ANALYSIS OF THE ACUTE AND CHRONIC EFFECT OF ANTIDEPRESSANTS ON MICE WITH EXPERIMENTAL LEARNED HELPLESSNESS

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The experimental model of behavioral depression known as "learned helplessness" is based on psychophysiological [8] and neurochemical [7] studies on different species of animals. Preliminary unavoidable exposure to an aversive (emotional-stress) situation has been shown to give rise to a long-lasting deficiency of behavior, manifested as difficulty in learning conditioned-reflex or operant escape and (or) avoidance reactions. The phenomenon of learned helplessness in rats can be abolished by the tricyclic antidepressants imipramine and desipramine [11].

The absence of adequate behavioral models of depression in animals suitable for the detection and study of specific activity not only of the tricyclic (classical), but also of so-called atypical antidepressants, especially for chronic administration, when the pharmacologic effect is the result of adaptive reorganization of neurochemical receptor systems [3], justified a more detailed study of a wide group of antidepressants. Preliminary observations [2] showed that this is a promising behavioral model for the discovery of potential antidepressants of the atypical series.

EXPERIMENTAL METHOD

Experiments were carried out on male tetrahybrid CAWA mice weighing 20-23 g (from the "Svetlye Gory" Nursery, Academy of Medical Sciences of the USSR), kept in the animal house under standard conditions with water and food ad lib. The animals were sent to the laboratory 2 h before testing and housed in individual cages. Preliminary unavoidable aversive stimulation was applied in dark chambers (30 × 15 × 15 cm), to the floor of which electric shocks (150 μA, 50 Hz, duration 6 sec, interval 30 sec) could be applied. Exposure lasted 40 min. The avoidance reaction was tested in a shuttle box (30 × 15 × 15 cm) consisting of two compartments, through the floor of which electric shocks could be applied, connected by an opening with automatically controlled shutter. Shocks (150 μA) were applied to each compartment in turn with an interval of 30 sec, and after a delay of 4 sec the shutter was opened and the animal allowed to escape into the safe compartment. In the case of nonavoidance after 20 sec the shutter was closed and the shocks discontinued. In the course of one session the animal received 10 series of shocks. The latent period and total number of escape reactions were estimated.

All drugs were administered enterally through an atraumatic tube in a volume of 0.3 ml, 40 min before testing in the shuttle box to assess their acute effect. During chronic administration (twice a day at 10 a.m. and 6 p.m.) testing was carried out 24 h after the last dose on the 7th and 15th days. Desipramine and chlorimipramine (from Germed, East Germany), amitryptaline (from Spofa, Czechoslovakia), pyrazidol (from the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute), trazodone (from Trittico, Italy), zimelidine (from Astra, Sweden, supplied by Professor Ross), and befuraline and original derivatives of benzofuran and morpholine synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR (Professor V. I. Zagorevskii), were used. Control animals received the same volume

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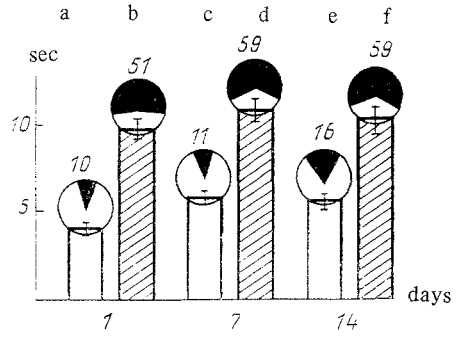

Fig. 1

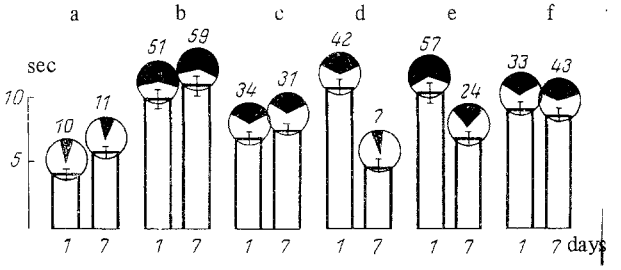

Fig. 2

Fig. 1. Time course of parameters of behavioral depression in mice. a, c, e) Control 1; b, d, f) control 2. Sector shaded black and numbers denote number of unperformed avoidance reactions (in % of total number of tests). Abscissa, duration of observation (in days); ordinate, latent period of avoidance reaction (in sec).

Fig. 2. Effect of antidepressants on parameters of behavioral depression after administration for 6 days. a) Control 1; b) control 2; c) desipramine 10 mg/kg; d) zimelidine 10 mg/kg; e) trazodone 10 mg/kg; f) befuraline 50 mg/kg. Remainder of legend as to Fig. 1.

TABLE 1. Effect of Acute and Chronic Administration of Antidepressants on Parameters of Avoidance Reaction in Mice Exposed Previously to Unavoidable Stress (M ± m)

Substance	Single dose			Administration for 14 days		
	nonavoidance	avoidance	Mean latent period of avoidance, sec	nonavoidance	avoidance	Mean latent period of avoidance, sec
	% of total number of tests			% of total number of tests		
Control 1	10	90	4,1±0,3	16	84	5,8±0,4
Control 2	51	49	10,0±0,7	59	41	10,6±0,9
Desipramine (10 mg/kg)	34[a]	66	7,1±0,4[b, c]	18[d]	82	7,1±0,4[a, d]
Chlorimipramine (10 mg/kg)	31[a]	69	8,5±0,4[b, c]	21[d]	79	8,3±0,5[b, c]
Amitryptaline (5 mg/kg)	34[a]	66	6,6±0,3[b, d]	26[d]	76	7,2±0,4[a, c]
Pyrazidol (10 mg/kg)	38[b]	62	5,5±0,6[d]	16[d]	84	5,3±0,9[d]
Zimelidine (10 mg/kg)	42[b]	58	11,0±0,5[b]	13[d]	87	5,4±0,3[d]
Trazodone (10 mg/kg)	57[b]	43	10,7±0,8[b]	20[d]	80	6,0±0,5[d]
Trazodone (2,5 mg/kg)	58[b]	42	8,9±0,3[b]	42	58	7,4±0,8[c]
LIS-30 (1 mg/kg)	40[a]	60	4,3±0,9[d]	14[d]	86	5,4±0,5[d]
Befuraline (50 mg/kg)	33[a]	67	9,5±0,5[b]	12[d]	88	8,2±0,4[b, c]
DZ K-153 (10 mg/kg)	44[b]	56	9,4±0,5[b]	7[d]	93	7,9±0,3[b, c]

Legend. a) $P < 0.05$, b) $P < 0.01$ compared with control 1; c) $P < 0.05$, d) $P < 0.01$ compared with control 2.

of solvent (water). In control 1 group the rats were not subjected to unavoidable stimulation, whereas in control 2 group the animals were subjected to preliminary unavoidable aversive stimulation but did not receive antidepressants.

EXPERIMENTAL RESULTS

Animals previously subjected to a series of unavoidable stimuli, when subsequently (24 h later) tested in the shuttle box exhibited a significant behavioral deficiency. The authors who first described the method [6-8] adopted an increase in the mean latent period of the avoidance reaction as the only parameter of development of learned helplessness. We took into account both the latent period and the number of completed avoidance reactions (relative to the total number of tests). Comparison of the two parameters (Fig. 1) gives a clearer picture of the behavioral deficit relative to control 1. Essentially even after completion of avoidance in some tests the animal did not repeat the skill in response to repeated presentations of the stereotype. The phenomenon of learned helplessness lasted a long time. On retesting

on the 7th and 15th days after exposure to stress the behavioral deficit remained at the same level (Fig. 1) even though the animal responded with separate avoidance reactions to three repetitions of the stereotype (10 stimuli in each case). In other words, the animal did not learn the avoidance reaction. This stability of the behavioral model means that it can be used to estimate the effect of chronic administration of antidepressants.

Data showing the effect of a single dose of antidepressants (40 min before testing) are given in Table 1. None of the drugs increased the number of completed avoidance reactions up to the control 1 level (animals not exposed beforehand to stress). Significant shortening of the latent period was produced only by pyrazidol and LIS-30. No aftereffect of the antidepressants was found 24 h after administration. Retardation of avoidance in animals previously exposed to unavoidable stress (the latent period was more than doubled) corresponds to difficulty in initiation of a response which has been well studied previously in dogs and rats [8]. It is not due to any motor deficiency [6]. Behavioral manifestations of learned helplessness are based on changes in the state of the brain neurotransmitter systems [7], which are produced by a mechanism of a situational conditioned reflex to repetition of the stress situation. The leading role in the development of learned helplessness has been ascribed in recent years to serotonin deficiency, in particular in the septal zone of the brain [12]. This experimental model simulates to some degree the state of reactive depression and enables the relative rate of development of the effect of antidepressants to be estimated during chronic administration.

The effect of tricyclic antidepressants administered for a period of 6 days (testing 24 h after the last dose, i.e., on the 7th day) did not differ from their acute effect. As an example, data on the action of desipramine are given in Fig. 2. Amitryptaline had a similar effect. Atypical antidepressants, the spectrum of whose neurochemical action is dominated by the serotonin-positive component (zimelidine is a selective blocker of serotonin reuptake [10], trazodone in a large dose, with a serotonin-mimetic action [9], pyrazidol, and LIS-30 are type A MAO inhibitors [4, 5]; all significantly improved the behavioral parameters and brought them close to their levels in control 1. Befuraline and DZK-153, with a powerful action on catecholamine metabolism [1], did not abolish retardation of the avoidance reaction, although they increased the total number of completed reactions. This indicates, in particular, a difference in the functional organization of the two basic phenomena of learned helplessness: performance of the avoidance reactions and the rate of realization of the behavioral response.

The tricyclic antidepressants abolished the avoidance reaction deficit in stressed animals only after administration for 14 days (Table 1), which corresponds to the latent period of development of the effect in clinical practice. By this time all antidepressants tested reversed the manifestation of learned helplessness, so that the ratio between avoidance and nonavoidance was the same as in control 1, whereas the temporal parameter of avoidance was significantly shortened and came close to the control 2 level. The results of pharmacokinetic tests [11] indicate that 24 h after chronic administration of antidepressants only trace concentrations of them can be found in the brain and blood. Effects recorded after a course of antidepressants are due to adaptive changes in the neurochemical systems of the brain and in synaptic membrane receptors [3].

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EFFECT OF THE SPECIFIC BENZODIAZEPINE ANTAGONIST RO15-1788 ON INHIBITION OF HIPPOCAMPAL UNIT ACTIVITY EVOKED BY PHENAZEPAM

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High-affinity specific binding sites for benzodiazepines (BD), which function as receptors and mediate the pharmacologic activity of these compounds, have been found comparatively recently in the mammalian CNS [5, 12].

The writers previously described the effect of BD on the evoked potential (EP) of hippocampal neurons [1]. However, the question whether this effect is mediated through interaction of BD with a specific receptor remained unanswered.

The aim of this investigation was to test the hypothesis that the effect of BD on EP arising in hippocampal area CA1 in response to stimulation of Schaffer's collaterals (SC) is mediated through the benzodiazepine receptor (BDR). For this purpose the action of the specific BD antagonist, RO15-1788, on inhibition induced by phenazepam in the hippocampus was studied. The effect of RO15-1788 on EP also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices from Wistar rats aged 2-3 weeks by the method described previously [1]. The animals were decapitated, the upper cranial bones were removed, and a transverse slice of the hippocampus about 400 μ thick was removed, and transferred to a thermostatically controlled experimental chamber through which flowed balanced Hanks' solution. The solution was previously saturated with a gas mixture containing 95% O_2 and 5% CO_2 (pH 7.2-7.4), and for the next 30-40 min the temperature in the chamber was gradually adjusted to 25-30°C. Bipolar glass stimulating microelectrodes filled with Hanks' solution were introduced into the stratum radiatum, in which SC are located. Recording microelectrodes filled with Hanks' solution were led up to neurons in area CA1.

EP began to be recorded 1-1.5 h after preparation of the slice. Pulses of direct current 0.2 msec in duration, with a frequency of 0.1 Hz and a voltage of 10-20 V, were used for stimulation. The parameters of stimulation were chosen in order to evoke a population spike (PS), which is a synchronous discharge of the pyramidal neurons in this area. In the present experiments the amplitude of PS varied from 2 to 5 mV in different experiments. The results were recorded on photographic paper from an oscilloscope.

Solutions containing 2 μM phenazepam, 4 μM RO15-1788, and 2 μM hexobarbital were used. Phenazepam and RO15-1788 were dissolved beforehand in ethanol to a concentration of 10^{-2} M. From these solutions, working solutions to the required concentration in Hanks' solution were prepared. Appropriate quantities of ethanol were added to the flow of liquid in the control.

The experiments were conducted by the following scheme: phenazepam was added to the system and applied for 10-15 min; during application of phenazepam, the antagonist RO15-1788 was

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