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BRIEF COMMUNICATION

PD135158, a CCK-B Antagonist, Reduces "State," But Not "Trait" Anxiety in Mice

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BELZUNG, C., N. PINEAU, A. BEUZEN AND R. MISSLIN. *PD135158, a CCK-B antagonist, reduces "state," but not "trait" anxiety in mice.* PHARMACOL BIOCHEM BEHAV 49(2) 433–436, 1994. – It has recently been proposed that Balb/c neophobic responses in a free exploratory paradigm are related to "trait" anxiety, while the behavior of mice in the light/dark choice test paradigm is related to "state" anxiety. The purpose of this study was to assess the action of the CCK-B receptor antagonist PD135158 in both models. Results show that PD135158 was effective in the light/dark choice test but not on the Balb/c neophobic reactions in the free exploratory situation. It is suggested that PD135158 is specially effective in state anxiety induced by fear provoking situations.

Mice Light/dark choice test Neophobia Balb/c Free exploration test PD135158 CCK-B antagonists State anxiety Trait anxiety

SEVERAL lines of evidence suggest that selective cholecystokinin type B (CCK-B) receptor antagonists may elicit anxiolytic activity. For example, the CCK-B receptor antagonists L365260, CI988, and PD135158 reduced anxiety in mice confronted with a black/white box test and in rats confronted with an elevated plus-maze test, with the social interaction test, and with a model of conditioned suppression of drinking (5,10,11,14–17). By contrast, CCK-B agonists elicit anxiogenic effects (7,8). However, other authors failed to find any effect of L365260 in the black/white chamber model in mice (9), while others, using punished responding tests, reported the effect of CCK-B receptor antagonists to be weak when compared to benzodiazepines (8).

Recently, it has been shown that Balb/c mice exhibited strong neophobic responses when confronted simultaneously with a familiar and a novel compartment in a free exploratory pardigm (6). Drugs that bind in a nonselective manner to benzodiazepine recognition sites, such as chlordiazepoxide, diazepam, and RO198022, were able to completely abolish neophobia in Balb/c mice, while the 5-HT_{1A} receptor agonist, 8-OH-DPAT and the 5-HT_3 antagonist, zacopride, were ineffective in reducing neophobic responses of these mice (6). As no neurovegetative changes appear in mice that have a free access to novelty when compared to the modifications induced by situations in which these animals are forced (13), the free exploratory situation can be considered to be devoid of stressfull components. Therefore, the neophobia of Balb/c mice can be considered as a constant feature of their behavior. It has been suggested that the neophobia of this strain of mice is related to the type of anxiety termed by Lister (12) as "trait" anxiety which is different from the "state" anxiety induced by stressful situations (2).

The present study was designed to compare the pharmacological effects of a selective CCK-B receptor antagonist, PD135158, on the behavior of Balb/c mice confronted with the light/dark choice paradigm especially conceived to test state anxiety (1,2) (Experiment 1) and with the free exploratory test that reveals trait anxiety (Experiment 2).

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METHOD

Animals

Male Balb/c By JIco mice from Centre d'Elevage Iffa Credo (France), 10 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12 h reversed light : dark cycle with light on at 2000 h so that we could observe animals in their active period, that is between 1400 and 1700 h. Each mouse was tested only once in one experiment.

Experiment 1

Apparatus. The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One of these boxes was darkened; a light from a 100 W desk lamp above the other box provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) connected the dark box to the lit one. During observation, the experimenter sat always at the same place, next to the apparatus.

Procedure. The subjects were individually tested in 5-min sessions in the apparatus described above. Testing was performed between 1400 and 1600 h. Mice were placed in the lit box to start the test session. The amount of time spent in the lit box (TLB) and the number of transitions through the tunnel were recorded, minute per minute, during 5 min, after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes. Mice were naive to the apparatus.

Experiment 2

Apparatus and Procedure. The apparatus constisted of a polyvinylchloride box (30 × 20 × 20 cm) covered with Plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The apparatus was kept on a stand in the mouse room. The experimenter always stood next to the box in the same place. Approximatively 24 h before testing, each subject was randomly placed in one-half of the apparatus with the temporary partitions in place, to be familiarized with it. The floor of this half only was covered with fresh sawdust and the animal was given unlimited access to food and water during the familiarization phase. The duration of this period was 24 h. On the test day, the temporary partitions between the familiar and novel compartment were removed and the subject was then observed, under red light, for 10 min. The number of units entered (locomotion), the number of rears made by the animals (rears), the number of stretched postures that mice exhibit toward the novel place before their first entry into it (avoidance toward novelty), and the time spent in the novel area were recorded.

Experiment 1

Balb/c mice were randomly allocated to the following groups: PD135158 (0, 0.01, 0.1, and 1 mg/kg; $n = 12$).

Experiment 2

Mice were randomly allocated to the following groups: PD135158 (0, 0.01, 0.1, and 1 mg/kg; $n = 10$).

Drugs

PD135158 (RBI, France) was dissolved in saline and injected in concentrations giving an injection volume of 10 ml/kg. The solution was administered subcutaneously, 40 min before testing.

Statistical Analysis

Comparisons between groups were made using a combined ANOVA and the Duncan's a posteriori test.

RESULTS

Experiment 1

ANOVA revealed significant differences among groups for TLB, $F(3, 44) = 4.95$, $p < 0.01$, and transitions, $F(3, 44) = 5.29$, $p < 0.01$. Figure 1 shows that PD135158 significantly increased TLB at doses of 0.1 and 1 mg/kg and the number of

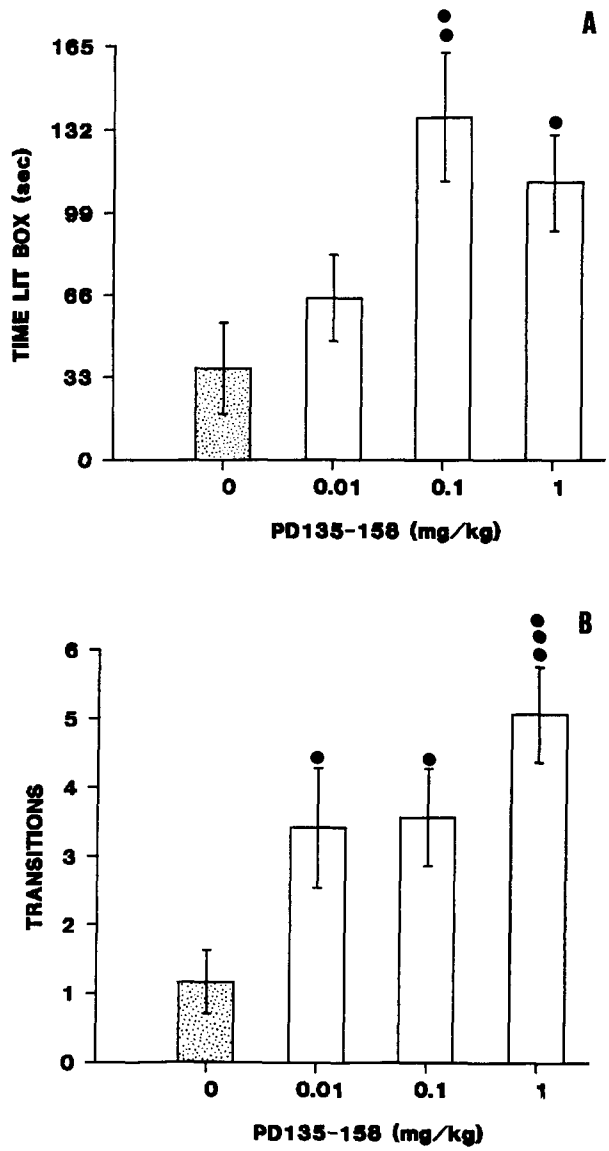

FIG. 1. Effects of PD135158 on time in lit box (TLB) (A) and number of transitions in the light/dark choice test (B) (mean ± SEM); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Duncan a posteriori t-test).

transitions at doses of 0.01, 0.1, and 1 mg/kg (Duncan a posteriori test).

Experiment 2

ANOVA revealed that PD135158 had no effects on the behavior of mice in the free exploratory paradigm: [locomotion, $F(3, 36) = 0.745$; rears, $F(3, 36) = 0.98$; avoidance toward novelty, $F(3, 36) = 0.187$; and time spent in the novel area, $F(3, 36) = 1.10$].

DISCUSSION

This study shows first that the CCK-B receptor antagonist PD135158 markedly increased the time spent in the lit box as well as the number of transitions between the two boxes in Balb/c mice confronted with the light/dark choice test.

This result confirms the anxiolytic properties of CCK-B receptor antagonists (11,17). However, it is to be noted that Hendrie et al. (9) did not find such effects with CCK-B antagonists. This discrepancy can be explained by the fact that these

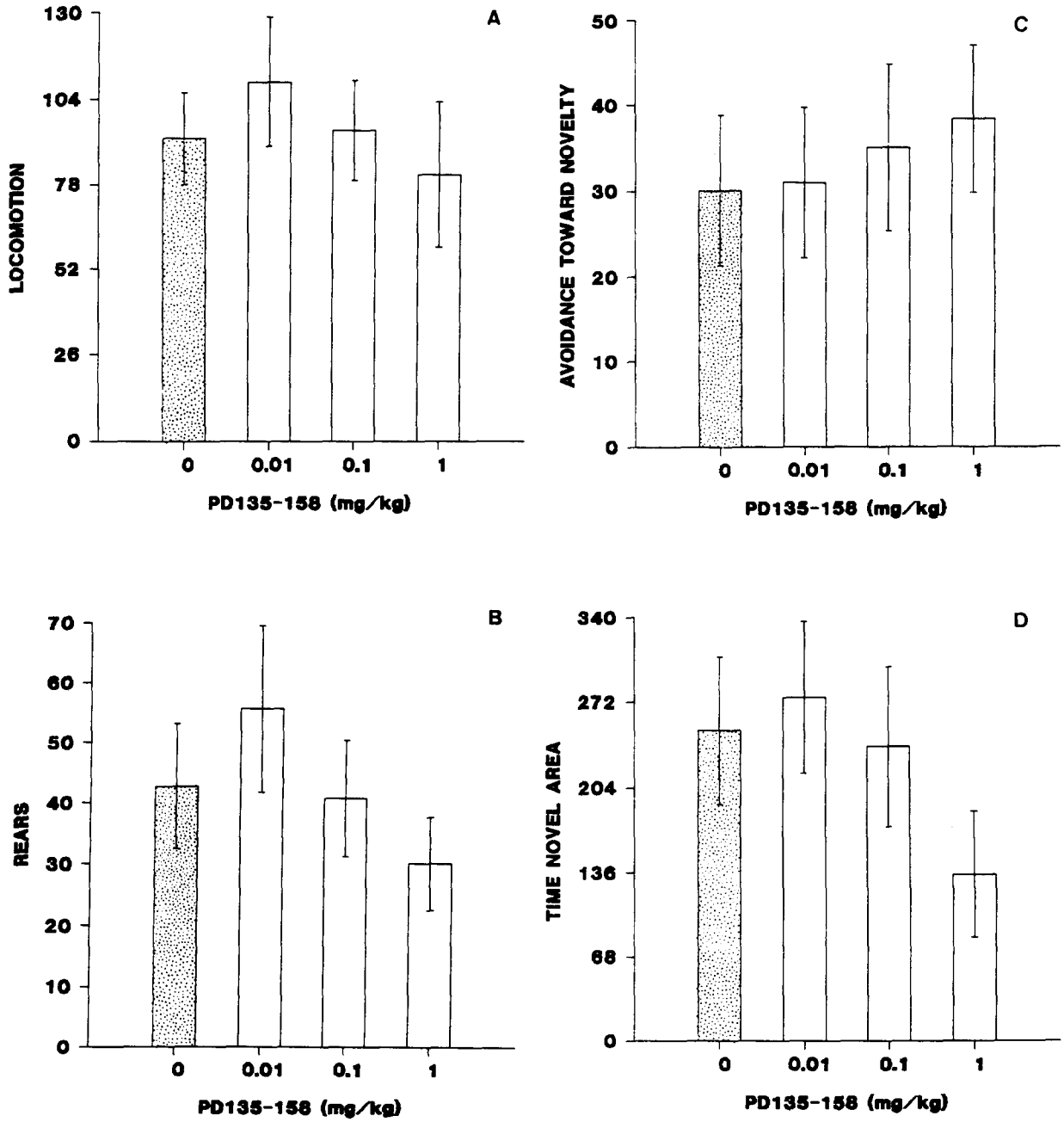

FIG. 2. Effects of PD135158 in the free exploratory test on locomotion (A), rears (B), avoidance toward novelty (C), and time spent in the novel area (D) (mean ± SEM).

authors used a different strain of mice (DBA/2 vs. Balb/c mice) and another type of apparatus. Indeed, their apparatus consisted of a box divided into two unequal parts, one-third being black painted and two-thirds being white painted and brighly illuminated. Crawley et al. (4) have shown that in such an apparatus no anxiogenic effects of benzodiazepine receptor inverse agonists have been found, whereas in the present test procedure, these effects were obvious (1).

Furthermore, this study shows that the CCK-B receptor antagonist PD135158 had no effect on the neophobia in Balb/c mice confronted with the free exploratory test paradigm. This lack of action of this drug closely resembles that observed with the 5-HT_{1A}receptor agonist, 8-OH-DPAT, and with the 5-HT_3 receptor antagonist, zacopride, in Balb/c mice confronted with the same experimental design (6). Because the free exploratory test has been found to be devoid of stressful components (13), it has been sugggested that neophobic reactions of Balb/c mice are revealed rather than induced by this situation, and, thus, they are related to the so-called trait anxiety (12). It is to be noticed here that, when the Balb/c mice were confronted to the light/dark choice situation, their trait anxiety still exists because it is a constant feature of is strain, but it is not measured by the parameters that are presented here. Indeed, in this apparatus, the time spent in lit box and number of transitions may model state anxiety while other variables, such as time spent in the tunnel, model trait anxiety (3). The CCK-B receptor antagonist PD135158 acts on the two first parameters (Experiment 1) but not on the time spent in the tunnel (results not shown). Taken together, these data lead to the suggestion that CCK-B receptor antagonist, PD135158, is active only in particular experimental conditions, i.e., in models of fear-motivated and constraining situations referred by Lister (12) as provoking state anxiety. For example, Haro et al. (8) demonstrated that extremely low doses of CCK agonists, ceruletide and pentagastrin, were active in rodents housed under overcrowded conditions, while the same doses were ineffective in rats housed in normal conditions. It has also been proposed by Griebel et al. (6) that the lack of 5-HT drugs in reducing neophobia of Balb/c mice is probably related to the fact that these compounds are only effective in stressful situations.

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