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Pharmacological evaluation of the stress-induced social avoidance model of anxiety

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Abstract

We have shown earlier that mild electric shocks induce a lasting social avoidance in male rats. Here we investigated whether shock-induced social avoidance can be developed into a laboratory model of stress-induced anxiety. The putative new model would assess sub-chronic, stress-induced anxiety (as opposed to tests based on natural fear) in a heterologous context (as opposed to classical fear conditioning). A single exposure to mild electric shocks induced a robust social avoidance that lasted more than 5 days. Low doses of chlordiazepoxide (0.5, 1 mg/kg), diazepam (0.5, 1, 5 mg/kg), buspirone (0.3, 1 mg/kg), and fluoxetine (1, 3, 5 mg/kg) abolished this effect, whereas the anxiogenic compound *m*-chlorophenylpiperazine (0.5–3 mg/kg) induced social avoidance in unshocked rats. These effects were produced at doses that did not affect locomotion in the open field. Haloperidol (0.05, 0.1, 1, 5 mg/kg) influenced social avoidance at sedative doses only. The sensitivity of the model to anxiolytic agents was compromised at high (sedating) doses. Taken conjointly, these data show that shock-induced social avoidance can be used to assess the anxiolytic potential of compounds. In addition to predictive validity, the model appears to show construct and face validity as well: stress is among the etiological factors of, whereas social avoidance simulates the social deficits seen in, a variety of anxiety disorders. The model may be used to study the effects of anxiolytics on sub-chronic states of stress-induced anxiety.

Keywords: Stress; Anxiety; Benzodiazepines; Buspirone; Fluoxetine; *m*-Chlorophenylpiperazine

1. Introduction

We have shown previously, that strong acute stressors (defeat in aggressive encounters, electric shocks) lead to a lasting social avoidance which could be reduced by low doses of chlordiazepoxide and buspirone [27,30]. Stress is among the etiological factors of a variety of anxiety disorders (e.g., separation anxiety, generalized anxiety, social phobia, acute stress disorder, post-traumatic stress disorder) [4,13,14,18,32,34,36,40,45,46]. In addition, social avoidance is a symptom of many anxiety disorders including generalized anxiety [35,47,54]. Therefore, we hypothesized that the lasting effects of strong stressors on social avoidance can be developed into a model of anxiety.

The available tests of anxiety are of three major types. One type is based on the unconditioned fear of rodents from electric shocks (punished drinking test [53]), unknown social partners (social interaction test [21]), strong light (light/dark test [15]), open spaces/height (plus-maze test [44]), and predators (anti-predator defensive behaviours in a visible burrow system [7]). These tests exploit natural fears. The second type of anxiety tests is based on conditioned fear responses (e.g. fear-potentiated startle [11], contextual fear-induced freezing [10], conditioned defensive burying [51], anticipatory fear [39], conditioned emotional response [17], mouse defence test battery [8]). In these tests, subjects express fear when facing cues or contexts associated earlier with aversive experiences. The third type of tests also involves aversive experiences, but testing is performed in a context that is different from the stressing context. For example Korte and De Boer [38] showed that the exposure of rats to a context that was previously paired with electric shocks induced anxiety-like behaviour in the elevated plus-maze. Anxiety lasted

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up to 90 min. Similarly, the short lasting exposure of rats to a cat results in – this time long lasting – anxiety in the elevated plus-maze [1,2]. Cat exposure was proposed to model post-traumatic stress disorder.

The shock-induced social avoidance test would belong to the third type (stress-induced anxiety in heterologous contexts), but would be different from the model of both Adamec et al. [1] and Korte and De Boer [38]. It differs from the latter as shock-induced social avoidance lasts days, whereas the re-exposure of rats to aversive stimuli affects plus-maze anxiety for about 90 min. The difference from the model of Adamec et al. [1] is that shock-induced social avoidance (at the shock intensities applied so far) does not seem to model post-traumatic stress disorder. This is suggested by pharmacological evidence: both chlordiazepoxide and buspirone reduced shock-induced social avoidance [27,30], yet neither compound is effective against post-traumatic stress disorder. Thus, shock-induced social avoidance would potentially model sub-chronic generalized anxiety.

The pharmacological responsiveness of rats exposed to the shock-induced social avoidance model was insufficiently characterized so far. To extend disparate earlier observations, we assessed here the effects of two benzodiazepines (chlordiazepoxide and diazepam), two serotonergic anxiolytics (buspirone and fluoxetine), an anxiogenic compound (*m*-chlorophenylpiperazine, *m*-CPP), and the dopamine antagonist haloperidol. Noteworthy, haloperidol is frequently used as a negative control in anxiety test validations (see e.g. [19,42,52]), whereas *m*-CPP is well known for its anxiogenic effects in both rodents and humans [6,25,43]. In earlier experiments, buspirone and fluoxetine provided inconsistent effects in classical tests of anxiety, despite their clinical usefulness [9,48]. Therefore, their assessment in the new model appeared especially important.

2. Methods

2.1. Animals and housing

Male Wistar rats (Charles–River Laboratories, Hungary) weighing approximately 250–300 g were used. Rats were maintained in a 12:12 h reverted day–night schedule (lights on at 07:00 h) under standard laboratory conditions (temperature: 21 ± 2 °C; humidity: 60 ± 10%). Standard laboratory food (Charles–River Laboratories, Hungary) and tap water were freely available. Animals were isolated 5 days before stress application and housed individually. Cages size was 20 cm × 40 cm × 25 cm (height). All studies were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committees of the Institute of Experimental Medicine and EGIS Pharmaceuticals Ltd.

2.2. Shock exposure

Electric shocks were delivered 1 day before the social avoidance test, via the grid floor of a transparent Plexiglas box (25 cm × 25 cm × 25 cm). An alternating current of 100 V and 3 mA was applied. Shock duration was 0.01 s; shocks were delivered in trains lasting 1 s with an inter-shock interval of 0.02 s. Two trains per min were delivered over 12 min.

2.3. The social avoidance test

The test was performed in two plastic cages connected by a sliding door as shown in Fig. 1 (upper panel). The cages were open in the upper part; their walls were 40 cm high. The experimental room was lit by dim red light. The subject was placed in the smaller cage (surface: 15 cm by 50 cm) for a 3 min habituation period. The larger cage (surface: 40 cm by 40 cm) was divided into two equal compartments by a transparent, perforated plastic wall. The distant compartment contained a large unfamiliar male. This unfamiliar stimulus opponent was unshocked. After the habituation period, the sliding door was removed, and the subject was allowed to explore the cage for 5 min. The test apparatus did not permit physical contact between the experimental and stimulus animals. It should be noted that subjects had a clear view of the confined opponent when the door was removed, but not before. Despite the fact that smell is more important for rats than vision, the visibility of the opponent proved important in these experiments (see also Section 4). Behaviour was video-recorded from above and later analysed. Two variables were recorded: the number and duration of visits made to the compartment containing the opponent ("opponent entries" and "%opponent time", respectively). Behavioural scoring was done by experimenters blind to treatment conditions.

2.4. Locomotor activity

Locomotor activity was measured in an open field equipped with a photobeam system (Biochemical Laboratory Service Inc., Hungary). Rats were placed into the corner of a Plexiglas chamber (57 cm × 20 cm × 28 cm) and were allowed to explore it for 5 min. Horizontal activity was recorded as a consecutive break of two photo beams, spaced at 190 mm from the walls and each other, and placed 40 mm above the floor.

2.5. Drugs and doses

The following compounds were assessed in the social avoidance model: chlordiazepoxide (0, 0.5, 1, 2.5, 5 and 10 mg/kg), diazepam (0, 0.5, 1, 5 and 10 mg/kg), buspirone (0, 0.3, 1, 3 and 10 mg/kg), fluoxetine (1, 3, 5, 10 and 20 mg/kg), haloperidol (0, 0.05, 0.1, 1 and 5 mg/kg), and *m*-CPP (0, 0.5, 1 and 3 mg/kg). In the open field, we assessed the effects of chlordiazepoxide (0, 5, and 10 mg/kg), diazepam (0, 5, and 10 mg/kg), buspirone (0, 3, and 10 mg/kg), fluoxetine (0, 1, 5, and 10 mg/kg), and *m*-chlorophenylpiperazine (0, 0.5, 1, and 3 mg/kg). Chlordiazepoxide and *m*-CPP came from SIGMA (St. Louis, USA). Other compounds were synthesised by the EGIS Pharmaceuticals. Diazepam and chlordiazepoxide were dissolved in 0.4% methylcellulose, whereas other compounds were dissolved in saline. All compounds were injected intraperitoneally in a volume of 2 ml/kg.

2.6. Experimental design

Different groups of naive rats were used in each of the following experiments.

Experiment 1 tested the time-curve of shock-induced social avoidance. Rats were either left undisturbed or shocked 10, 5, or 1 days before behavioural testing ($N = 8$ per group). Rats were tested in the social avoidance paradigm in a random order.

Experiment 2a, 2b, and 2c assessed the effects of chlordiazepoxide, diazepam, and buspirone in animals that were exposed to electric shocks 1 day earlier. Vehicle-treated, not shocked rats were used as controls. Pharmacological treatments were administered 30 min before behavioural testing. Sample size was 8 per group throughout. The three compounds were assessed in separate experiments; and doses were randomized within each experiment.

Experiments 3a and 3b assessed the effects of fluoxetine under conditions similar to experiment 2. In experiment 3a, we assessed doses that are usually applied with this compound (5, 10, and 20 mg/kg; sample size was 8 per group). As the lowest dose abolished the effects of shock exposure, whereas the larger doses appeared to inhibit locomotion, we have assessed lower doses in experiment 3b (1, 3 and 5 mg/kg; sample size was 8 per group). Thus, the 5 mg/kg dose of fluoxetine was assessed twice (in experiments 3a and 3b, respectively).

In experiment 4, we assessed the effects of haloperidol 1 day after shock exposure. Testing was performed 30 min after the administration of vehicle or haloperidol. Experiment 4a assessed the effects of large doses (0, 1 and 5 mg/kg), whereas experiment 4b assessed the effects of lower doses (0, 0.05, and 0.1 mg/kg). Sample size was 8 in both cases, whereas treatments were randomized.