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ANALYSIS OF THE BRAIN ACTH-IMMUNOREACTIVE PEPTIDE SPECTRUM IN INBRED MICE

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Mice of the BALB/c (C) and C57BL/6 (B6) strains, characterized by high and low emotionality respectively in open field (OF) tests, have been shown to differ considerably in both the initial level and the time course of changes in the plasma ACTH concentration after exposure to stress in an OF and after administration of a benzodiazepine tranquilizer [1]. The ACTH concentration in the pituitary gland of animals of these lines also differs [3]. We know that the ACTH molecule contains regions with neurotropic activity [4]. It can therefore be postulated that differences in the level of this hormone and (or) its biotransformation products in the brain are an essential factor in the mechanisms of formation of hereditary features of emotional behavior.

In the first stage of the investigation, undertaken to test this hypothesis, spectra of ACTH-immunoreactive peptides (ACTH-IP) were studied in chromatographic fractions of an acid brain extract, and also in the blood plasma of mice belonging to B6 and C lines and their F_1 hybrids.

EXPERIMENTAL METHOD

The B6 and C mice and their F_1 hybrids were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. The animals were kept 10 to a cage on a standard diet with 12 h of daylight and 12 h of darkness. A model of emotional stress in the OF test was used [2]. The mice were decapitated immediately after the OF experiments. The brain was quickly extracted and the medulla, cerebellum, and cerebral cortex removed. The rest of the brain was used for subsequent study. The material was homogenized in 9 ml of 1 M acetic acid, containing 0.1% (by volume) of thiodiglycol, and heated to 90°C. To each sample of blood plasma 9 ml of this same solution was added. Samples were heated for 15 min on a water bath at 90°C. They were then centrifuged at 37,000g in the 50Ti rotor on an L5-50 centrifuge (Beckman, USA). The resulting supernatant was decanted into polyethylene flasks, frozen to −40°C, and lyophilized. The lyophilized samples were then dissolved in 0.1% (by volume) trifluoroacetic acid (TFA, from Fluka, Switzerland) and filtered through nitrocellulose filters (0.45 μ, Nucleopore, USA). The resulting peptide extract, in a volume of 1 ml, was fractionated on an "Analyst" high-pressure liquid chromatograph (LDC, England), using a Partisil PXS 10/25 ODS column, (Whatman, England). A spectrophotometric detector (λ 210 nm, sensitivity 0.2) was used for scanning. Elution was carried out under gradient conditions with two solutions. Solution A, 0.1% (by volume) TFA—acetonitrile (17:3); solution B, the same components in the ratio of 1:4. A gradient of 0 to 7% of solutions B and A was used. Fractionation continued for 1 h at a flow rate of 1 mm/min. The shape of the gradient is illustrated in Fig. 1. The column was cleaned by passing solution B through it for 20 min. To determine the ACTH-IP concentration the eluate was collected in separate fractions each of 2 ml. The acetonitrile was evaporated *in*

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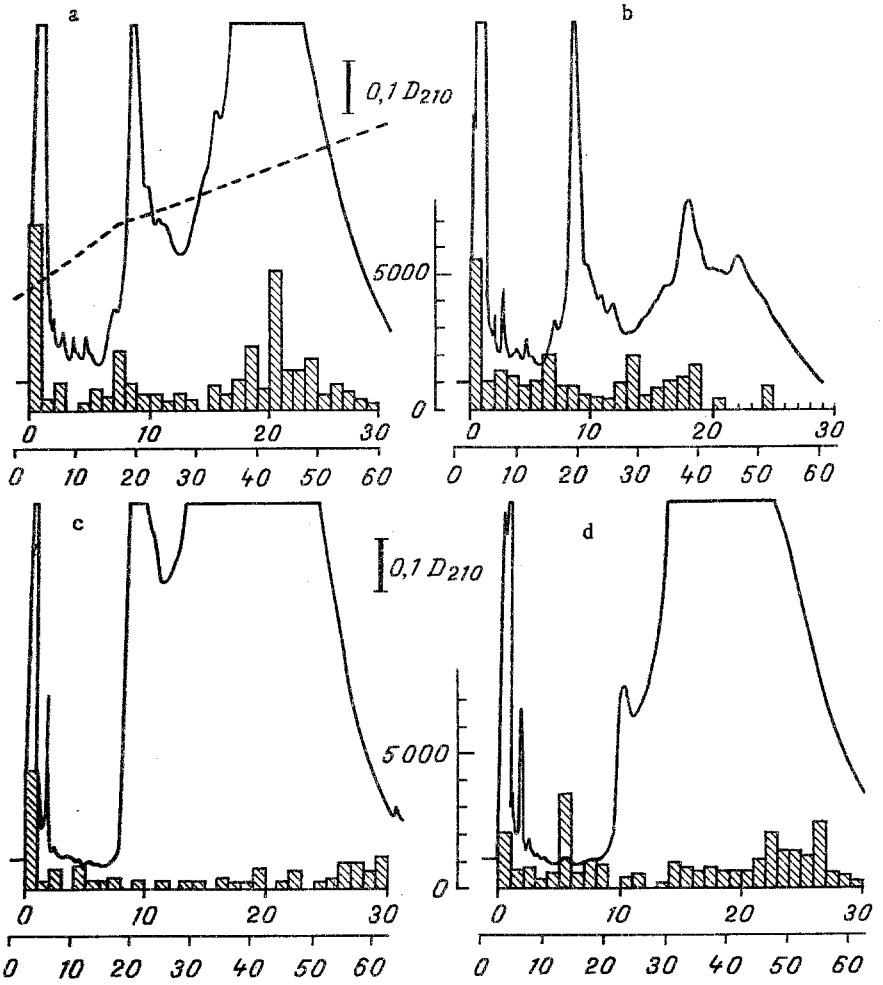

Fig. 1. Chromatogram of acid brain extract and blood plasma of B6 mice and ACTH-IP concentration in separate fractions before and after exposure to stress in the OF test. Here and in Figs. 2 and 3: horizontal axis, time (in min); vertical axis, ACTH-IP level (in pg ACTH/g tissue): a) control chromatogram of brain extract (broken line shows shape of gradients); b) chromatogram of brain extract after OF test; c) control chromatogram of extract of blood plasma; d) chromatogram of extract of blood plasma after OF test. Numbers beneath columns show Nos. of fraction.

vacuo at 37°C and the residue lyophilized. The test peptides were identified by radioimmunoassay using kits from CIS Internationale (France).

Typical chromatographic curves were obtained, and their reproducibility confirmed in 5-7 experiments. ACTH-IP was determined in products of chromatographic fractionation of pooled acid brain extracts from 6 animals. The results of two repeated experiments were identical. Average values of the parameters studied are given below.

EXPERIMENTAL RESULTS

Results of determination of ACTH-IP in the fraction of acid brain extracts of B6, C, and F_1 mice are given in Figs. 1-3. It will be evident that the test material was present in larger quantities in the B6 than in the C mice in fractions 1, 11, 18, 19, and 21-24. Conversely, the ACTH-IP level was higher in the C mice in fractions 2, 3, 5, 7-10, 12, 13, 17, 20, 25, and 27-29 than in the B6 mice. In some fractions differences were very small or absent altogether (Figs. 1-3, a).

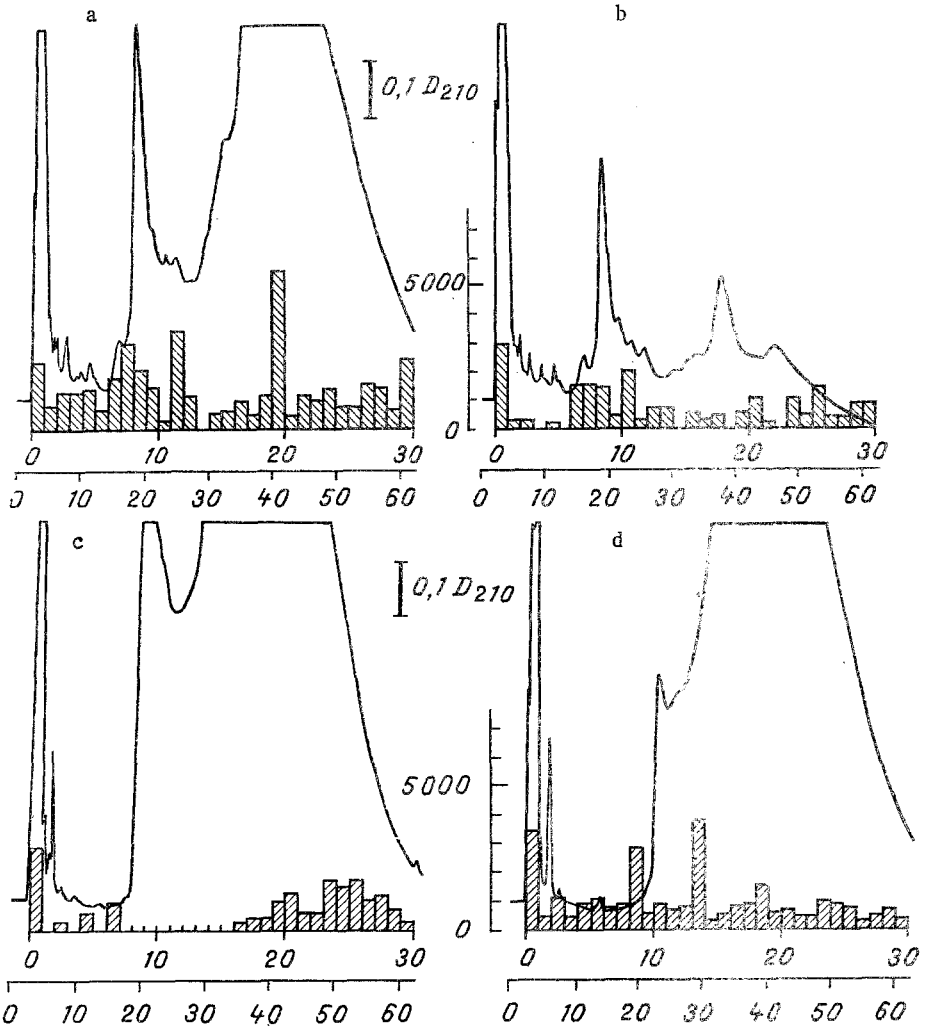

Fig. 2. Chromatogram of acid brain extract and blood plasma of C mice and ACTH-IP concentration in separate fractions before and after exposure to stress in OF test.

Analysis of the chromatograms reveals qualitative differences in the distribution of ACTH-IP. In fractions 4, 15, and 30, for instance, these peptides were found only in C mice, and the opposite pattern was observed in fraction 14.

In the F_1 hybrids the ACTH-IP level in fractions 3-5, 7, 8, 11, 18, 19, 21, 23, 25, and 27 was similar to that in the C mice, whereas in fractions 1, 2, 9, 10, 15, 20, 28, and 29 the ACTH-IP level was the same as B6 mice. In fractions 12 and 17, no ACTH-IP could be found in F_1 mice.

After exposure to stress in the OF test all the experimental animals showed a tendency for the level of immunoreactive material to decrease (Figs. 1-3, b). The ACTH-IP concentration fell most noticeably in the C mice in fractions 2, 3, 5, 8-10, 12, 13, 17, 18, 20, 22, 24, 25, 27, 28, and 30, and none were found in fractions 4, 6, 15, 19, and 23. Similar changes in the B6 mice were recorded in fractions 8, 19, and 21, and no ACTH-IP could be found in fractions 20, 22-24, and 26-30. In the F_1 hybrids the quantity of test peptides was reduced in fractions 1, 4, 7, 8, 16, 20, 22, 27, and 30, and none could be found in fractions 5, 6, 10, 14, 18, and 23-26. Meanwhile, the ACTH-IP concentration was increased in fractions 11 and 26 in C mice, in fractions 5, 6, 13, 14, and 17 in B6 mice, and in fractions 2, 3, 9, 11, 13, 19, 21, 28, and 29 in the F_1 mice. In fraction 14 of the B6 mice, fractions 4 and 15 of the C mice, and 12, 15, and 17 of the F_1 mice, the test peptides were identified only after the OF test.

Chromatographic analysis of ACTH-IP in peptide extracts from the animals' blood also revealed marked interlinear differences (Figs. 1-3, c). A higher level of immunoreactive material was found in B6 mice than in C mice in fractions 1, 3, 5, 17, and 30, and the opposite picture was observed in fractions 7, 18, 19, 22, 25, and 26. In fractions 2, 8, 10, 12, 14, 15, and 27,

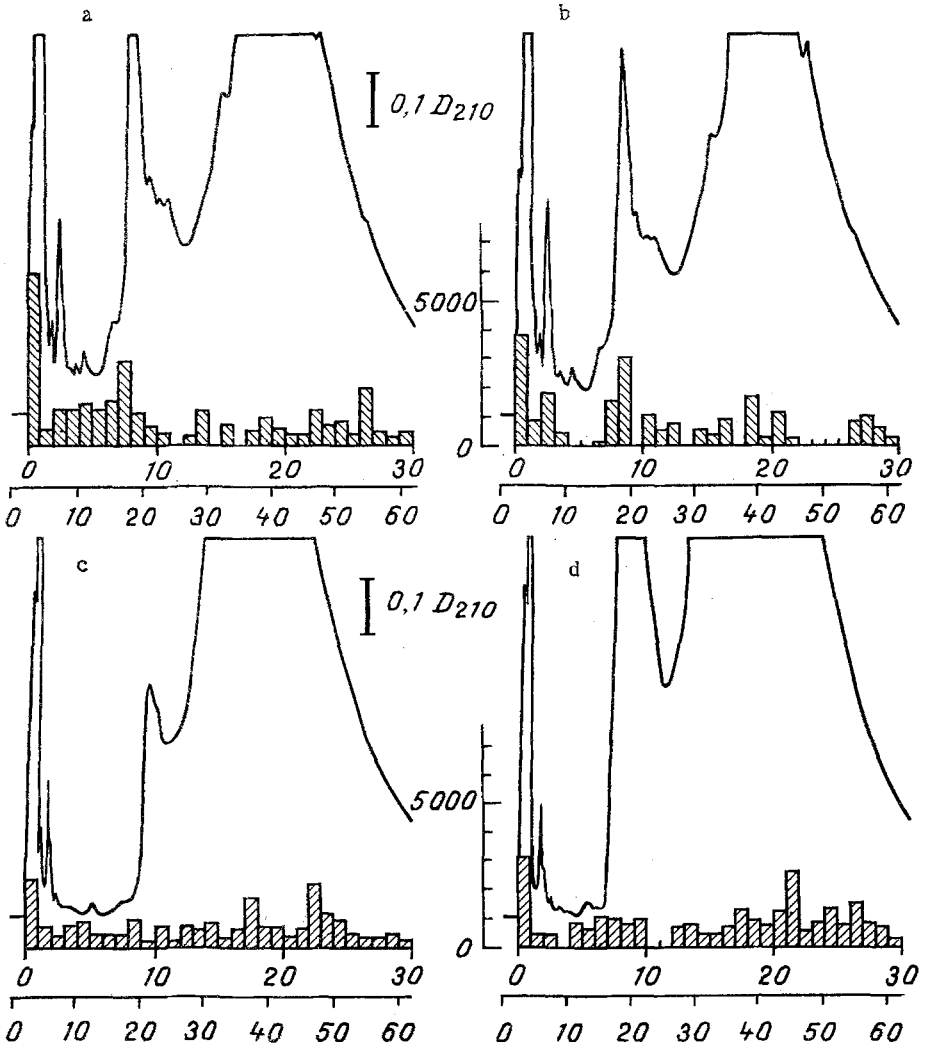

Fig. 3. Chromatogram of acid brain extract and blood plasma of F_1 hybrids and ACTH-IP concentration in separate fractions before and after exposure to stress in OF test.

obtained from C mice, and in fractions 21 and 24 obtained from B6 mice, no ACTH-IP could be detected. The hybrid animals were similar to C mice with respect to fractions 1, 3, 22, and 24 and similar to B6 mice with respect to fractions 5 and 12.

As a result of exposure to stress the ACTH-IP concentration in the blood plasma rose both in the parental lines and in the F_1 mice, in agreement with data in the literature [1]. However, the changes observed differed from each line. For instance, the ACTH-IP level rose in C mice in fractions 3, 5, and 17-19, but in the B6 mice, in fractions 2, 6-8, 12, 15, 17-19, 22, 23, and 25-27. In some fractions peptides were found only after the OF test: in C mice in fractions 2, 4, 6, 8-16, and 27, and in B6 mice in fractions 4, 9, 16, 21, and 24. The ACTH-IP concentrations in F_1 mice rose after exposure to stress in fractions 2, 6-8, 10, 14, 18, 21, 22, and 25-28 (Figs. 1-3, d).

The results show that, first, the distribution of ACTH-IP in chromatographic fractions of the acid extract is specific for mice with different genotypes, and second, in animals with different hereditarily determined types of behavior, characteristic changes in the ACTH-IP level take place after emotional stress resulting from the OF test. Moreover, F_1 hybrids, which inherited the B6 type of investigative activity of OF, and which occupy an intermediate position between the B6 and C lines for emotionality, possessed both similar and different features compared with the two parental lines as regards the presence of ACTH-IP.

The ACTH-IP concentration in fractions from blood plasma differed from that in the brain. However, specificity for the line was preserved as regards both the initial level and the time course of its changes after the OF test.

It will also be noted that after chromatographic fractionation of the peptide extracts of the brain, ACTH-IP were found in many regions of the chromatogram, in agreement with data in the literature [5]. To elucidate the causes of this phenomenon, the isolated fractions must be subjected to further experimental fractionation and analysis.

Thus the ACTH-IP spectrum in the brain and the qualitative character of changes in their concentration after exposure to emotional stress are both genetically dependent, and this is an important basis for future research to study the functional role of ACTH-IP in the formation of hereditary features of emotional behavior.

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EFFECT OF MYELOPEPTIDES ON PHYSIOLOGICAL AND PATHOLOGICAL PAIN

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Intact human and animal bone marrow cells produce low-molecular-weight peptides with various bioregulatory properties (myelopeptides) [8]. Peptides with mol. wt. of 1.3-2 kilodaltons cause a two-threefold increase in antibody formation at the peak of the immune response [4, 5]. Myelopepties have not only immunostimulating activity, but also analgesic properties. It has been shown that injection of myelopeptides into animals causes changes in bioelectrical responses to nociceptive stimulation characteristic of analgesics of the morphine and endogenous opiate type [1, 6]. It has also been found that myelopeptides displace labeled opiates competitively from specific binding sites on lymphocytes and brain nerve cells [7].

The aim of this investigation was a further study of the analgesic action of myelopeptides on models of physiological and pathological pain.

EXPERIMENTAL METHOD

Myelopeptides were isolated from the supernatant of cultures of hog bone marrow cells by gel-chromatography on Sephadex G-25 (fine), equilibrated with physiological saline, pH 7.2. The dose of the myelopeptides was estimated on the basis of their protein content, which was determined by Lowry's method.

Noninbred and Wistar rats (males) weighing 200-220 g were used.

An experimental model of pathological pain was produced by creating a generator of pathologically enhanced excitation (GPEE) in the dorsal horn of the lumbosacral segments of the spinal cord by means of penicillin. Penicillin was applied to the dorsal surface of the lumbosacral segments on the left side in an agar wafer (10 × 4 × 1.5 mm). To 1 ml of 1% agar, 15,000 U of penicillin in a volume of 1 ml was added. The method of production of a pain syndrome of spinal origin, and also the method of assessing the separate components of development of the pain syndrome were described previously [2, 3].

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