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Potentiation by Propranolol of Stress-Induced Changes in Passive Avoidance and Open-Field Emergence Tests in Mice

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STONE, E. A., M. NAJIMI AND D. QUARTERMAIN. *Potentiation by propranolol of stress-induced changes in passive avoidance and open-field emergence tests in mice.* PHARMACOL BIOCHEM BEHAV 51(2/3) 297-300, 1995.—Noradrenergic and serotonergic systems are known to be stimulated during various forms of stress. The present study examined the effect of the β-adrenergic serotonin$_{1A}$ receptor blocker propranolol on the ability of stress to elicit behavioral inhibition in mice. Mice were given the drug before immobilization or tube-restraint stress, and then were tested for either passive avoidance performance or time to emerge into an open field. Propranolol markedly potentiated stress-induced increases in latency in both of these tests, suggesting that it exacerbated reactions to stress. These results agree with previous data indicating that under certain conditions, propranolol can potentiate the effects of stress in rodents. The results support the hypothesis that the response of the noradrenergic and/or serotonergic systems to stress may have anxiolytic or antistress effects.

Stress Mouse Fear Passive avoidance Emergence test Propranolol

PROPRANOLOL, the β-adrenergic and serotonin$_{1}$ receptor antagonist, has been shown to reverse the behavioral effects of several types of stress and stress-related substances (2,3,6,13). However, the drug has also been found to exacerbate certain of these effects. Microinjections of propranolol into the amygdala has been shown to lead to an increased degree of gastric pathology folowing restraint (10). This has suggested that the noradrenergic and serotonergic responses to stress may have antistress effects under some conditions. We recently found in preliminary experiments that systemic propranolol potentiated the effect of immobilization stress on passive avoidance behavior. Because this effect appeared to be further evidence of a protective effect of aminergic responses, we undertook the present experiments to replicate and extend these preliminary studies.

METHOD

Animals

Subjects for this study were male Swiss-Webster mice (Harlan Hsd : ND4) 6–8 weeks of age and 20–30 g body wt. Animals were housed five per cage on a 12 L : 12 D cycle (lights on 0700 h) with food and water available ad lib.

Induction of Stress

Two forms of stress were employed: immobilization, in which the animal's paws were taped to a platform, and tube-restraint, in which the animal was enclosed in a conical tube 7.5 cm long and 3.5 cm diam. at its widest point, tapering to a blunt end of 1.5 cm. The stressors lasted 1 h. Neither stress produced signs of pain in the animals, as judged from the lack of vocalization. The animals, however, were emotionally aroused and showed struggling, urination, and defecation. The stress protocols had been approved by the New York University School of Medicine IUCAC.

Apparatus and Procedures

Passive avoidance learning and retention was studied in a standard mouse shuttle-box (BRS/LVE MSC-002, Laurel, MD) adapted for step through passive avoidance. The safe compartment, which was 13 × 13 × 9 cm, was constructed from clear Plexiglas with one wall painted white and a solid floor made from a white cardboard insert. The compartment was covered with a white plastic lid with a 28-V lamp in the center that was illuminated during training and testing. The shock chamber was the same size as the safe side with a floor

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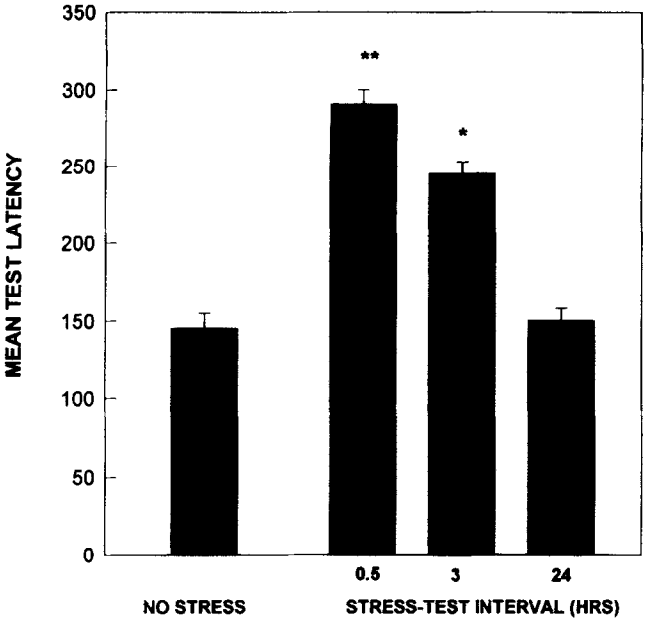

FIG. 1. Mean passive avoidance test latencies (±SEMs) for mice tested either 0.5, 3, or 24 h after 1 h of immobilization stress. The nonstress control group indicates the amount of avoidance in nonimmobilized mice. Groups were composed of 13–15 animals. *$p < 0.02$; **$p < 0.001$ compared to nonstress controls.

constructed from stainless-steel rods through which a scrambled shock (0.2 mA, 0.5 s duration) could be delivered from a constant current shock source (Coulbourn Instruments, Allentown, PA). The walls were painted flat black and the compartment was covered with a black Plexiglas lid. The two compartments were separated by a dividing wall that contained a guillotine door. The procedure was as follows: Mice were placed in the safe compartment, and after 10 s the guillotine door was raised. When the animal crossed into the dark side the door was lowered and a single 0.2-mA, 0.5-s duration foot-shock was automatically delivered. Retention of the avoidance response was tested 24 h after training and 1 h after immobilization stress. Animals not crossing into the dark side within 300 s were assigned that latency as a test score.

Emergence into an open field was examined in a standard mouse photoactometer. The arena was 46 cm in diameter with walls 41 cm high. The floor of the apparatus was white and was illuminated by a 90-W lightbulb positioned 50 cm above the floor. A metal container, 10 cm long and 6.5 cm in diam., mounted on a Plexiglas base, was placed in the center of the arena. The procedure was as follows: 30 min after immobilization or control treatment, mice were introduced individually to the photoactometer. Mice were placed in the container and time to emerge into the open field was recorded. Mice failing to emerge within 400 s were given this latency as a test score. Control mice were placed in the container directly from the home cage.

Drug Administration

L- and D-Propranolol HCl (Sigma) were freshly dissolved in isotonic saline and injected subcutaneously (SC) in a volume of 10 ml/kg body wt.

RESULTS

Effects of Immobilization Stress on Passive Avoidance and Emergence Test

The first experiment was performed to verify the effectiveness of the stressor and characterize its temporal effects. Groups of mice were trained in passive avoidance and subjected 24 h later to immobilization stress. Passive avoidance was tested 0.5, 3, and 24 h postimmobilization. Latencies are shown in Fig. 1.

Stress significantly increased latency [one-way analysis of variance (ANOVA): $F(3, 52) = 8.35$, $p < 0.001$]. Multiple comparisons by the Newman-Keuls method showed that the 0.5-h ($p < 0.001$) and 3-h groups ($p < 0.02$) were significantly different from the control group, but the 24-h group was not.

The effect of stress on emergence into an open field was examined in two groups of mice. One group was subjected to immobilization stress for 1 h and tested 30 min later. A control group that was not immobilized was tested directly from the home cage. Mean ± SEM latency for stressed mice was 165.87 ± 35.0 s ($n = 15$) and for nonstressed, 56.93 ± 22.3 s ($n = 15$). This difference was statistically significant [$t_{28} = 2.624$, $p = 0.014$].

Effects of Propranolol on Immobilization Stress

Passive avoidance. Mice were first given passive avoidance training and then injected 24 h later with either saline or L-propranolol 30 min before being subjected to either control conditions or immobilization stress. They were tested for avoidance retention 30 min after the stress. Table 1 shows the results. A two-way ANOVA revealed a nonsignificant effect of stress [$F(1, 46) = 3.62$, $p = 0.06$], a significant effect of L-propranolol [$F(1, 46) = 9.82$, $p = 0.003$], and a nonsignificant interaction of stress × propranolol [$F(1, 46) = 2.95$, $p = 0.09$]. Planned comparisons using the Newman-Keuls test indicated that the stressed group given L-propranolol differed significantly from the stressed group given saline ($p = 0.014$), but that the nonstressed group given L-propranolol did not differ significantly from the nonstressed group given saline ($p = 0.89$). An additional group of mice given D-propranolol and subjected to stress did not differ significantly from the saline-stressed group (D-propranolol, 289.3 ± 17.2 ($n = 10$).

This experiment was replicated using a lower dose of L-propranolol (2.5 mg/kg) with only two groups, the saline stress and the propranolol stress groups. Propranolol at this lower dose also resulted in a significant increase in latency

TABLE 1

EFFECT OF L-PROPRANOLOL AND IMMOBILIZATION STRESS ON PASSIVE AVOIDANCE

Group	*N*	Stress	Drug	Test Latency
1	13	No	Sal	173.0 ± 48.5
2	10	Yes	Sal	258.0 ± 77.0
3	12	No	Prop 10	184.0 ± 60.6
4	15	Yes	Prop 10	475.8 ± 55.5*

Propranolol or saline was injected 30 min before immobilization and a passive avoidance retention test was conducted 30 min poststress. Values are mean latencies + SEMs.

*$p < 0.02$ vs. group 2.

TABLE 2

EFFECT OF L-PROPRANOLOL AND IMMOBILIZATION STRESS ON EMERGENCE INTO OPEN FIELD

Group	N	Stress	Drug	Test Latency
1	16	No	Sal	64.4 ± 22.2
2	10	No	Prop	96.6 ± 55.2
3	15	Yes	Sal	165.9 ± 35.0
4	12	Yes	Prop	338.7 ± 41.5*

Propranolol or saline was injected 30 min before immobilization and the animals were tested for emergence into an open field 30 min poststress. Values are mean latencies ± SEMs.

*$p \leq 0.005$.

[saline, 239.1 ± 57.7 (n = 9), propranolol, 521.4 ± 52.7 (n = 11), $t_{18} = 3.61, p < 0.01$].

Emergence test. Mice were injected with either saline or propranolol (2.5 mg/kg) and subjected 30 min later to either control conditions or immobilization stress for 1 h. The animals were tested for emergence into an open field 30 min after the stress. The results are shown in Table 2. Two-way ANOVA revealed a significant effect of stress [$F(1, 51) = 26.13, p < 0.0001$], a significant effect of propranolol [$F(1, 51) = 9.54, p = 0.003$], and a significant stress × propranolol interaction [$F(1, 51) = 4.62, p < 0.05$]. Planned comparisons (Newman-Keuls) revealed that the propranolol nonstressed group was significantly different from the saline stressed group ($p = 0.0004$), but that the propranolol nonstressed group was not significantly different from the saline nonstressed group ($p = 0.53$).

Effect of Propranolol on Tube-Restraint Stress

Mice that had been trained in the passive avoidance task were given either saline or L-propranolol 10 mg/kg 30 min before tube restraint and were tested for retention 30 min afterward. The propranolol-treated group (196.5 ± 52.5, n = 11) did not differ significantly from the saline-treated group (103.3 ± 43.2, $n = 11, t_{20} = 1.36, p < 0.2$), although the difference was in the same direction as that for the tape restraint procedure.

DISCUSSION

As discussed earliier, there have been reports that propranolol can potentiate the effects of stress in rodents. The present results appear to be further evidence of this phenomenon. The drug clearly increased poststress measures of behavioral inhibition in the present studies. This was found to hold over a range of doses (2.5–10 mg/kg) and for two behaviors that are known to be sensitive to stress, passive avoidance and emergence into an open field. Although propranolol did not give a statistically significant increase with tube restraint, the direction of the effect was the same as that found for the tape immobilization procedure, thus suggesting that the effect was probably not peculiar to the latter form of stress.

Our study does not agree with previous findings of decreased behaviorally inhibiting effects of stress after propranolol (13). The reason for this discrepancy is not clear at present. It does not seem to be the result of differences in the behaviors studied, because the emergence task was used in previous studies of the effects of propranolol that showed opposite results from ours. It also does not seem to be related to the type of the stressor used, as restraint in a plastic tube was also used in these studies. Furthermore, it does not seem to be related to the dosage of propranolol or to any sedative or neuromuscular effect of the drug, as both the earlier and present studied employed the 2.5-mg/kg dose, and no sedation or incoordination was observed in any animal at the highest dose used (10 mg/kg). Although the propranolol-treated mice did not emerge from the start compartment, they were observed to be quite active. One factor that we have not excluded is a species or strain difference. Most previous studies with propranolol used rats, whereas the present one employed mice. Furthermore, the only previous study in mice used a different mouse strain (6). Further research will be necessary to assess the role of this factor.

The effect of propranolol on the behavioral effects of stress may shed light on the function of brain or peripheral monoaminergic responses to stress. Stress is known to evoke increases in the release of norepinephrine (1,7,11) and serotonin (4,8) in most brain regions and in the periphery. The fact that propranolol, which blocks β-noradrenergic and serotonergic$_{1A}$ receptors, increased behavioral inhibition after stress suggests that one or both of these aminergic responses to stress may have antistress effects. In support of this we have recently shown that blockade of brain β-1 adrenoceptors enhances hypoactivity after stress (12), and Kennett et al. (9) found that blockade of serotonergic receptors enhances behavioral depression after stress. Glavin (5) also found that selective depletion of norepinephrine potentiates gastric pathology to restraint stress. Further studies on the effects of selective noradrenergic and serotonergic receptor blockers on reactions to stress are therefore warranted.

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