EFFECT OF CHRONIC PSYCHOGENIC STRESS ON SOME BEHAVIORAL AND NEUROCHEMICAL CHARACTERISTICS OF RATS

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Unavoidable painful electrical stimulation, reinforcing a conditioned stimulus in accordance with a randomized program, with a probability of presentation of 0.5, is known to be a highly effective psychotraumatic agent, causing the development of neurosis in animals [1]. In particular, on the model suggested in 1976 by Hecht et al. [5], daily exposure of short duration (each session lasted 13 min) induced a marked neurosis-like state on the 15th day in rats, accompanied by characteristic changes in autonomic and electrographic parameters. The present writers showed previously [4] that neurosis development of this kind in rats is accompanied by depression of the animals' behavior, expressed as a reduction of all behavioral parameters in "maze" and "open field" tests. The mechanism of these disturbances has not yet been explained. It is likewise not clear to what extent the changes observed are caused by repeated painful electric shocks and are not the result of psychogenesis, as a response to an acute deficit of information on the possibility of avoidance.

In the investigation described below behavioral, somatic, and certain neurochemical parameters were studied in rats under conditions of unavoidable chronic stress, according to Hecht et al. (with the same parameters) in a situation of possible avoidance, with the same total number of aversive stimuli.

EXPERIMENTAL METHOD

Noninbred male rats weighing 180-220 g were kept in the animal house under natural conditions of light and darkness and with free access to water and food. A slightly modified technique of Hecht et al. was used for 15 days to produce neurosis [4]. An alternative series of experiments was set up to present an equivalent (60 sec) number of painful electric shocks, combined with flashes, in a shuttle box, with the possibility of avoidance. A day before the beginning of the 15-day-long experiment, and again 1 day after it, animals of both experimental and control groups were tested in a maze and open filed. The animals were decapitated and the brain membranes isolated shortly after the end of the last test [3]. The suspension of membranes in appropriate buffers (1 mg protein in 1 ml) was kept at -20°C for not more than 2 weeks. Specific binding of ^{3}H-flunitrazepam (86 Ci/mmole) and ^{3}H-dihydroalprenolol (21 Ci/mmole) [3, 7] (both compounds were from Amersham Corporation, England) was studied. The dissociation constant (K_d) and the maximal concentration (B_{max}) of ligand–receptor complexes were determined in Scatchard plots by means of an HP-33E computer (USA). The protein concentration in the samples was determined by Perterson's method [8].

EXPERIMENTAL RESULTS

As Table 1 shows, neurosis formation for 15 days in rats was accompanied by a significant decrease in all parameters of behavioral activity; as was shown previously, moreover, the changes were very persistent in character [4]. In the alternative experiments with continuous electric shock reinforcement, under conditions of possible avoidance, the animals divided spontaneously into two groups: group 1) nonavoiding animals, which completely abondoned all attempts to avoid the shocks after the 2nd-3rd session, and throughout the session of painful electrical stimulation (12 shocks for 5 sec each time, with intervals of 30 sec) they remained in a fixed posture in the starting compartment; group 2) evading animals, which left the starting compartment after varied latent periods, but, irrespective of this, received electrical

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TABLE 1. Behavioral and Somatic Parameters of Rats after Chronic (continuous or randomized) Painful Electrical Stimulation (M ± m)

Group of animals	Maze	Open field				Relative weight of adrenals	Increase in body weight over 15 days, g
	Total activity	Horizontal activity	Vertical activity	Investigative activity	Visits to central zones		
1: Control ($n = 30$)	17,5±2,0	29,0±4,0	7,2±2,0	10,5±2,0	3,0	0,13±0,08	32±2,8
2: Evading ($n = 5$)	15,1±3,2	15,0±3,6 $P_{1-2} \leqslant 0,01$	6,9±2,1	6,7±1,9	1,0	0,14±0,07 $P_{1-2} \leqslant 0,05$	22±1,3 $P_{1-2} \leqslant 0,01$
3: Nonevading ($n = 9$)	4,7±1,1 $P_{1-3} \leqslant 0,01$ $P_{3-4} \leqslant 0,05$	12,0±2,3 $P_{1-3} \leqslant 0,01$ $P_{3-4} \leqslant 0,05$	4,0±1,0	3,4±1,3 $P_{1-3} \leqslant 0,05$	0	0,15±0,06	16±2,5 $P_{1-3} \leqslant 0,01$ $P_{3-4} \leqslant 0,05$
4: Developing neurosis (n = 8)	1,5±0,3 $P_{1-4} \leqslant 0,01$	5,6±2,1 $P_{1-4} \leqslant 0,01$	1,8±0,8 $P_{1-4} \leqslant 0,05$	1,6±0,5 $P_{1-4} \leqslant 0,01$	0	0,15±0,08 $P_{1-4} \leqslant 0,05$	3,6±0,5 $P_{1-4} \leqslant 0,01$

Legend. Behavioral parameters expressed in absolute mean values for 5 min of observation. Here and in Table 2: n) number of animals.

TALE 2. Effect of Chronic (continuous or randomized) Painful Electrical Stimulation on Characteristics of Specific ^{3}H-Dihydroalprenolol and ^{3}H-Flunitrazepam Binding by Rat Brain Membranes (M ± m)

Group of animals	^{3}H-dihydroalprenolol		^{3}H-flunitrazepam	
	K_d	B_{max}	K_d	B_{max}
1: Control (n = 3)	100±9	100±12	100±10	100±3
2: Evading (n = 5)	96±9	105±10	101±8	101±4
3: Nonevading (n = 9)	108±5	95±7	122±12	93±3
4: Developing neurosis (n = 7)	162±6	64±3*	84±2	87±3

Legend. Relative values (in % control). Characteristics of specific binding in control group for ^{3}H-dihydroalprenolol K_d = 3.08 ± 0.36 nM, B_{max} = 110 ± 10 fmoles/mg protein; for ^{3}H-flunitrazepam K_d = 1.36 ± 0.04 nM, B_{max} = 1165 ± 116 fmoles/mg protein. Isotherms of specific binding obtained by the use of six concentrations of ligands; each point in three repetitions. Three independent experiments with each ligand carried out for each group. *$P < 0.05$ compared with control.

stimulation for a total of 60 sec during each session. The evading rats differed significantly from the control rats only in their index of horizontal activity which, moreover, was significantly higher than that of animals in which neurosis was induced by the method of Hecht et al. In rats which abandoned attempts at avoidance (group 3) depression of behavior was even deeper and affected all characteristics, including vertical activity, which did not differ significantly from that in the control. According to the parameter of investigative activity, animals of group 3 did not differ significantly from those in which neurosis was induced by the method of Hecht et al. (group 4). Nevertheless, the total activity of the nonevading rats in the maze and their horizontal activity in the open field were significantly higher than that of neurotic rats, evidence of the milder degree of the pathological disturbances of their behavior. The increase in body weight of the animals of all three experimental groups over the period of 15 days was significantly less than that of the control animals, but with respect to this parameter also the group of nonevading animals differed significantly from the neurotic rats, and occupied an intermediate position between them and the control animals. The relative weight of the adrenals of animals of all three experimental groups was significantly greater than in the control, but no significant difference was found between groups 2, 3, and 4 with respect to this parameter. On the whole, analysis of the data in Table 1 confirms the two basic assertions made previously: First, ability to evade a conditioned aversive stimulus is a factor which prevents the development of behavioral disturbances [9]; second, under conditions of unavoidable aversive stimulation the most marked disturbances of behavior were observed in the case of randomized presentation of the stimulus [5].

Analysis of the state of the benzodiazepine receptors and β-adrenoreceptors of the brain membranes of the same animals showed (Table 2) that the characteristics of these targets for

the evading animals did not differ significantly from the control, but in the group of non-evading animals there was only a tendency for K_d for specific binding of ^{3}H-flunitrazepam to increase. Marked changes in the receptors studied were found only in the group of neurotic animals, in which a significant decrease was found in the affinity (an increase in K_d) and concentration of β-adrenoreceptors, and also a decrease in the concentration of benzodiazepine receptors, with a tendency toward an increase in their affinity. A compensatory change in brain β-adrenoreceptors in response to increased catecholamine secretion in stress has been described more than once previously [2]. We also know that in different types of stress, depending on its duration and intensity, a decrease in the concentration of brain benzodiazepine receptors may be observed [6]. In the present case, the most important fact from our point of view is that the change in receptor characteristics took place only in animals with the severest and most lasting behavioral disturbances, induced by the psychogenic character of the stress situation due to the randomized aversive reinforcement rather than to the actual physical factors of stress.

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