A New Natural Model of Increased Anxiety in Rats

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The availability of a suitable model is a prerequisite for the experimental study of the pathogenesis of anxiety and phobia. Experimental simulation of anxious-phobic disturbances is performed by exposure to systemic or central pharmacological influence, electolytic or surgical damage to certain structures of the brain, electrical stimulation of emotiogenic structures, exposure to stress, and other methods [3,7].

In the present work we attempted to identify individuals with an initially high level of anxiety in a natural rat population by using a novel method of assessing anxious-phobic states in rats, based on a battery of tests characterizing species-specific responses of the animal to ethologically appropriate test stimuli provoking manifestations of anxiety and fear [1], and to analyze the changes of the level of anxiety and phobia in such animals for the effect of different psychotropic preparations.

MATERIALS AND METHODS

The study was carried out on 210 male albino Wistar rats weighing 230-300 g. The animals were kept under the usual conditions in the vivarium (5-7 rats in a cage, natural dark-light cycle) and permitted free access to food and water.

The level of anxiety and phobia in the animals was determined with the aid of the multiparametric method of assessing states of anxiety and phobia in points, which was developed by us [1] and slightly modified (the dimensions of the parallelepiped in the "step-down" test were 17×17×10 cm). The animals, grouped in fours, were examined from 10:00 to 12:00 h.

The following pharmacological preparations were used in the study: pentylenetetrazole (ampoules, Tallinn Khimfarmzavod, Estonia) in a dose of 10 mg/kg, diazepam (ampoules, Gedeon Richter, Hungary) in doses of 0.1 and 0.6 mg/kg, phenazepam (tablets, Minmedbioprom, Russia) in a dose of 0.05 mg/kg, sodium valproate (powder, Leiras, Finland) in a dose of 200 mg/kg, haloperidol (ampoules, Gedeon Richter, Hungary) in doses of 0.01 and 0.5 mg/kg, melipramine (ampoules, Egis, Hungary) in a dose of 10 mg/kg, and sodium lactate (powder, Serva, DL-isomer, Germany) in a dose of 600 mg/kg. The preparations were diluted in pharmacopea physiological saline (ampoules, Voronezh Khimfarmzavod, Russia). Phenazepam was dissolved using TWEEN-80 (Serva, Germany) in a ratio of 0.5 mg of tablet preparation to 5 ml of TWEEN-80. All preparations and physiological saline were injected intraperitoneally in a volume of 0.1 ml/100 g animal weight. The animals were examined 20-30 min after a single injection of each of the preparations and 40 and 20 min after the injections of diazepam and pentylenetetrazole, respectively, for the combined administration of these compounds.

Different rat groups were used to study the effect of each preparation in each dose studied. The initial mean sum of the points according to the data of the primary examination of the rats in each group was taken as 100%. The interval between the primary and the experimental (postinjection) examination of the animals was 5-7 days.

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The results were statistically processed using the paired nonparametric Wilcoxon's T test and nonpaired parametric Student's t test with the aid of STATGRAPH software on an IBM PC/AT.

RESULTS

It was established in the preliminary experiments that the initial mean number of points characterizing the level of anxiety and phobia constituted 6.9±0.3 points (n=112) in the rats of the population studied (which comprised animals of different batches). Based on these data and taking into account the fact that normal point distribution is not rejected in the natural population of the animals [1], after the initial examination the rats were arbitrarily divided into "not anxious" (with an initial mean number of points less than 7.0) and "anxious" (with an initial mean number of points more than 7.0) animals. Further investigations on the effects of all preparations were carried out in the groups comprising initially "not anxious" and initially "anxious" animals.

Injection of physiological saline raised the level of anxiety and phobia in the group of initially "not anxious" rats (approximately by 40%, p<0.05, n=12) (Fig. 1, *2*) and did not alter the level of anxiety and phobia in the group of initially "anxious" rats (Fig. 2,*2*). Since the procedure of sham injection, including all the operations usually performed by the researcher down to the prick in the abdomen (without actual injection), did not change the level of anxiety and phobia either in "not anxious" or in "anxious" animals (Figs. 1, *1* and 2, *1*), this effect was produced by the physiological saline per se, and not simply by exposure to stress during the injection procedure.

Administration of preparations with anxiolytic properties (sodium valproate in a dose of 200 mg/kg, phenazepam in a dose of 0.05 mg/kg, and diazepam in doses of 0.1 and 0.6 mg/kg) to rats reduced the level of anxiety and phobia in the animals with an initially high level of anxiety (in the experimental groups n=7, n=8, and n=13, respectively; in all groups p<0.05) (Fig. 2, *3-6*). Injections of the same preparations in the above doses did not change the initial level of anxiety in the animals with an initially low level of anxiety and phobia (Fig. 1, *3-6*).

Administration of the anxiogen pentylenetetrazole in a dose of 10 mg/kg caused a marked (approximately by 170%) increase of the level of anxiety and phobia in "not anxious" animals (n=9, p<0.01) (Fig. 1, *7*) and a comparatively lesser increase of the level of anxiety and phobia

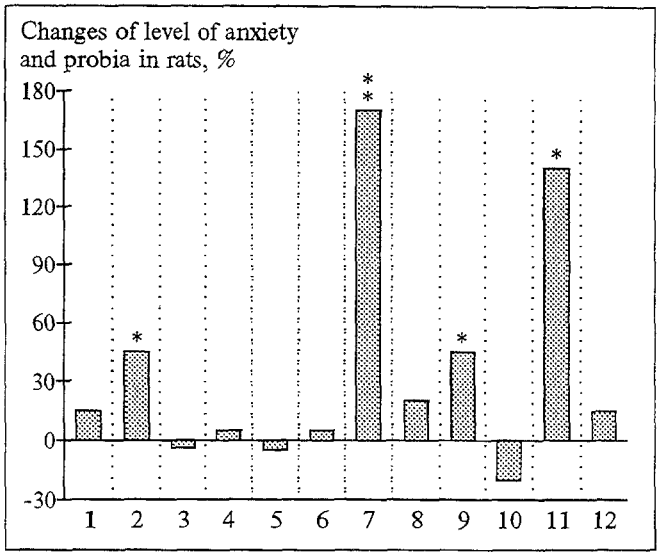

Fig. 1. Deviations (%) from initial mean sum of points (within the group) according to the scale for assessment of anxious–phobic states in rats with an initially low level of anxiety and phobia after injections of preparations producing various effects. Here and in Fig. 2: average control value of mean sum of points obtained during primary examination of each experimental group is taken as 100%; *1*) sham injection; *2*) physiological saline; *3*) sodium valproate, 200 mg/kg; *4*) phenazepam, 0.05 mg/kg; *5*) diazepam, 0.1 mg/kg; *7*) pentylenetetrazole, 10 mg/kg; *8*) diazepam, 0.6 mg/kg + pentylenetetrazole, 10 mg/kg; *9*) sodium lactate, 600 mg/kg; *10*) haloperidol, 0.01 mg/kg; *11*) haloperidol, 0.5 mg/kg; *12*) melipramine, 10 mg/kg; the investigation was started 20–30 min after a single injection of all preparations; in the case of combined administration of diazepam and pentylenetetrazole, the examination was started 40 and 20 min after injections of diazepam and pentylenetetrazole, respectively; ordinate: changes of level of anxiety and phobia in rats, %; asterisks indicate reliable differences as compared with the initial level of anxiety and phobia in the group (paired nonparametric Wilcoxon T test); one asterisk: p<0.05; two asterisks: p<0.01. *1*) n=8; *2*) n=12; *3*) n=7; *4*) n=7; *5*) n=9; *6*) n=9; *7*) n=9; *8*) n=8; *9*) n=8; *10*) n=7; *11*) n=9; *12*) n=12.

(approximately by 20%) in initially "anxious" animals (n=10, p<0.05) (Fig. 2, *7*).

Preliminary injection of diazepam in a dose of 0.6 mg/kg 20 min before injecting pentylenetetrazole in a dose of 10 mg/kg prevented the pentylenetetrazole-induced increase of the level of anxiety and phobia in both "not anxious" and "anxious" animals (Figs. 1, *8* and 2, *8*).

Injection of sodium lactate in a dose of 600 mg/kg raised the initial level of anxiety and phobia in both "anxious" and "not anxious" animals (approximately by 40% for n=8, p<0.05 in both groups) (Figs. 1, *9* and 2, *9*).

Injection of the neuroleptic haloperidol in a low dose (0.01 mg/kg) did not alter the initial level of anxiety and phobia either in "not anxious" or "anxious" rats (Figs. 1, *10* and 2, *10*). Administration of haloperidol in a high dose (0.5 mg/kg) caused an increase of the initial level of

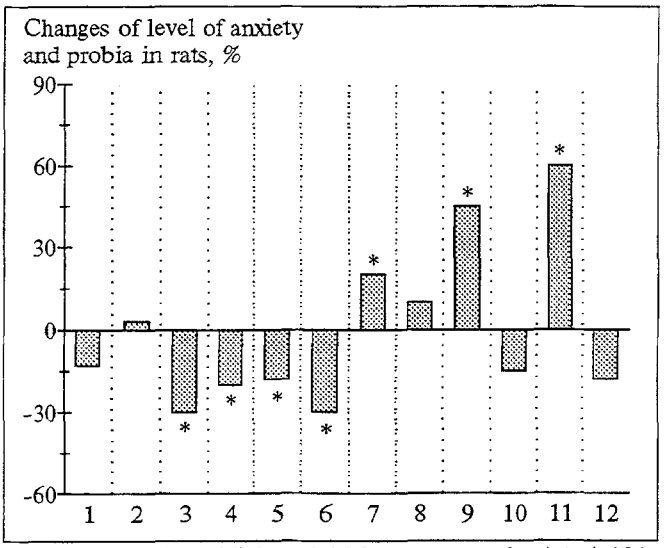

Fig. 2. Deviations (%) from initial mean sum of points (within the group) according to the scale for assessment of anxious–phobic states in rats with an initially high level of anxiety and phobia after injections of preparations producing various effects. *1)* $n=8$; *2)* $n=9$; *3)* $n=7$; *4)* $n=8$; *5)* $n=13$; *6)* $n=9$; *7)* $n=10$; *8)* $n=8$; *9)* $n=8$; *10)* $n=9$; *11)* $n=5$; *12)* $n=8$.

anxiety and phobia in both "not anxious" (approximately by 140%, $n=9$, $p<0.05$) (Fig. 1, *11*) and "anxious" rats (approximately by 60%, $n=5$, $p<0.05$) (Fig. 2, *11*).

Injection of the tricyclic antidepressant melipramine in a dose of 10 mg/kg did not alter the initial level of anxiety and phobia in either "not anxious" or "anxious" rats (Figs. 1, *12* and 2, *12*).

The additional statistical analysis performed showed that the average increase of the level of anxiety and phobia caused by pentylenetetrazole (10 mg/kg) in "not anxious" rats constituted 194.1±50.0% ($n=8$), while that caused by haloperidol (0.5 mg/kg) was 165±35.1%, these values exceeding the average increase of the level of anxiety and phobia caused by injection of physiological saline in such animals (54.2±13.4%) ($n=9$, $p<0.05$ and $p<0.01$, respectively). The average increase of the level of anxiety and phobia for injection of sodium lactate in initially "not anxious" rats (41.0±13.4%, $n=8$) did not exceed that level after injection of physiological saline ($p>0.05$).

The data obtained provide evidence that the rats with an initially different (low or high) level of anxiety and phobia respond differently to injections of the same psychotropic preparations.

The elevated level of anxiety and phobia in "not anxious" rats after injection of physiological saline may be due to long-term changes of the level of GABA in response to injection of the preparation, because it is known that as soon as 20 min after intravenous injection of saline, changes are observed in the content of different amino acid neurotransmitters in the mouse brain, these changes including a decrease in the GABA content [5]. GABA plays an important role in interrupting anxious states, as has been demonstrated on different experimental models of anxiety [4]. Harro et al. [2] established that the number of GABA-benzodiazepin binding sites is reduced in the cerebral cortex of rats regarded by the authors as anxious. One may assume that the reason why physiological saline had no effect upon the level of anxiety and phobia in initially "anxious" rats is that there is an initial deficiency of the processes of inhibition and of GABA in the cerebral cortex of such animals, this, in turn, being determined by a reduced number of GABA-benzodiazepin binding sites.

The anxiolytics sodium valproate, phenazepam, and diazepam lowered the initially high level of anxiety in "anxious" rats and prevented an anxiogenic effect of saline in "not anxious" animals; this is evidently also due to a different efficacy of the inhibitory processes in the cerebral cortex of the two types of animals, and it must be taken into account during screening of potential preparations with anxiolytic properties.

Subseizure doses of the analeptic corasole provoke uneasy anxiety and fear in human beings and behavioral changes (aggravated anxiety) in animals [3,6]. It is known from clinical practice that a state of anxiety and fear is one of the possible adverse reactions to high therapeutic doses of haloperidol. In our studies, pentylenetetrazole and haloperidol in a high dose (0.5 mg/kg) raised the level of anxiety and phobia in animals with different initial levels of anxiety. However, sodium lactate (a preparation provoking panic attacks in patients with spontaneous panic disorders [8]) elevated the level of anxiety and phobia just in initially "anxious" animals, because the average increase was the same in "not anxious" rats given sodium lactate and in those given physiological saline.

Administration of the neuroleptic haloperidol in a low dose (0.01 mg/kg) and of the antidepressant melipramine did not alter the level of anxiety and phobia in rats with an initially high level of anxiety.

The results of our findings attest to the validity of using rats with an initially high level of anxiety and phobia in model studies of the basic mechanisms of anxiety by comparing electrophysiological and neurochemical specificities of animals with initially low and high levels of anxiety and phobia. In addition, the use of the rats with an initially high level of anxiety and phobia opens up novel possibilities in the screening of preparations

with potential anxiolytic properties and in the development of pathogenetic therapy of states of anxiety and phobia.

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Individual Differences of Responses to Acute Stress Associated with Type of Behavior. Resistance (Liability) to Disturbances of Behavior and Sleep

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The resistance of the organism to stress is known to be associated with individual specificities. For instance, it has been shown that under conditions of chronic stress the resistance of the cardiovascular system of rats of various genetic strains is different [9]. A different level of behavioral resistance to neurotizing effects has been discovered in the open field in rats with different levels of motor activity [7] and with different capabilities for elaborating the response of emotional resonance after P. V. Simonov [1].

The aim of the present work was to study resistance to acute stress (AS) in rats with different types of behavior in the open-field test and endurance swimming. The degree of stress resistance was assessed as the intensity of changes of the parameters of behavior and of the sleep-wakefulness cycle.

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MATERIALS AND METHODS

The experiments were carried out on 136 male albino rats. The type of animal behavior was determined with the aid of two tests: open field [2] and endurance swimming [10]. The rats were divided into groups as described previously [5]. Nine groups of rats were distinguished: two extreme groups with the active and passive type of behavior, one intermediate group, and 6 mixed groups (not used in the subsequent investigation). Within each of the three chosen groups (the two extreme groups and the intermediate one) the rats were divided into two subgroups: control and experimental. The rats of the control subgroups were subjected to two-time behavioral testing. Before the second test, the rats of the control subgroups were subjected to AS caused by unpredictable and ines-

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