 8 min of the test, after an acclimation period of 2 min, the time a mouse spent floating almost motionless in the water was scored by an observer unaware of the drug treatment, using a keyboard interfaced with PDP-11 microcomputer running SKED-11 software (State Systems, Kalamazoo).

Tryptophan.

Experiment 1. The control and swim- and fight-stressed mice were given an intraperitoneal injection of 0, 12.5, 50.0, 100.0 and 200.0 mg/kg L-tryptophan (Sigma, St. Louis) dissolved in distilled water 60 min prior to the swim test. The injection volume was 10 ml/kg body wt. Each group contained 8–43 mice.

Experiment 2. Because of the U-shaped dose-response relationship found for tryptophan (see results of Experiment 1), additional doses of tryptophan near to 100 mg/kg dose were given to the control mice. In this experiment, the nonstressed mice received an intraperitoneal injection of 0, 50, 75, 100 and 125 mg/kg of tryptophan. The number of animals in each group was 10–12. The swim test was carried out as described above.

Holeboard

The holeboard apparatus was made of Plexiglas (40 × 40 × 30 cm) and had four holes, 3 cm in diameter, equally spaced in the floor. Infrared photocells in the walls of the box and directly beneath each hole provided automated measures of locomotor activity (number of beam interruptions), of the number of exploratory head-dips made and the duration of head-dipping.

Mice, either control or fight-stressed (n = 8–11 naive animals per group), were injected intraperitoneally with 0, 12.5, 50, 100, 200 or 300 mg/kg tryptophan 60 min prior to the holeboard test. The injection volume was 10 ml/kg body weight, except for animals which were injected with 200 or 300 mg/kg tryptophan as well as appropriate controls, which received 20 ml/kg of solution due to tryptophan's limited solubility in water. The testing took place in a dimly lit room, and involved placing a mouse in the center of the floor, and allowing it to explore for 5 min. At the end of the test, the mouse was removed and the floor of the box cleaned.

RESULTS

Swim Test

Tryptophan.

Experiment 1. Analysis of variance showed a main effect of stress on immobility in the swim test, $F(2,286) = 6.05$, $p<0.005$, stressed mice having higher immobility times than the control mice. In post hoc tests (Fisher's least significant difference), however, the mice receiving saline did not differ from one another.

In the control mice tryptophan showed a dose-related effect on the duration of immobility between the doses of 0–100 mg/kg: 100 mg/kg tryptophan significantly shortened immobility ($p<0.05$) (Fig. 1a). At the 200 mg/kg tryptophan dose, control mice showed immobility times not significantly different from those following saline injection. In the swim-stressed mice, 50 mg/kg tryptophan significantly shortened the period of immobility ($p<0.05$), while the other doses did not have a significant effect (Fig. 1b). Tryptophan had no significant effect on behavior in the swim test

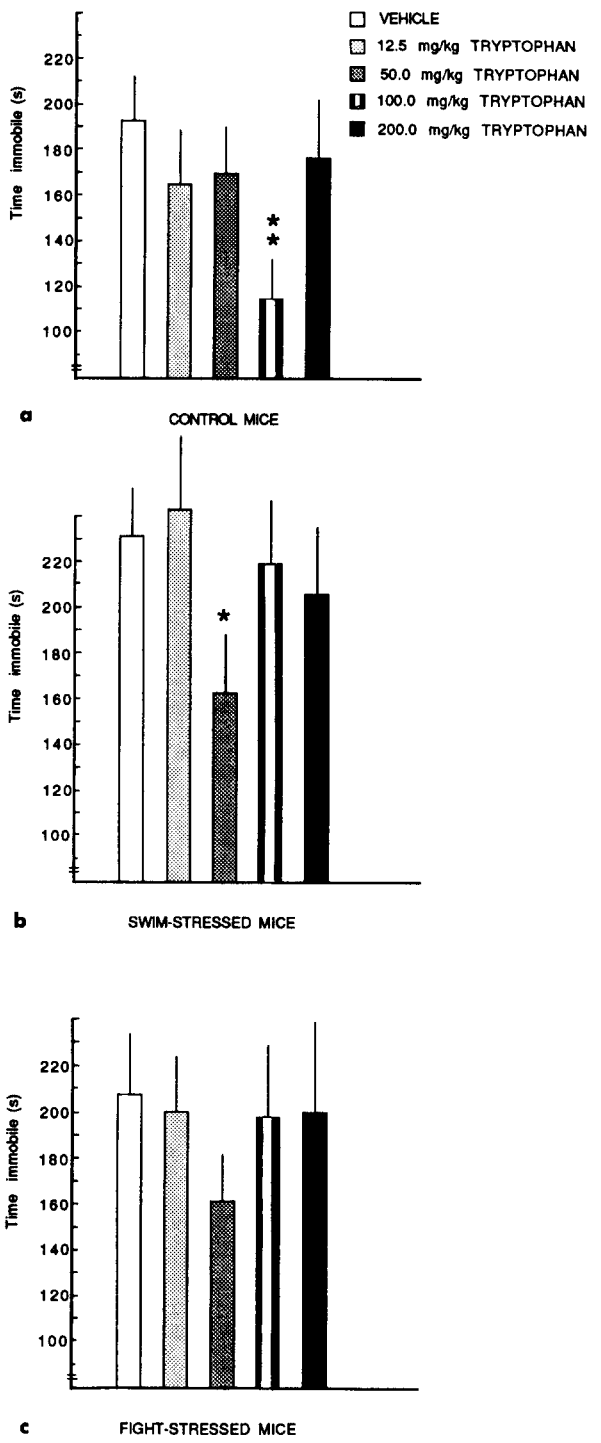

FIG. 1. The effect of 0, 12.5, 50, 100 and 200 mg/kg tryptophan on duration of immobility in the swim test of control (a), swim-stressed (b) and fight-stressed (c) mice. Means+SEM of 8–43 mice per group are shown. $^{**}p<0.01$; $^{*}p<0.05$.

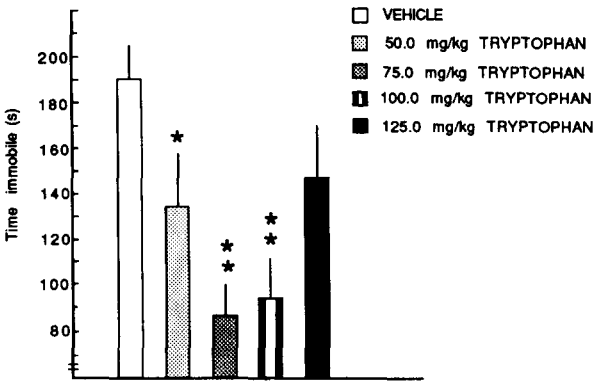

FIG. 2. The effect of 0, 50, 75, 100 and 125 mg/kg tryptophan on duration of immobility in the swim test of nonstressed control mice. Means+SEM of 10–12 mice per group are shown. $^{**}p<0.01$; $^{*}p<0.05$.

of the fight-stressed mice at any dose tested (Fig. 1c).

Experiment 2. As in Experiment 1, tryptophan had an U-shaped dose-response relationship on immobility in the control mice in the swim test, $F(4,45)=5.35$, $p<0.001$ (Fig. 2). In this experiment 50 mg/kg ($p<0.05$) and 75 and 100 mg/kg ($p<0.001$) tryptophan significantly shortened immobility. However, 125 mg/kg tryptophan did not significantly alter the immobility times in the swim test.

Holeboard

The number of head-dips, the time spent head-dipping or locomotor activity scores did not differ between the control mice receiving saline or 12.5 or 50 mg/kg tryptophan (Table 1a). Similarly, 100, 200 or 300 mg/kg tryptophan did not affect these holeboard measures either in the control or the fight-stressed mice (Table 1b). The fight-stressed mice, however, showed lower overall locomotor activity scores than the nonstressed mice, $F(1,68)=4.76$, $p<0.05$.

DISCUSSION

The doses of tryptophan given in the swim test study varied from 12.5 to 200 mg/kg. This dose range was chosen because in rats 25 mg/kg tryptophan is reported to cause a 100% increment in brain tryptophan, and a 50% increment in brain 5-HT concentration (3). A maximal (approximately 80%) increase in brain 5-HT is reported following administration of 100 mg/kg tryptophan (3). In the present study, tryptophan (100 mg/kg) significantly short-

TABLE 1

(a) THE EFFECT OF 0, 12.5 AND 50 mg/kg TRYPTOPHAN ON THE HOLEBOARD MEASURES IN THE CONTROL MICE

	Number of Head-Dips	Time Spent Head-Dipping (sec)	Locomotor Activity Scores
Tryptophan			
0 mg/kg	73.9 ± 5.5	35.5 ± 2.6	164.7 ± 7.2
12.5 mg/kg	62.9 ± 5.4	32.0 ± 4.4	167.5 ± 12.0
50.0 mg/kg	72.8 ± 5.6	32.9 ± 2.7	173.0 ± 7.5

Means ± SEM of 10 animals per groups are shown.

(b) THE EFFECT OF 0, 100, 200 AND 300 mg/kg TRYPTOPHAN ON THE HOLEBOARD MEASURES BOTH IN THE CONTROL AND FIGHT-STRESSED MICE

	Number of Head-Dips	Time Spent Head-Dipping (sec)	Locomotor Activity Scores
Tryptophan			
0 mg/kg			
C*	52.5 ± 4.1	26.0 ± 3.1	163.3 ± 7.2
FS	52.3 ± 6.7	27.6 ± 4.8	142.5 ± 7.0
100 mg/kg			
C	50.0 ± 3.8	27.3 ± 4.2	161.5 ± 8.8
FS	46.8 ± 6.3	24.1 ± 4.1	147.0 ± 11.4
200 mg/kg			
C	56.9 ± 4.1	28.2 ± 4.1	150.8 ± 5.8
FS	47.8 ± 5.5	29.7 ± 4.3	153.0 ± 12.0
300 mg/kg			
C	51.0 ± 5.0	25.2 ± 4.6	163.3 ± 5.0
FS	39.7 ± 4.6	22.7 ± 5.2	143.6 ± 10.3

*C = control mice.
FS = fight-stressed mice.
Means ± SEM of 8–11 animals per group are shown.

ened the immobility time of nonstressed mice in the swim test. Fifty and 75 mg/kg tryptophan also shortened immobility time (although this effect for 50 mg/kg was not statistically significant in Experiment 1). Following administration of doses of tryptophan greater than 125 mg/kg no significant change in immobility time was seen compared to saline injected controls. Thus tryptophan had an U-shaped dose-response relationship on immobility in the swim test.

For the effects of tryptophan to be observed in the swim test, preexposure to a stressor was not necessary. In fact, tryptophan changed immobility less clearly in the swim- or fight-stressed mice than in the controls. The results, however, do not indicate that the nonstressed and stressed mice responded to tryptophan in a different manner. Tryptophan thus seems to differ from the 5-HT uptake inhibiting compounds in its efficacy to shorten immobility of nonstressed animals in the swim test (2,5).

It has been reported that 5-HTP, which is synthesized from tryptophan and is an immediate precursor of 5-HT, reduces the duration of immobility in the swim test; however, this is seen at doses that increase locomotor activity (9). In the present study tryptophan shortened immobility time in a dose range which did not alter locomotor activity as measured in the holeboard test.

The cause of the U-shaped dose-response relationship for tryptophan on the duration of immobility in the swim test is unclear. The lack of efficacy of higher doses of tryptophan does not appear to be due to the emergence of sedative effects (which might mask any shortening of immobility time), since none of the doses tested showed any significant effect on locomotor activity in the holeboard test. Interestingly, while tryptophan showed no effects on locomotor activity in either control or fight-stressed mice, the latter did show an overall relatively low level of locomotor activity, as has been previously noted (6).

It is, of course, possible that the effects seen here are due to some intrinsic effect of tryptophan unrelated to its 5-HT elevating properties. Furthermore, it remains unclear whether the effects of tryptophan on immobility in the swim test are mediated by factors within or outside the central nervous system. For example, elevation of peripheral 5-HT levels has been reported to induce hypoglycemia, which could affect the duration of immobility in the swim test (11).

In conclusion, tryptophan was found to have an U-shaped effect on immobility in the swim test: 0–100 mg/kg tryptophan caused a dose-related shortening and doses higher than that did not affect the immobility times. The results show that tryptophan's effect resembles those of a number of antidepressant drugs in the swim test [for review, see (13)].

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