GENETIC CONTROL OF THE PLASMA ACTH LEVEL IN MICE

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Previous investigations showed that the response of animals to emotional stress is genetically dependent [1, 4]. The object of this investigation was to study one of the most important parameters of the stress response, namely the plasma ACTH level, in mice differing in their behavior in an open field (OF) test.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 (B6) and BALB/c (C) mice from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, and reciprocal F_1, F_2, and F_1 × C hybrids, obtained in the laboratory, weighing 18-20 g; the animals were kept on a standard diet with 12 h daylight and 12 h darkness, 10 in a cage at a time, in a separate room in the animal house for 1 month before the experiment began.

A stress situation was simulated in an OF test [4]. Blood obtained after decapitation was collected in cold tubes with EDTA solution (1 mg/ml). Plasma was obtained by centrifugation in an I-6B centrifuge (from Beckman, USA) at 2000g for 15 min at 0-4°C.

The plasma ACTH concentration was determined by a radioimmune method, using reagents from kits from International CIS (France). The radioactivity of the samples was measured on a Gamma-4000 counter (from Beckman, USA). All experiments were carried out from October through January. Blood was taken daily between 10 a.m. and 2 p.m.

In the control experiments no significant differences were found in the ACTH concentration during this time interval. The results of analysis of ACTH levels in reciprocal F_1 hybrids were similar, and accordingly the data obtained in experiments on the B6 × C combination are given below.

EXPERIMENTAL RESULTS

In the experiments of series I a hybridologic analysis was made of the plasma ACTH concentration in B6 and C mice and their hybrids. The results showed (Table 1, Fig. 1) that the ACTH level is under genetic control and is inherited in accordance with the basic rules established for quantitative characters [2]. Calculation by Serebrovskii's equation [2] of the minimal number of genes (n) controlling this character showed that $n = 0.6$, i.e., $n \approx 1$. This gives good grounds for considering that inheritance of the initial ACTH level in mice of the lines tested is unifactorial in type.

The absence of characteristic Mendelian ratios in F_2 and in the progenies of batch-crossings can be explained by the continuous spectrum of distribution of the character in the parental lines, accompanied by a considerable influence of environmental factors. The results agree well with modern views on the molecular mechanisms of ACTH synthesis in the composition of one large precursor molecule [8] and with other data in the literature [9].

In the experiments of series II the plasma ACTH concentration was studied in B6 and C mice and their F_1 hybrids at different time intervals after exposure to stress in OF, and in control animals after identical manipulations accompanying the experiment (handling), except placing them in the open field.

In B6 mice handling did not cause any significant changes in the ACTH concentration for 1 h. Conversely, placing in OF led to a sharp rise in the hormone level immediately after

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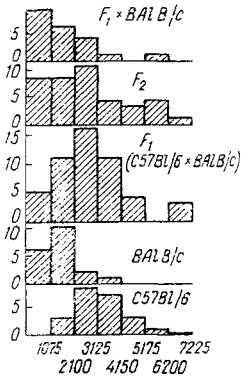

Fig. 1

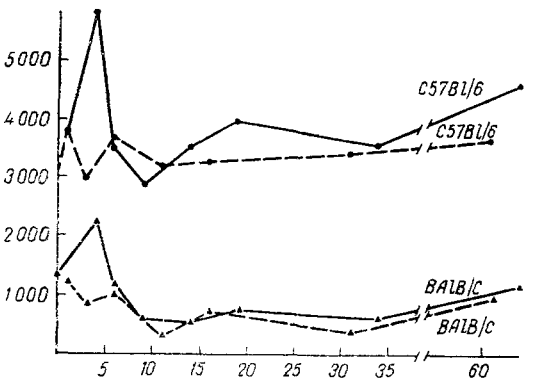

Fig. 2

Fig. 1. Inheritance of initial plasma ACTH level during crossing of C57BL/6 and BALB/c mice. Abscissa, ACTH concentration (in pg/ml); ordinate, number of animals.

Fig. 2. Plasma ACTH concentration in C57BL/6 and BALB/c mice after exposure to stress in OF and after handling. Abscissa, time (in min); ordinate, ACTH concentration (in pg/ml). Continuous line — OF, broken line — handling.

the end of the experiment, followed by a rapid fall. The next significant increase in the ACTH concentration occurred 15 min after the experiment in OF (Fig. 2).

A different time course was observed in the C mice. In control animals there was a continuous decline in the ACTH concentration starting with the first minute after the mice were picked up, and not until 1 h later did its level rise very slightly.

Placing the C mice in OF led to a significant rise in the hormone level immediately after the end of the procedure. However, the same continuous fall in the ACTH concentration as in the control animals was then observed (Fig. 2). The results agree with those of an investigation of the corticosterone concentration in mice of the same lines under analogous conditions; the corticosterone concentration increased rapidly in C mice after handling alone, whereas in B6 mice the corticosterone response was weaker, and in the absence of OF, it disappeared after 10 min.

Thus in B6 and C mice, characterized by different behavior in a stress situation, the hormonal response, which lies at the basis of the stress response, differed sharply.

Investigation of the ACTH concentration in F_1 hybrids revealed changes similar on the whole to those found for the parental B6 line. The initial hormone level, the temporal and quantitative characteristics of the periodic rises of its concentration in the blood plasma were similar (Table 2).

The character of inheritance of the ACTH concentration during crossing of B6 × C lines was thus similar to that of inheritance of the principal parameters of behavior in OF [3]. However, other features were present in the F_1 hybrids: in them, unlike in B6, handling caused significant changes in ACTH (Table 2). Nevertheless, examination of the B6 and C animals and their F_1 hybrids as a model with a genetically determined response to primary stress in OF leads to the conclusion that the inherited variants of function of the ACTH-corticosteroids system play an important role in and are interconnected with the formation of the type of behavior in a stress situation.

Taken as a whole, the results demonstrate genetic control over the plasma ACTH concentration and, in light of modern data on the biological activity of oligopeptides found in the composition of the molecule of this hormone, they may prove useful to the explanation of physiological and behavioral differences between B6 and C mice such as emotionality [5], ability to form conditioned reflexes and memory [6], preference for alcohol [10], and other differences discovered during the course of many investigations on animals of these lines [11].

TABLE 1. Statistical Parameters of Initial ACTH Concentration in Blood Plasma of Parental Lines B6 and C and in F_1, F_2, and F_1 × C Hybrids

Generations	Number of animals (n)	Mean values ($\bar{x} \pm S\bar{x}$)	Dispersion (σ_x)
B6	22	3100±215*	1007
C	19	1350±162	707
F_1	50	2747±214*	1511
F_2	38	2636±284	1749
F_B (F_1×C)	21	1682±351 †	1608

*P < 0.001 compared with mice of the C line.
† P < 0.05 compared with mice of the B6 line.

TABLE 2. Plasma ACTH Concentration in (C-57BL/6 × BALB/c)F_1 Hybrids after Exposure to Stress in the Open Field (OF + t*) and after (—OF + t) Handling

Experiment	Number of animals	ACTH concentration, mg/ml	P compared with initial level	P compared with «handling»
Initial level	50	2747±214		
First minute inCF†	13	3604±462	>0,05	
OF +0	30	2565±144	>0,1	<0,1
—OF +0	30	2602±196	>0,1	
OF +2	8	3463±362	>0,1	<0,1
—OF +2	8	3675±188	>0,1	
OF +5	8	2581±397	>0,1	<0,1
—OF +5	9	2844±344	>0,1	
OF +10	9	5672±667	<0,001	<0,1
—OF +10	9	5594±630	<0,001	
OF +15	9	4494±513	<0,01	<0,1
—OF +15	6	3775±304	>0,1	
OF +30	8	3513±455	>0,1	<0,1
—OF +30	8	3000±355	>0,1	
OF +60	9	2222±474	>0,1	<0,1
—OF +60	9	2944±1118	>0.1	

*t) Time (in min) after experiment lasting 3 min in OF.
†ACTH concentration was determined 1 min after placing in OF.

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