

EFFECT OF PHENAZEPAM ON PLASMA ACTH LEVEL OF INBRED MICE  
EXPOSED TO STRESS

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It was shown previously that inbred mice of the C57BL/6 (B6) and BALB/c (C) strains, in the open field (OF) test, give either an active response with predominance of investigative behavior (B6) or a passive (freezing) response (C) [3].

Benzodiazepine tranquilizers, phenazepam and diazepam, given to B6 mice had a mainly sedative effect, whereas given to C mice in low doses they had an activating effect [3, 4]. It has also been observed that B6 and C mice differ considerably both in the initial plasma ACTH level and in the time course of its changes after handling in the OF test [7]. (C57BL/6 × BALB/c)<sub>F1</sub> hybrids (<sub>F1</sub>) inherited investigative behavior in OF and a high ACTH level, characteristic of the parental B6 strain [3, 7].

The aim of this investigation was to study hereditary control of changes in the ACTH level after administration of phenazepam.

#### EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C mice weighing 18-20 g from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, kept on a standard diet, with a 12-h day-light and 12-h darkness schedule, with 10 animals per cage, in a separate room of the animal house for 1 month before the experiment began. Phenazepam was injected intraperitoneally in doses of 0.05, 0.1, and 0.5 mg/kg 30 min before the OF test began. The animals were exposed to stress and ACTH levels were determined by the method described previously [4]. The numerical data were subjected to statistical analysis by Student's *t* test.

#### EXPERIMENTAL RESULTS

Control injection of physiological saline led to a marked rise of the serum ACTH level in B6 mice after only 30 sec, whereas in the C mice at this time the hormone concentration was lower than initially (Table 1). This last fact is evidently linked with the stronger corticosteroid response of C mice than of B6 mice to handling, observed previously [5]. It is also evidence that the procedure of injection was a stress-inducing factor for the B6 mice. During the next 30 min the ACTH level returned to normal in the B6 mice and remained below the initial level in C mice (Table 1). In the period before the OF test, phenazepam, depending on the dose, lowered the ACTH level in B6 mice. In C mice the minimal ACTH level was observed 30 min after injection of the tranquilizer in a dose of 0.05 mg/kg (Table 2). Neither handling nor OF, preceded by injection of physiological saline, caused any additional rise of the ACTH level in the B6 mice, which must probably be attributed to an increase in the corticosterone concentration as a result of the injection. In B6 mice, after injection of phenazepam a tendency was preserved in all the experiments for the ACTH level to fall, by an amount which depended on the dose. Its concentration 15 min after the OF test was low in the control series also (Table 2).

The C mice reacted in the first minute of the OF test with a transient rise of the ACTH level, but after 15 min, it fell just as in the B6 mice. Phenazepam in most cases lowered the ACTH concentration, but without any dose dependence (Table 1).

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TABLE 1. Effect of Phenazepam on Plasma ACTH Level (in ng/ml) in B6, C, and F<sub>1</sub> Hybrid Mice after Open Field Test (M ± m)

Strain of mice	Dose of drug, mg/kg	Time after injection, min			
		1/2	1	15	30
B6	Control	7067±471 (9)*	3885±653 (10)	2563±247 (8)	2863±365 (15)
	0,05	—	—	1994±204 (9)	1782±312 (15)**
	0,10	—	—	1481±289 (8)**	1093±186 (14)**
	0,50	—	—	963±176 (8)**	713±89 (15)**
C	Control	678±89 (6)*	416±89 (6)*	454±88 (8)*	653±139 (8)*
	0,05	—	—	397±118 (6)	200±50 (6)**
	0,10	—	—	603±69 (10)	621±235 (7)
	0,50	—	—	752±110 (10)	813±167 (8)
F <sub>1</sub>	Control	2574±325 (10)	2347±248 (9)	1236±483 (8)*	900±178 (9)
	0,05	—	—	1091±156 (8)	900±169 (9)
	0,10	—	—	957±107 (9)	531±99 (10)
	0,50	—	—	738±173 (7)	284±115 (10)**

Legend. \*P < 0.05 compared with initial level, \*\*P < 0.05 compared with control. Number of animals given in parentheses.

TABLE 2. Effect of Phenazepam on Plasma ACTH Level (in ng/ml) in B6, C, and F<sub>1</sub> Hybrid MICE After Open Field Test (M ± m<sub>x</sub>)

Strain of mice	Dose of drug, mg/kg	-OF + 0'	OF + 1' in OF	OF + 0'	-OF + 15'	OF + 15'
B6	Control	2863±365 (15)	2746±262 (17)	2190±298 (15)	2337±386 (8)	1043±321 (8) <sup>a</sup>
	0,05	1782±312 (14) <sup>b</sup>	—	1404±236 (12)	1402±179 (10) <sup>b</sup>	906±122 (8) <sup>a</sup>
	0,10	1093±186 (15) <sup>b</sup>	—	1360±265 (15) <sup>b</sup>	895±152 (10)	1294±188 (8)
	0,50	713±89 (15) <sup>b</sup>	—	727±178 (15) <sup>b</sup>	439±133 (7) <sup>b</sup>	771±213 (7)
C	Control	653±139 (8) <sup>c</sup>	1720±205 (8) <sup>d</sup>	814±157 (9)	938±165 (9)	447±114 (8) <sup>a</sup>
	0,05	200±50 <sup>b</sup>	—	378±145 (10)	732±196 (9)	394±81 (9)
	0,10	621±235 (7)	—	380±100 (10) <sup>b</sup>	304±57 (10) <sup>b</sup>	438±94 (9)
	0,50	813±167 (8)	—	419±47 (9) <sup>a,b</sup>	412±105 (5)	459±84 (8)
F <sub>1</sub>	Control	900±178 (9)	—	2400±335 (10) <sup>d</sup>	—	—
	0,05	900±169 (9)	—	950±243 (10) <sup>b</sup>	—	—
	0,10	531±99 (10)	—	587±124 (10)	—	—
	0,50	284±115 (10) <sup>b</sup>	—	347±73 (10) <sup>b</sup>	—	—

Legend. <sup>a</sup>P < 0.05 compared with series (-OF + t'), <sup>b</sup>P < 0.05 compared with control, <sup>c</sup>P < 0.05 compared with initial level, <sup>d</sup>significance compared with series (-OF + 0').

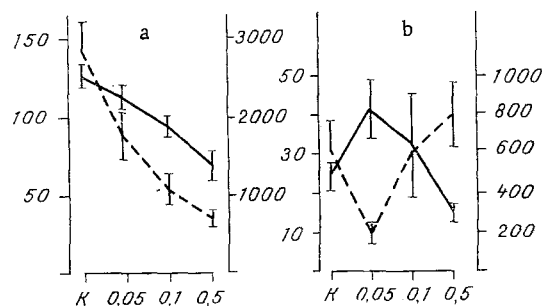


Fig. 1. Effect of phenazepam on plasma ACTH level (continuous line) and motor activity (broken line) of B6 (a) and C (b) mice in OF test. Abscissa, dose of phenazepam (in mg/kg); ordinate: on left - motor activity, on right - ACTH level (in ng/ml). K) Control.

Dose-dependent changes in the ACTH concentration in the hybrid animals after injection of the tranquilizer resembled more closely those in the parental line B6, but at the same time, after injection of physiological saline the hormone level fell in the F<sub>1</sub> hybrids, a characteristic feature of C mice.

The results show that changes in the ACTH-corticosterone system specific for genotype take place in inbred animals with different types of stress response, not only in response to stress [5, 7], but also in response to injection of a benzodiazepine tranquilizer. In this case also responses observed in B6 mice are characterized by stronger inertia than in C mice.

Connection between phenazepam-induced changes of behavior and ACTH concentration also will be evident. In B6 and F<sub>1</sub> hybrids the dose-dependent lowering of the ACTH level before the start of the OF test corresponded to diminution of motor activity, which was similar in character. Under these same conditions, in C mice the tranquilizer induced a significant fall of the ACTH level only in a dose of 0.05 mg/kg (Fig. 1). It was in this dose that phenazepam had an activating effect on their motor activity [6].

Assuming that the behavior of B6 and C mice in OF represents different versions of manifestation of an anxiety response [11, 12] it can be considered that the results confirm the hypothesis of functional antagonism between benzodiazepines and ACTH [8-10]. At the same time, they show clearly that these relationships are represented differently, depending on the hereditarily controlled type of response to stress.

Since changes in the ACTH-corticosteroid system are a component, that is universal for all mammals, in the development of responses to stress, in which marked differences are observed in the human population [1, 2], it can be concluded from these results that there are good grounds for determining the ACTH concentration for clinical and pharmacological monitoring during benzodiazepine therapy.

#### LITERATURE CITED

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