INHERITED DIFFERENCES IN PLASMA CYCLIC NUCLEOTIDE LEVELS OF MICE EXPOSED TO STRESS AND TREATED WITH PHENAZEPAM*

S. B. Seredenin and B. A. Badyshtov

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Changes in the cAMP and cGMP concentrations in the blood plasma, on the one hand, are known to reflect the functional state of the catecholaminergic and cholinergic systems [11, 12] and, on the other hand, they can be used as parameters of the response to stressors [13].

The aim of the present investigation was to study plasma cAMP and cGMP levels in C57BL/6 (B6) and BALB/c (C) mice and their F_1 hybrids, which respond differently to stress and to benzodiazepine tranquilizers [5, 6].

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C mice and their reciprocal F_1 hybrids, weighing 18-20 g. The animals were kept under laboratory animal house conditions on a standard diet, with 10 mice per cage, 12 h of daylight and 12 h of darkness for 1.5 months before the experiment began. The open field test (OF) was used as model of emotional stress [2]. Phenazepam, in doses of 0.05 and 0.1 mg/kg, was injected intraperitoneally 30 min before the experiment began. The concentrations of cAMP and cGMP were determined in the blood plasma by methods described previously [8, 15]. The initial level of the nucleotides (the animals were killed immediately after removal from the cage) and their concentrations 15 min after the experiment in OF (the OF + 15 series) were investigated. When the effects of phenazepam were analyzed, nucleotide concentrations were determined at the same times. In control experiments the animals were subjected to the same manipulations except for being placed in the OF (the handling series). Instead of phenazepam they were given an injection of physiological saline (0.9% NaCl solution), and an OF test was carried out after the injection.

EXPERIMENTAL RESULTS

The initial plasma cAMP level was found to be higher in B6 mice than in C mice. The cGMP concentration was similar in animals of the two strains. In the handling series the

*7-Bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

TABLE 1. Plasma cAMP and cGMP Concentrations (in pmoles/ml) of B6 and C Mice and Their F_1 Hybrids in Handling and OF + 15 Series (M ± m)

Strain of mice	Initial level		Handling series		OF+15 series	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
C57BL/6	45,0±2,6 (n=6)	24,9±0,8 (n=5)	39,6±4,8 (n=5)	21,6±1,4 (n=5)	25,6±3,4* (n=5)	21,4±0,7 (n=4)
BALB/c	23,6±1,3 (n=27)	27,0±1,1 (n=45)	28,4±1,9* (n=27)	26,8±3,5 (n=25)	35,4±1,2* (n=25)	32,4±1,6* (n=24)
F_1	58,8±8,8 (n=10)	20,1±0,9 (n=9)	52,4±1,4 (n=5)	27,6±0,8* (n=6)	22,8±1,4* (n=5)	24,8±1,6* (n=5)

Legend. Cyclic nucleotide concentrations in reciprocal F_1 hybrids were similar. Data given for B6 × C combination. *P < 0.05 compared with initial level. Here and in Table 2, n denotes number of experiments.

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TABLE 2. Effect of Phenazepam on Plasma Cyclic Nucleotide Levels (in pmoles/ml) in B6 and C Mice in Different Series of Experiments (M ± m)

Strain of mice	Dose of phenazepam, mg/kg	Handling series		OF+15 series	
		cAMP	cGMP	cAMP	cGMP
C57BL/6	Control (0.9% NaCl solution)	66,0±5,8[a] (n=5)	23,1±0,37 (n=5)	37,6±5,8[B] (n=5)	24,3±1,0 (n=6)
	0,05	60,0±4,2 (n=5)	18,0±1,9[6] (n=5)	38,0±2,6[B] (n=4)	29,3±1,6 (n=4)
	0,1	56,0±2,2 (n=6)	29,4±1,0 (n=5)	42,0±4,0 (n=5)	25,8±2,0 (n=5)
BALB/c	Control (0.9% NaCl solution)	55,2±2,2[a] (n=7)	27,2±0,4 (n=7)	56,0±3,6 (n=6)	26,8±1,1 (n=6)
	0,05	12,8±0,5 (n=5)	29,7±1,9 (n=5)	36,0±1,6[6,B] (n=6)	35,0±1,8[6,B] (n=6)
	0,1	11,0±0,6[6] (n=4)	25,0±1,8 (n=6)	50,0±8,0[B] (n=5)	39,8±2,3[6,B] (n=6)

Legend. a) $P < 0.05$ compared with initial level, b) the same compared with control, c) the same compared with OF + 15 series.

cAMP concentration in B6 mice had a tendency to fall, but after OF the fall was significant. Conversely, in C mice, the cAMP level rose both in the handling series and after OF (Table 1). The cGMP concentration remained unchanged in the B6 mice after all procedures, but in the C group it rose after OF. Thus in B6 animals, which respond actively in OF with predominance of investigative behavior, and in C mice, characterized by a marked anxiety reaction in OF [2], significant differences were discovered in the initial plasma cyclic nucleotide concentrations, together with opposite changes in the levels under the conditions of this test.

The F_1 hybrids, resembling B6 in their investigative activity in OF, and occupying an intermediate position between B6 and C as regards emotional make-up, inherited the original nucleotide ratio and changes in the cAMP level after handling and the OF test of the B6 type. Meanwhile, the increase in the cGMP concentration observed in F_1 after OF corresponded to the reaction observed in the C group (Table 1).

Interlinear differences in the original cAMP level in the B6 and C groups agree with data on the brain adrenergic activity of these animals, revealing a higher level of this activity in B6 mice, and inherited as a dominant trait by their F_1 hybrids [11, 14]. The cGMP concentration in the majority of experiments was higher in the C mice, also in agreement with data indicating greater cholinergic activity in the C mice than in the B6 group [10, 14].

The results of the present investigation thus confirm conclusions [9, 12] indicating that the plasma cAMP and cGMP levels reflect activity of the catecholaminergic and cholinergic systems.

Meanwhile correlation of the original ratio between the concentrations of cyclic nucleotides and the specific character of changes in their concentrations, on the one hand, with the various forms of stress response characteristic of B6, C, and F_1 hybrid mice, on the other hand, demonstrates hereditary control over these biochemical changes and justifies a further study of these parameters as possible criteria for use in distinguishing between different types of stress states.

The data in Table 2 show that after a control injection of physiological saline the cAMP level in B6 mice rose to 146%, and in C mice to 239% of the initial level, whereas the cGMP concentration was unchanged. Phenazepam did not affect the cAMP concentration in the B6 mice but sharply reduced it in the C group.

As a result of exposure to stress in OF the cAMP level in the B6 group fell after the control injection and after injection of the tranquilizer, just as in the handling and OF + 15 series (Tables 1 and 2). Phenazepam evidently does not modify the changes in the plasma cAMP concentration in B6 mice due to handling and the OF test. Meanwhile in C animals, the tranquilizer in a dose of 0.05 mg/kg partly prevented the rise in the cAMP concentration observed after the OF experiment, preceded by injection of 0.9% NaCl solution (Table 2). Incidentally, in this same dose phenazepam stimulated the behavior of C mice in OF [5].

Changes in the cGMP level in the B6 animals in experiments with phenazepam were opposite in direction, and in the C group, the OF test after injection of the tranquilizer caused the cGMP concentration to rise (Table 2).

Analysis of the experimental results shows that the action of phenazepam on animals with different types of stress reaction also is accompanied by specific changes in the cyclic nucleotide concentrations.

Without dwelling in this paper on the mechanisms of the observed effects, it will be noted that benzodiazepines differ in their effect on animals with an active and animals with a passive type of stress response. When the response is active phenazepam and diazepam have a mainly sedative action, whereas if the response if passive (inactivity), their action is anxiolytic [5, 7]. Similar effects have been found in man [1, 4].

If the homologous character of the basal mechanisms of development of the stress reaction in man and other mammals is accepted [3], there is reason to suppose that determination of changes in plasma cyclic nucleotide concentrations induced by benzodiasepines will prove useful both for the differentiation between stress states and for clinical and pharmacologic screening of tranquilizers.

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