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Blockade of Effect of Stress on Risk Assessment Behavior in Mice by a Beta-1 Adrenoceptor Antagonist

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STONE, E. A., J. RHEE AND D. QUARTERMAIN. *Blockade of effect of stress on risk assessment behavior by a beta-1 adrenoceptor antagonist.* PHARMACOL BIOCHEM BEHAV **55**(2) 215–217, 1996.—Previous studies have shown that acute stress impairs risk assessment behavior in mice. The present study was undertaken to determine the role of beta adrenoceptors, which are known to be stimulated by stress, in this effect. Mice were treated with either a beta-1 antagonist, betaxolol, a beta-2 antagonist, ICI 118551, an alpha-1 antagonist, prazosin, or an alpha-2 antagonist, yohimbine, and 30 min later were subjected to a 1-h session of restraint stress. Thirty minutes after the stress the animals were tested for the entry latency, number of headpokes prior to entry, and the path of entry into a white open field from a small dark box. In agreement with previous findings, stress was found to markedly reduce risk assessment behaviors as reflected by a reduced entry latency, a reduced number of headpokes and a changed entry path from wall hugging to central entry. Betaxolol was found to prevent all of the above effects of stress dose dependently, whereas ICI 118551, prazosin, and yohimbine had no reversal effects. It is concluded that beta-1 receptor activation and possibly brain glycogen depletion is involved in the effects of stress on risk assessment behavior. **Copyright © 1996 Elsevier Science Inc.**

Risk assessment Stress Beta-1 adrenoceptor Betaxolol ICI 118551

WHEN rodents are confronted with a novel environment they engage in a variety of information gathering behaviors presumably to assess the danger of the situation. These behaviors, which include visual scanning and stretch and attend responses along with a reluctance to enter the new environment, have been termed risk assessment behaviors (1). Risk assessment can be readily measured using a novel open field in mice. Nonstressed mice will enter the field only after repeated (30–40) stretch and attend responses occurring over a 10–20-min period and will enter close to the walls. We and others have shown that this risk assessment behavior is grossly impaired by stress (1,7). We have found that acutely stressed mice enter the field after much fewer responses in about 1/10th the time as nonstressed animals and walk directly into the center of the field. How stress produces this dramatic behavioral change is not known. Because stress potently activates the central noradrenergic system, we have measured the effect of noradrenergic receptor blocking agents on this phenomenon. In the present article we report that blockade of beta-1 receptors with betaxolol selectively blocks this effect of stress.

METHOD

Male Swiss–Webster mice 6–8 weeks of age were used. The animals were housed in groups of five per cage and were maintained on a 12 L:12 D cycle (lights on 0700 h) with ad lib food and water. On the day of the experiment the animals were transported from the animal facility to the lab at 0730 h and given 3 h to habituate. At 1100 h they were administered one of several drugs or saline SC and 30 min later were subjected to either restraint stress or control conditions (undisturbed). The stress was administered by placing the animal for 1 h in a plastic tube 3.5 × 7.5 cm with air holes. Immediately after the stress the animals were returned to their home cages. Thirty minutes later they were placed individually in a small black box (21 × 12.5 × 12.5 cm, length × width × height) adjoining a larger white open field (45 × 42 × 22 cm, length×width× height) through a small opening (3.5 cm^2). The floor of the field by the opening was marked off into six equal angles radiating from the opening. The two closest to the walls (0–30° and 150–180°) were labeled 1, the two closest

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TABLE 1

EFFECT OF BETA ADRENERGIC RECEPTOR ANTAGONISTS ON EFFECT OF STRESS ON RISK ASSESSMENT BEHAVIORS

Pretreatment	Stress	Latency (s)	Headpokes	Entry Path
Saline	No	652 ± 64	24.3 ± 2.7	1.14 ± 0.97
Betaxolol 20 mg/kg	No	622 ± 97	26.1 ± 5.0	1.20 ± 0.20
ICI118551 2 mg/kg	No	612 ± 63	16.1 ± 3.2	1.33 ± 0.21
4 mg/kg	No	347 ± 217	7.3 ± 4.9	—
Prazosin 1 mg/kg	No	397 ± 110	7.6 ± 2.3†	—
Yohimbine 2 mg/kg	No	186 ± 73*	4.0 ± 1.0†	—
Saline	Yes	117 ± 46‡	4.8 ± 1.1‡	2.41 ± 0.21‡
Betaxolol 10 mg/kg	Yes	523 ± 134§	6.1 ± 2.0	—
20 mg/kg	Yes	457 ± 89#	17.4 ± 3.3¶	1.33 ± 0.33§
ICI118551 2 mg/kg	Yes	318 ± 93§	7.7 ± 2.4	1.88 ± 0.20
4 mg/kg	Yes	61 ± 32	1.5 ± 0.9§	—
Prazosin 1 mg/kg	Yes	321 ± 112	4.4 ± 1.6	—
Yohimbine 2 mg/kg	Yes	116 ± 87§	2.1 ± 1.6§	—

Values are means and SEMs. Groups ranged in size from 10–20 animals except for the ICI118551 4 mg/kg nonstressed group, which contained four animals.

*< 0.05, †< 0.005, ‡< 0.001 vs. nonstressed saline group, §< 0.05, ¶< 0.005, #< 0.001 vs. corresponding nonstressed drug treated group.

to the midline (60–90° and 90–120°) were labeled 3, and the two intermediate angles (30–60° and 120–150°) were labeled 2. The behaviors recorded were a) the number of times the animal stuck its head out prior to entry (head pokes), b) the latency to enter the field with all four paws, and c) the path by which the animal entered the field with a 1, indicating a path close to the walls, and a 3 indicating a path close to the midline.

Data were analyzed nonparametrically by the median test evaluated by Fisher's exact probabilities.

Drugs used were betaxolol (Synthelabo), ICI 118551 (Imperial Chemical Industries), prazosin (Pfizer), and yohimbine (Sigma).

RESULTS

The results are presented in Table 1. In agreement with our previous studies, restraint stress produced a marked reduction in risk assessment behaviors. Saline-injected stressed mice had a significantly shorter latency and significantly fewer head pokes than saline-injected control mice. Moreover, stressed mice entered the field directly into the center, whereas nonstressed mice stayed close to the wall. Betaxolol at 20 mg/kg significantly prevented the effects of stress on latency, head pokes, and on entry path. The drug did not affect these behaviors in nonstressed animals. Betaxolol at 10 mg/kg reversed the effect of stress on latency but not on head pokes (entry path was not measured in at this dose). ICI 118551 at 2 mg/kg did not affect latency, head poke number, or entry path in either the stressed or nonstressed groups. At 4 mg/kg there was also no effect on latency in either group but there was a reduction in head poke number in the stressed group. Entry path was not tested for this dose of ICI 118551. Prazosin had no effect on latency but reduced head poke number in nonstressed mice. Yohimbine reduced both latency and head poke number in both the nonstressed and stressed mice. Entry path was not tested for prazosin and yohimbine.

DISCUSSION

The present results show that the beta-1 selective antagonist, betaxolol, reverses the effect of stress on risk assessment behavior. In agreement with our previous study, we found that stress markedly reduced risk assessment behaviors as evidenced by a reduced latency to enter the field, a reduced head poke number, and an altered path of entry. Betaxolol reversed each of these effects of stress and did so dose dependently. At 10 mg/kg the drug reversed the latency decrease, and at 20 mg/kg it reversed both the latency and head poke decreases. The 20 mg/kg dose is known to selectively block brain beta-1 receptors, allowing for differences in dosage between rats and mice (12). These effects of betaxolol constitute a true reversal of the stress effects because they were only found in the stressed animals.

The reversal effect appears to be selective to betaxolol. ICI 118551, which is a beta-2 selective antagonist, did not have this effect at either 2 or 4 mg/kg. The 2 mg/kg dose has been shown to selectively block beta-2 receptors in the brain, allowing for differences in dosage between rats and mice (12). Although ICI 118551 at 4 mg/kg did have an effect on head poke number in the stressed mice, this change was in the direction of an exacerbation of the stress effect in contrast to the ameliorating action of betaxolol. The alpha-1 antagonist, prazosin, and the alpha-2 antagonist, yohimbine, also did not reverse the effects of stress on latency or head poke number. In contrast, yohimbine shortened latency and reduced head pokes in the nonstressed animals and exacerbated these effects in the stressed animals whereas prazosin reduced head pokes in the nonstressed mice.

How betaxolol reverses the effect of stress on risk assessment behavior is not yet entirely clear. A possible nonspecific sedative effect can be ruled out because in a previous study the drug had no effect on rotorod performance (10). Several findings suggest that betaxolol may act by preventing a decrease in beta-1 receptor function caused by excessive beta-1 receptor activation during stress. First, in previous research we have found evidence that acute stress produces a transient decrease in beta-1 receptor function in that both stress and beta-1 blockers produce similar decreases in open-field behavior (10) and arousal from anesthesia (9). Second, the beta-1 deficit after stress may be due to either a desensitization of beta-1 receptors (11,13) or a depletion of glycogen in beta-1

receptor occupied cells (4), effects known to occur after stress. If one assumes that risk assessment behavior is subserved by brain circuits containing beta-1 receptors or cells that are dependent for energy on glycogenolysis, then either of these effects could explain the stress-induced impairment of risk assessment. Betaxolol, by blocking beta-1 receptor activation during stress, would prevent both the receptor desensitization and the glycogen depletion (2,3,5,6,8) and, thus, would reverse the impairment. A contrary finding is that betaxolol had no effect on risk assessment behavior in nonstressed animals; however, it cannot be excluded that the drug was excreted before the behavioral test (2 h postinjection) and, therefore, only acted during the application of stress. The present results, thus, warrant further investigation of the roles of beta-1 receptor function and brain glycogen in risk assessment behavior and its impairment by stress.

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