FOURTH SESSION

Environmental, maternal and genetic determinants in neuropsychopharmacology

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PHARMACOGENETIC ANALYSIS OF MECHANISMS OF EMOTIONAL STRESS: EFFECTS OF BENZODIAZEPINES

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Summary. – The effects of the benzodiazepine tranquillizer phenazepam (0.05, 0.075, 0.1 mg/kg) in the open field test of the mice C57Bl/6, BALB/c strains and their F_1 hybrids have been studied. The tranquillizer produced a dose-dependent suppression of the locomotor activity of C57Bl/6 mice, while 0.05 mg/kg activated the behaviour of the BALB/c mice. The response to phenazepam of F_1 hybrids resembled that of their C57Bl/6 parental strain, also in their pattern of the open field behaviour. Interstrain differences in the plasmatic levels of ACTH, corticosterone, cAMP and cGMP after the open field test have been detected. Interstrain differences in the modifications of [^{3}H]–diazepam and [^{35}S]–TBPS binding to brain membranes between C57Bl/6 and BALB/c mice, dependent on the $^-$Cl ions addition, have been also found.

Riassunto (Analisi farmacogenetica dei meccanismi dello stress emotivo. Effetti delle benzodiazepine). - *Sono stati studiati gli effetti del fenazepam (tranquillante benzodiazepinico) alle dosi di 0,05, 0,075, 0,1 mg/kg mediante open field test su topi dei ceppi C57Bl/6 e BALB/c e su loro ibridi F_1. Il tranquillante causava una riduzione dell'attività locomotoria dose-dipendente nei topi C57Bl/6 mentre la dose di 0,05 mg/kg provocava un aumento dell'attività motoria nei topi BALB/c. La risposta al fenazepam degli ibridi F_1 rassomigliava a quella del ceppo C57Bl/6, anche nel profilo di comportamento in open field test. Dopo il test sono state osservate le differenze nei livelli plasmatici di ACTH, corticosterone, AMPc e GMPc nei due ceppi. Vi erano inoltre differenze nelle modificazioni cerebrali, in seguito all'aggiunta di ioni Cl^-, tra i topi C57Bl/6 e BALB/c.*

Since benzodiazepine tranquillizers were introduced into clinic, the problem of individual response to these drugs has been widely discussed [1, 2]. There are reasons to hypothesize that there is a hereditary disposition to benzodiazepines. The stress reaction, target of benzodiazepine action is known to be differently controlled in animals of different species [3-8] and in humans as well [9].

The purpose of our study was to investigate hereditary disposition to the effects of benzodiazepine tranquillizers.

Materials and methods

Male C57Bl/6 and BALB/c mice («Stolbovaya» animal farm of the USSR Academy of Medical Sciences) and their reciprocal F_1 hybrids, weighing 18–20 g, were used. The animals were housed in a separate animal room in groups of ten and maintained on 12 h light-dark cycle with free access to standard food and water. The investigations were carried out from October to January, from 10.00 up to 14.00 daily. Control tests failed to show any distinctions of the rated parameters during the mentioned intervals of time. Since the results of the tests on the reciprocal F_1 hybrids were similar, we present data obtained on the combination C57Bl/6 x BALB/c.

The following drugs were used in the research: phenazepam (Physico-Chemical Institute, Odessa USSR), ^{3}H–diazepam (N–methyl [^{3}H], 71 Ci/mMole, Amersham, England), t[^{35}S]–butylbicyclo-phosphorothionate (^{35}S–TBPS), 60 Ci/mMole, New England Nuclear).

The behaviour of the animals was studied in the open field test (OF), according to the modifications described by Borodin *et al.* [3]. The general, horizontal, peripheral, central and vertical locomotor activity (LA) levels were estimated. Phenazepam was dissolved in Tween 80 and injected i.p. in doses of 0.05, 0.075 and 0.1mg/kg.

The biochemical indicators of the stress reaction determined in the plasma of the animals were: ACTH - by RIA method [10] using reagents by CIS

Intern Company (France), corticosterone - by the Murphy method [11], cAMP - by the method suggested by Brown *et al.* [12], cGMP - by RIA method [13] (Amersham, England). The basal concentrations of hormones and nucleotides in the plasma were measured immediately after decapitation of the animal, and at different intervals after the OF experiment, and in the special series - after handling. Radioligand analysis of the benzodiazepine receptor complex was carried out according to the methods described previously [14,15]. Statistical analysis of the data was based on Student's t-test.

Results and discussion

The results obtained in the OF test show that C57Bl/6 mice appear to possess an active response to a stress-inducing action, while a pronounced «freezing» reaction is observed in the BALB/c mice (Table 1). Differences between the strains in their response to tranquillizers have also been noticed. In the C57Bl/6 mice phenazepam was found to cause a dose-dependent LA decline. In the BALB/c mice a double-phase action was observed: 0.05 mg/kg of the drug induced stimulation, while 0.1 mg/kg - decreased the LA level (Table 1).

The behaviour of the F_1 hybrids in OF resembled that of the parental strain C57Bl/6, their reaction to phenazepam being inherited in a similar way: the tranquillizer acted to decrease the LA of the F_1 hybrids dose-dependently (Table 1).

Thus, action of the benzodiazepine seems to differ in animals with different genotype and to depend on the type of their reaction to stress. The crossing of C57Bl/6 with BALB/c mice results in the inheritance of the active behaviour in OF test and quality of the tranquillizer effect typical of the C57Bl/6 mice (Table 1).

Some biochemical characteristics of the emotional-stress reaction proved specific for the strains of mice. Fig. 1 shows differences between the strains in ACTH contents in the plasma. In the C57Bl/6 animals phenazepam supressed the locomotor activity and decreased the hormone level in plasma in a dose-dependent manner. In BALB/c mice the tranquillizer had an anxiolytic effect only at the dose of 0.05 mg/kg, and only this dose decreased the ACTH level (Fig. 2). Animals displaying active behaviour in the OF test, i.e. C57Bl/6 and F_1 hybrids, showed similar

Table 1. - *Effects of phenazepam on the behaviour of mice C57Bl/6 and BALB/c strain and their F_1 hybrids (C57Bl/6 × BALB/c) in the open field test (M ± SEM)*

Strain of mice	Locomotor activity	Control	Dose (mg/kg)		
			0.05	0.075	0.1
C57Bl/6	General	126.4 ± 7.8	111.5 ± 7.9 (*a*)	92.4 ± 6.7 (*b*)	58.5 ± 9.8 (*c*)
		no. = 25	no. = 15	no. = 15	no. = 15
	Horizontal	112.7 ± 7.3	101.1 ± 7.2 (*a*)	84.1 ± 8.6 (*b*)	55.6 ± 8.4 (*c*)
	Peripheral	83.8 ± 3.8	87.2 ± 6.9	72.5 ± 7.9 (*a*)	49.6 ± 7.7 (*c*)
	Central	28.9 ± 3.8	16.1 ± 2.4 (*b*)	10.6 ± 2.3 (*b*)	8.5 ± 1.7 (*b*)
BALB/c	General	24.5 ± 3.6	39.5 ± 6.9 (*a*)	32.7 ± 11.7	14.8 ± 1.8 (*b*)
		no. = 27	no. = 27	no. = 15	no. = 15
	Horizontal	24.5 ± 3.0	39.5 ± 6.9 (*a*)	32.7 ± 11.7	14.8 ± 1.8 (*b*)
	Peripheral	23.5 ± 4.0	38.9 ± 7.1 (*a*)	32.4 ± 11.6	14.4 ± 1.9 (*a*)
	Central	0.9 ± 0.5	0.5 ± 0.4	0.2 ± 0.1	0.4 ± 0.2
F_1 (C57Bl/6 x BALB/c)	General	106.2 ± 12.9	68.1 ± 16.3	60.9 ± 14.4 (*a*)	41.7 ± 10.8 (*c*)
		no. = 12	no. = 12	no. = 12	no. = 12
	Horizontal	99.4 ± 11.2	64.7 ± 13.1	58.4 ± 13.8 (*a*)	40.3 ± 9.9 (*c*)
	Peripheral	86.7 ± 9.8	57.7 ± 12.3	49.9 ± 9.6 (*a*)	34.0 ± 7.6 (*c*)
	Central	12.7 ± 3.3	7.0 ± 2.8	8.4 ± 4.2	6.1 ± 2.3
	Vertical	8.4 ± 1.7	3.4 ± 1.2 (*a*)	2.5 ± 1.2 (*a*)	1.3 ± 0.7 (*c*)

(*a*) Significant differences $p < 0.05$; (*b*) $p < 0.01$; (*c*) $p < 0.001$; no. number of animals

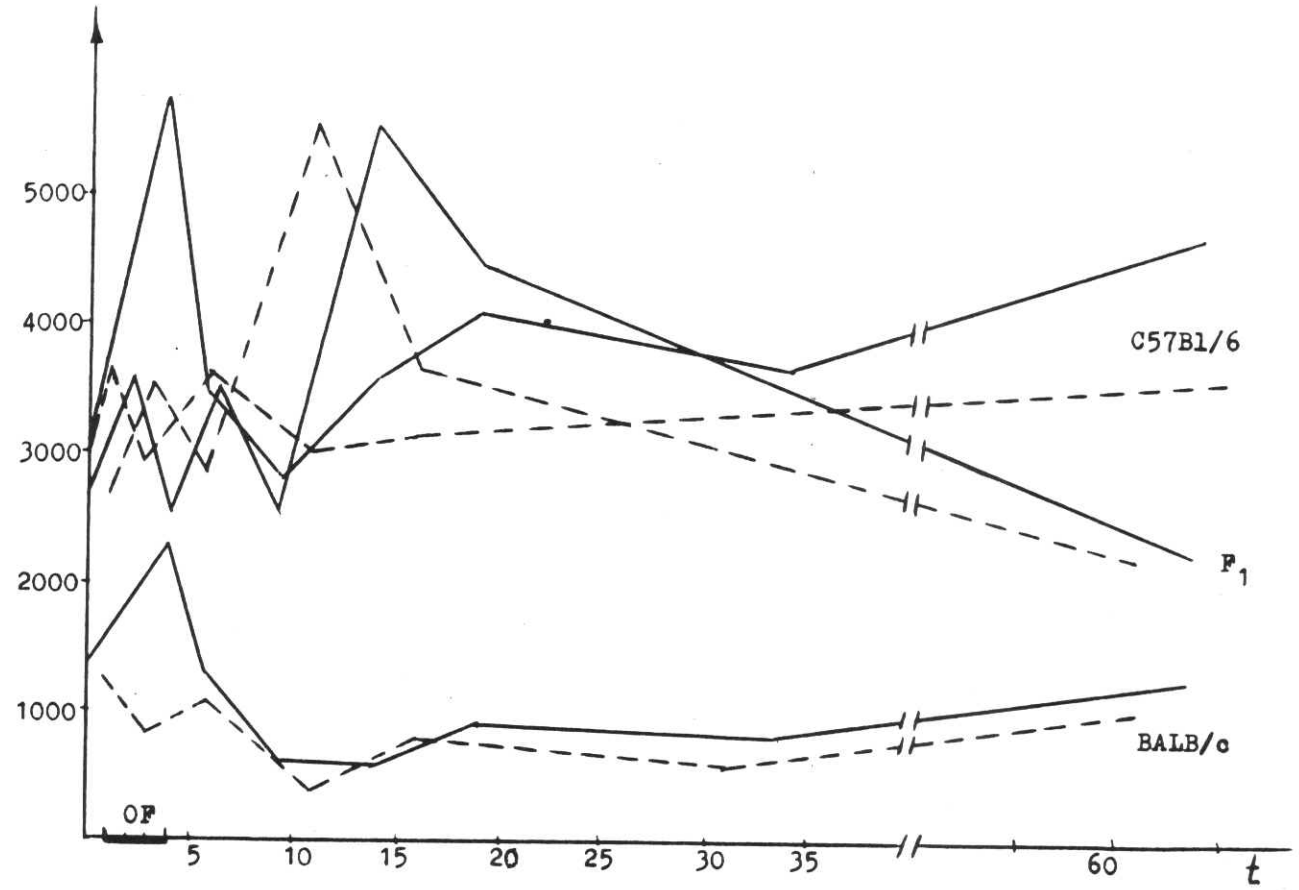

Fig. 1. - ACTH level in plasma of C57Bl/6 BALB/c, mice and F_1 hybrids (C57Bl/6 x BALB/c) after handling and open field stress. OF - open field - continuous line, handling - dotted line. Abscissa - time (min), Ordinate - ACTH level (pg/ml plasma).

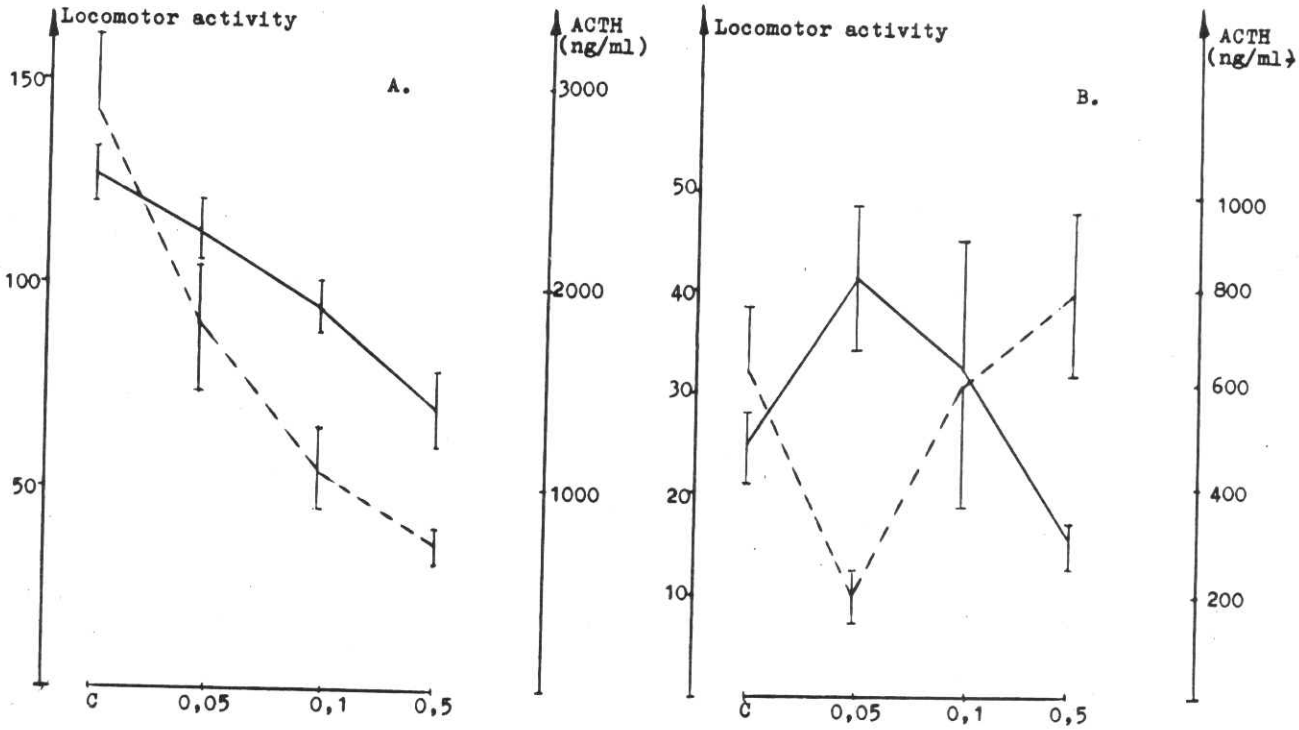

Fig. 2. - The effects of phenazepam on the locomotor activity in open field test and ACTH level in plasma of C57Bl/6 (A) and BALB/c (B) mice. Continuous line - locomotor activity, dotted line - ACTH level (pg/ml plasma). Abscissa - doses of phenazepam (mg/kg).

cAMP and cGMP plasma contents. Similar changes were also found after stress, while a different cyclic nucleotides correlation has been found in BALB/c mice (Table 2).

Of special interest are the differences observed in the changes of corticosterone in the plasma after handling and after OF test (Fig. 3). BALB/c mice were found to respond to handling with a sharp rise of the hormone level that did not change after the subsequent OF experiment. In C57Bl/6 mice a gradual increase of the corticosterone level was observed after handling and the subsequent OF test.

These results show that in the BALB/c mice the stress reaction develops in a labile way in contrast to a more gradual response of the C57Bl/6. Thus, we can conclude that different behaviour phenotypes in an emotional-stress situation correspond to different changes in the biochemical characteristics of emotional-stress reactions which proves the existence of unequal basal mechanisms involved in the response to stress.

At the next stage of the research, genetic differences in the regulation of the GABA–benzodiazepine receptor complex were studied. In the first series of experiments [^{3}H]–diazepam binding was measured in synaptosomal membranes using TRIS buffer. No differences were found between strains and the addition of GABA in the same conditions

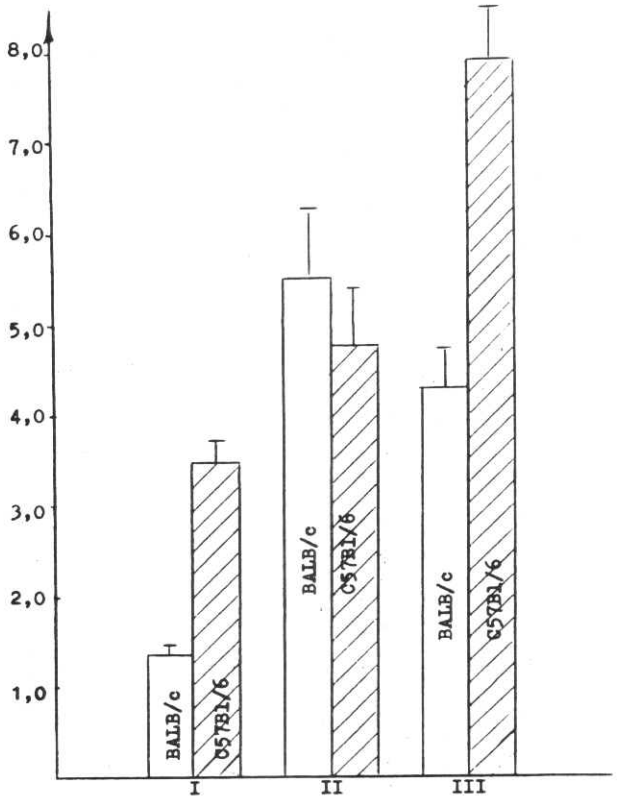

Fig. 3. - Corticosterone level in plasma of C57Bl/6 and BALB/c mice after open field test. Ordinate - corticosterone level (μg%). I) basal level; II) handling; III) open field.

Table 2. - *Concentrations of cAMP and cGMP in plasma of C57Bl/6, BALB/c mice and F_1(C57Bl/6 × BALB/c) hybrids after handling and open field test (pmole/ml plasma), (M ± SEM)*

Strain of mice	Basal level		Handling		Open field	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
C57Bl/6	45.0 ± 2.6	24.9 ± 0.8	39.6 ± 4.8	21.6 ± 1.4	25.6 ± 3.4	21.4 ± 0.7
	no. = 6	no. = 5	no. = 5	no. = 5	no. = 5	no. = 4
	$p_c < 0.001$	$p_{F_1} < 0.01$			$p_{bl} < 0.01$	
BALB/c	23.6 ± 1.34	27.0 ± 1.1	28.4 ± 1.9	26.8 ± 3.5	35.4 ± 1.22	32.4 ± 1.6
	no. = 27	no. = 45	no. = 27	no. = 25	no. = 25	no. = 24
	$p_{F_1} < 0.01$	$p_{F_1} < 0.05$	$p_{bl} < 0.05$		$p_{bl} < 0.001$	$p_{bl} < 0.01$
F_1 (C57Bl/6 x BALB/c)	58.8 ± 8.8	20.1 ± 0.86	52.4 ± 1.4	27.6 ± 0.8	22.8 ± 1.36	24.8 ± 1.6
	no. = 10	no. = 9	no. = 5	no. = 6	no. = 5	no. = 5
				$p_{bl} < 0.01$	$p_{bl} < 0.01$	$p_{bl} < 0.01$

p_c: Significant differences: between C57Bl/6 and BALB/c mice; p_{F_1}: between C57Bl/6 or BALB/c and F_1 mice; p_{bl}: between experimental and basal level; no.: number of animals.

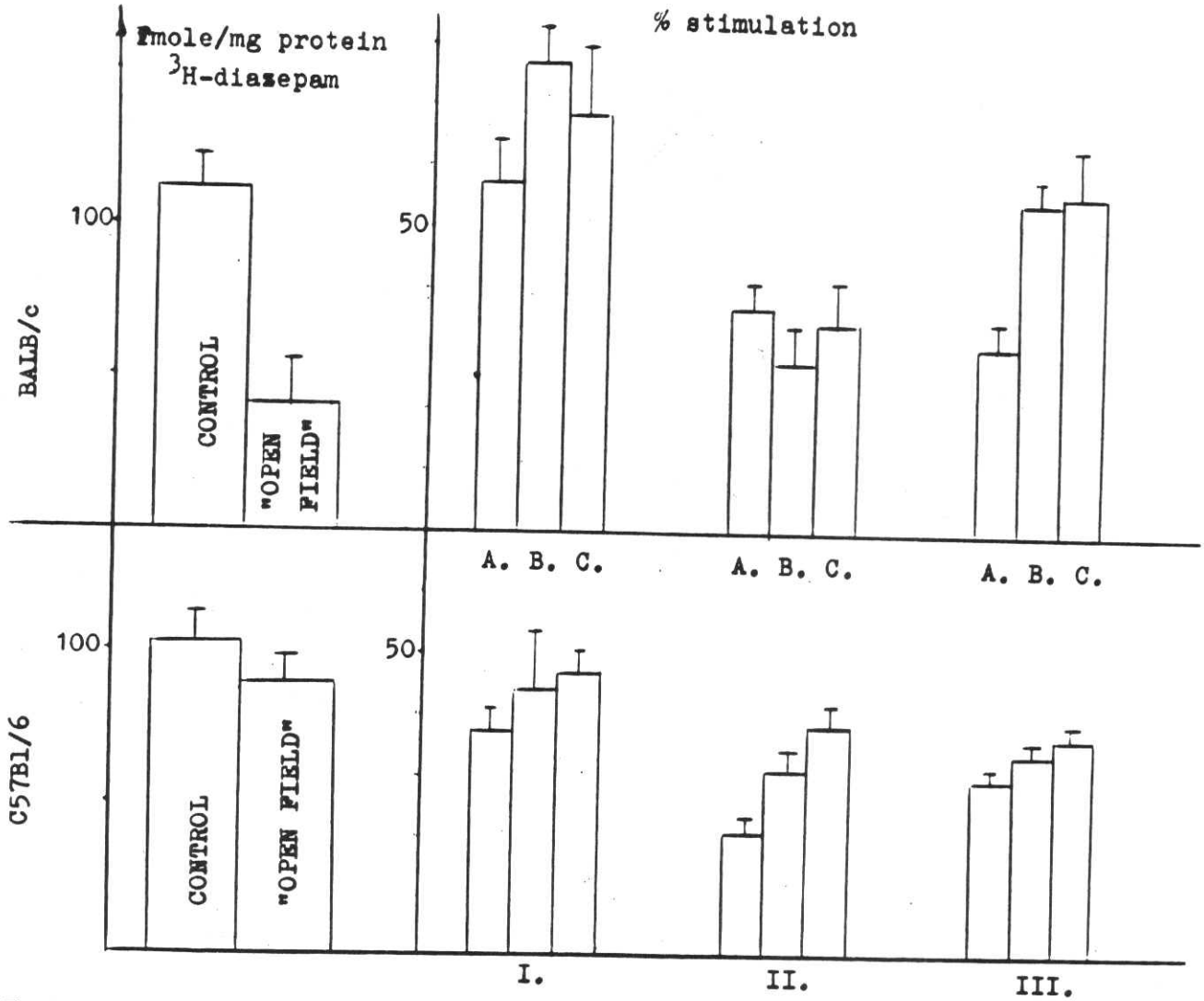

Fig. 4. - The influence of anxiolytic dose of diazepam on [^{3}H]–diazepam binding level and response to NaCl after open field stress. A: NaCl, 50 mM; B: NaCl, 100 mM; C: NaCl, 150 mM; I: control; II: open field; III: open field + diazepam, 0.075 mg/kg.

stimulated the [^{3}H]–diazepam binding to an equal extent in the two strains of animals.

The analysis of bicuculline-induced convulsions also failed to show any differences between the strains. However, the addition of NaCl into the incubation medium increased [^{3}H]-diazepam binding to BALB/c animals membranes in labile way, while the increase in the C57Bl/6 was more graduated and depended on the NaCl concentration (Fig. 4). Thus, the interstrain differences have been established in the regulation of the GABA-dependent Cl^- ionophore.

In order to analyse the state of $^-$Cl-ionophore *in vivo*, the dynamics of development of convulsions induced by picrotoxin were studied. As shown in Fig. 5, the transition from clonic seizures to tonic seizures was much more rapid in the BALB/c animals compared to that in the C57Bl/6 ones. The ability of NaCl to increase the benzodiazepine binding can be regarded as a functionally important feature. Fig. 4 shows that the brain membranes from the BALB/c mice following an emotional stress change their sensitivity to the stimulatory effect of NaCl. This effect is prevented by the injection of 0.075 mg/kg of diazepam 30 min prior to the OF experiment. On the other hand, the susceptibility of the membranes from the C57Bl/6 mice to the stimulatory effect of NaCl did not change after emotional stress.

The interstrain differences in the [^{3}H]–diazepam binding were not present when NaCl was substituted with $NaNO_3$. Studies on [^{35}S]–TBPS binding indicated that the interstrain differences were also due to NaCl content in the incubation medium (Table 3).

The above mentioned results suggest the existence of differences in functional activity of the GABA/benzodiazepine/chloride–ionophore receptor complex. The fact that the reported peculiarities of the radioligand binding in the C57Bl/6 and BALB/c mice were revealed, when the NaCl concentrations in the incubation medium varied, suggested that the differences between the strains are determined by

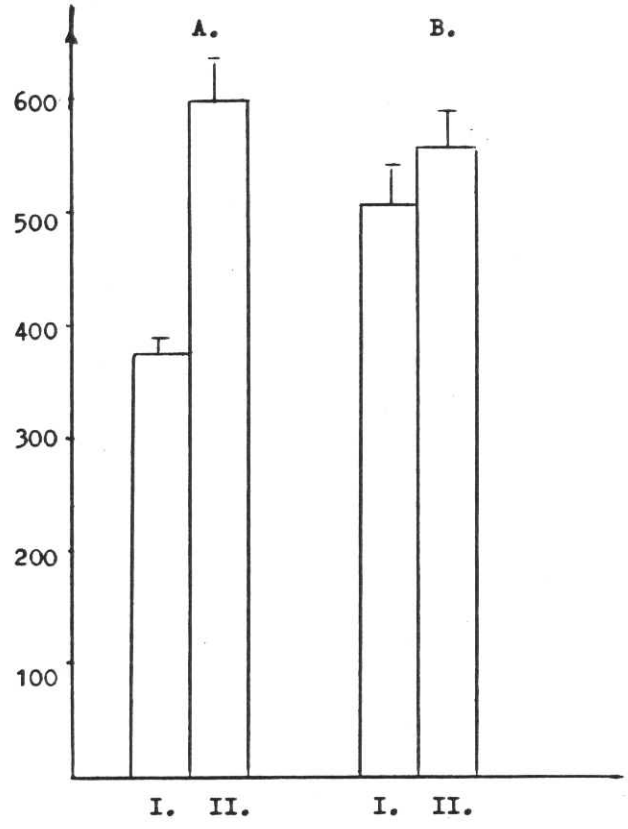

Fig. 5. - Convulsive effect of picrotoxin on C57Bl/6 and BALB/c mice. A: C57Bl/6; B: BALB/c; I: clonic seizures; II: tonic seizures. Ordinate - time onset (s).

Table 3. - *The analysis of curve inhibition of [^{35}S] -TBPS binding by picrotoxin in the membranes of inbred mice brain*

	C57Bl/6	BALB/c
IC_{50} (M)		
50 mM NaCl	$(3.43 \pm 0.24) \times 10^{-7}$ no. = 5	$(4.96 \pm 0.53) \times 10^{-7}$ no. = 5
	$p < 0.05$	
200 mM NaCl	$(2.3 \pm 0.23) \times 10^{-7}$ no. = 6	$(2.9 \pm 0.47) \times 10^{-7}$ no. = 6
Slope		
50 mM NaCl	-0.86 ± 0.03 no. = 5	-0.9 ± 0.04 no. = 5
200 mM NaCl	-1.03 ± 0.04 no. = 6	-0.88 ± 0.02 no. = 6
	$p < 0.05$	

$p < 0.05$ significant differences between C57Bl/6 and BALB/c mice; no.: number of animals.

unequal conformational reorganizations of the GABA/benzodiazepine receptor complex. Their more pronounced changes in the BALB/c mice as compared to the C57Bl/6 correspond to the differences between these strains in the reaction to emotional stress, in the benzodiazepine effects and in the dynamics of the changes of the ACTH and corticosterone levels in plasma.

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