INVESTIGATION OF THE BRAIN CYCLIC NUCLEOTIDE LEVELS IN INBRED MICE EXPOSED TO EMOTIONAL STRESS

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Data relating to the genetic control of the emotional-stress response [2, 3] attach particular importance to the elucidation of mechanisms of formation of differences observed in behavioral tests. It was shown previously that inbred C57B1/6 (B6) mice give an active response in the open field (OF) test, whereas BALB/c (C) mice give a passive response [2].

In view of the role of cyclic nucleotides in noradrenergic and cholinergic processes [6, 8], it was decided to study their concentrations in different parts of the brain of intact animals of the above-mentioned lines and in the same animals after exposure to stress in the OF test.

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C mice weighing 18-20 g. The animals were kept in the laboratory animal house on a standard diet, ten to a cage, with a 12-h period of daylight and darkness for three weeks until the beginning of the experiments. Emotional stress was simulated in the OF test [2]. cAMP and cGMP concentrations were determined in the hypothalamic region (I), the cortex (II), cerebellum (III), medulla and pons (IV), diencephalon and mesencephalon (V), using kits obtained from "Chemapol" (Czechoslovakia). Nucleotide levels were investigated initially, after decapitation of the animals immediately after removal from the cage, and also immediately after the end of the OF test (series OF + 0), and also 3, 20, and 120 min later (series OF + 3, OF + 20, and OF + 120, respectively).

EXPERIMENTAL RESULTS

The initial cGMP level in regions III and IV and the cAMP level in region IV of the brain was found to be higher in C than in B6 mice (Table 1). The character of distribution of cGMP in the region tested was virtually identical for both lines. The only exception was that the concentration of this nucleotide in B6 mice was higher in region II than in region IV, whereas the corresponding levels in C mice were equal.

Meanwhile, considerable interlinear differences were found in the distribution of cAMP among the brain structures. For instance, in brain region I of B6 mice the highest concentration of the nucleotide was recorded. In C mice the initial cAMP level in the hypothalamic region was similar to that in region II although, just as in B6, it was higher than in regions III, IV, and V. It was then found that the cAMP level in region II of the brain of C mice was higher than in region V, whereas in B6 mice the corresponding values were identical. It was also found that the cAMP concentration in region III of B6 mice was approximately twice as high as that in region IV, whereas in C mice there was no difference in the nucleotide concentrations in these regions. Finally, unlike in B6 animals, in which the initial cAMP level was the same in regions III and V, in C mice there was a statistically significant excess of the nucleotide concentration in region III.

Considerable interlinear differences also were demonstrated in a study of the time course of changes in the cyclic nucleotide concentrations in brain regions. The cAMP and cGMP concentrations in the regions of the brain investigated in C mice were constant (Table 1). Only a rapid (OF + 0) and transient rise of cAMP level in region III and a fall in the

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TABLE 1. Cyclic Nucleotide Concentrations in Brain Regions (in pmoles/mg wet weight) in C and B6 Mice at Different Times of the OF Test (M ± m)

Brain region	Initial level		OF + 0		OF + 3		OF + 20		OF + 120	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
					Mice of line C					
I	1,45±0,11 (32)	0,138±0,019 (29)	1,71±0,23 (13)	0,115±0,027 (13)	1,06±0,18 (10)	0,109±0,027 (13)	1,28±0,13 (16)	0,205±0,053 (15)	1,11±0,14 (14)	0,104±0,02 (15)
II	1,15±0,11 (35)	0,035±0,004 (35) a_1	1,55±0,18 (10)	0,018±0,004 (10)	0,93±0,17 (16)	0,025±0,004 (15)	1,04±0,13 (16)	0,031±0,005 (15)	1,01±0,18 (14)	0,035±0,007 (15)
III	0,88±0,07 (34) a_1	0,079±0,009 (33) $a_1b_1m_1$	1,25±0,14 (13) k_2	0,075±0,009 (13)	0,69±0,09 (15)	0,094±0,014 (15)	0,86±0,08 (16)	0,113±0,018 (16)	1,06±0,12 (15)	0,091±0,013 (16)
IV	0,74±0,07 (35) $a_1b_1m_1$	0,032±0,004 (33) $a_1c_1m_1$	0,83±0,15 (12)	0,025±0,006 (12)	0,56±0,08 (15)	0,024±0,005 (14)	0,89±0,10 (15)	0,036±0,009 (13)	0,63±0,07 (16)	0,035±0,006 (16)
V	0,64±0,07 (34) $a_1b_1c_2$	0,032±0,004 (34) a_1c_1	0,73±0,10 (14)	0,020±0,003 (14) k_2	0,68±0,13 (15)	0,021±0,004 (12)	0,69±0,08 (14)	0,027±0,006 (15)	0,68±0,12 (16)	0,026±0,005 (16)
					Mice of line B6					
I	1,56±0,15 (31)	0,125±0,021 (29)	1,79±0,20 (21)	0,110±0,022 (19)	1,52±0,15 (19)	0,117±0,021 (20)	1,17±0,13 (18)	0,103±0,017 (19)	2,52±0,24 (20) k_2	0,122±0,028 (20)
II	1,09±0,17 (34) a_2	0,030±0,003 (33) a_1	1,16±0,17 (20)	0,040±0,006 (21) k_2	1,02±0,12 (21)	0,035±0,006 (21)	1,33±0,18 (19)	0,034±0,006 (18)	1,14±0,08 (20)	0,028±0,004 (21)
III	0,90±0,10 (30) a_1	0,059±0,006 (30) $a_1b_1m_1$	1,08±0,11 (21)	0,094±0,013 (21) k_1	1,03±0,11 (21)	0,079±0,013 (21)	1,05±0,13 (20)	0,061±0,008 (20)	1,26±0,12 (20) k_2	0,085±0,011 (20) k_2
IV	0,50±0,05 (33) $a_1b_1c_1m_1$	0,022±0,003 (30) $a_1b_1c_1m_1$	0,79±0,13 (22) k_2	0,044±0,006 (21) k_1	0,64±0,07 (20)	0,033±0,006 (20) k_2	0,75±0,11 (20) k_2	0,029±0,004 (19)	0,81±0,07 (21) k_1	0,033±0,004 (22) k_2
V	0,70±0,09 (31) a_1	0,026±0,003 (34) a_1c_1	1,40±0,17 (21)	0,034±0,005 (19)	0,70±0,13 (22)	0,028±0,004 (22)	0,88±0,11 (20)	0,020±0,003 (19)	0,89±0,10 (20)	0,030±0,005 (20)

Legend. a_1) $p < 0.01$ compared with I; a_2) $p < 0.05$ compared with I; b_1) $p < 0.01$ compared with II; b_2) $p < 0.05$ compared with II; c_1) $p < 0.01$ compared III; c_2) $p < 0.05$ compared with III; k_1) $p < 0.01$ compared with initial level; k_2) $p < 0.05$ compared with initial level; m_1) significance of differences at $p < 0.01$ level between C and B6 mice. Number of experiments shown between parentheses.

cGMP level in region V were observed. Conversely, in B6 mice exposure to emotional stress in OF was accompanied by marked changes in the cyclic nucleotide concentrations in the brain structures. A slow (OF + 120) rise of the cAMP level was observed in regions I and III, and a rapid and prolonged rise in region IV. The cGMP concentration rose immediately after the OF test (OF + 0) in regions II, III, and IV. However, whereas in region II it was very short in duration, in regions III and IV it lasted for 2 h of the investigation.

We know that cyclic nucleotides mediate the action of many hormones and transmitters inside the cell. Accordingly, differences in their concentration assume importance on their own account, for they reflect integral differences in the processes of formation of the stress response.

It is worth noting that B6 and C mice, which in the OF test exhibited active and passive behavior respectively, exhibited differences in the initial level and distribution of cyclic nucleotides in the various brain regions, evidence of their participation in mechanisms of genetic control of emotional-stress responses of different types. The higher cGMP concentration in some of the brain structures of C mice, revealed by the present investigation, is in agreement with data in the literature, indicating a higher level of cholinergic activity in the brain of mice of this line than of B6 mice [7, 10]. The cAMP concentration was higher in C than in B6 mice only in region IV. This fact is in agreement with the results of investigations [1] showing that emotionally reactive Wistar rats, isolated from the general population on the basis of low motor activity in OF, are characterized by a higher concentration of adrenalin and dopamine, which are adenylate cyclase activators, in region A_1 of the medulla.

The greatest differences in the dynamics of the changes in cAMP and cGMP concentrations also were found in this same region. On the whole these results suggest that there are significant differences in the biochemical regulation of neurons in the brain stem and pons during the formation of different types of behavior of B6 and C mice in the OF test.

Specific differences in the initial level and time course of changes in cAMP concentration for the two lines also were discovered in the cerebellum. The possibility cannot be ruled out that these facts are linked with the different levels of motor activity of B6 and C mice. Elevation of the cGMP level in the cerebellum, coupled with increased motor activity during exposure to stress, were observed also by other investigators [5, 9].

In this study no correlation could be found between cyclic nucleotide levels in the brain and data obtained previously for blood plasma [4]. Nevertheless it is evident that the unequal and cGMP concentrations in the brain of the animals studied are evidence of genetic differences in the central neurochemical mechanisms of formation of responses to emotional stress, and in turn these differences may be responsible for the peripheral neurotransmitter and hormonal responses specific for the particular line, and thus for the time course of changes in nucleotide concentrations in the blood plasma characteristic of this type of response.

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