realized through a different anxiolytic system. The latter is evidently most sensitive to the action of ethanol. If the hypothetical "type 2 anxiolytic system" participates in the formation of alcohol motivation, these compounds will evidently reduce alcohol consumption in doses corresponding to ED_{50} for their anxiolytic effect. To test this hypothesis similar experiments were carried out using compounds in doses equivalent to ED_{50} for anxiolytic effect in animals after contact with ethanol for 8 months (by the test of competition for an area safe from electric shocks). It will be clear from Table 2 that these doses were an order of magnitude or more greater than the corresponding doses for intact animals. All the compounds, in these doses, effectively reduced voluntary consumption of ethanol by the animals at both the first and the third stages of experimental alcoholism. These data are evidence in support of the concept that a nonbenzodiazepine anxiolytic system also exists [10]. It is evidently this system which is highly sensitive to the action of ethanol and which participates in the control of its voluntary consumption.

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IMMUNOHISTOCHEMICAL DEMONSTRATION OF PRO-OPIOMELANOCORTIN PEPTIDE FRAGMENTS (β-ENDORPHIN AND ACTH) IN RAT AND MOUSE ADRENALS

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KEY WORDS: β-endorphin; ACTH; pro-opiomelanocortin; adrenals; immunohistochemistry.

It has been discovered in recent years that many neuropeptides, including opioid peptides, are formed not only in structures of the brain and hypothalamohypophyseal system, but also in many peripheral organs and tissues [7, 9]. For example, the mammalian adrenals have been shown to be the principal site of synthesis and secretion of endogenous opioid neuropeptides of the enkephalin group, formed from proenkephalin and prodynorphin [3, 4, 8]. Meanwhile data showing that the adrenal tissues contain neuropeptides formed from proopiomelanocortin (POMC), a high-molecular-weight polypeptide, and the precursor of several pituitary hormones and opioid peptides of the endorphin group (β-, γ-lipotrophins, α-, β-, and γ-melanocyte-stimulating hormone, ACTH, α-, β-, and γ-endorphins), are contradictory [2, 5, 6].

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The demonstration that peptide fragments of POMC can be produced in the adrenal tissues is particularly interesting, because this fact would enable the existence of mechanisms of regulation of pituitary function that are "independent of the pituitary gland" to be postulated.

The aim of this investigation was the immunohistochemical identification of peptide fragments of POMC (β-endorphin and ACTH) in the adrenals of rats and mice, and also to analyze their distribution in the adrenals in order to discover which concrete structures are involved in the biosynthesis of these POMC fragments.

EXPERIMENTAL METHOD

Experiments were carried out on the adrenals of noninbred male rats and mice weighing 180-220 and 16-18 g, respectively. After decapitation of the animals the adrenals were fixed in 5% glutaraldehyde solution in 0.15 M Na-phosphate buffer, pH 7.5, for 1 h at 20°C. After rinsing, sections 10-15 thick were cut on a freezing microtome.

Rabbit antisera against β-endorphin and ACTH, obtained previously, were used for the immunohistochemical analysis. To increase the specificity of analysis, corresponding high-affinity antibodies were obtained from the total antisera mentioned above by affinity chromatography on columns with immobilized antigens. After incubation of the sections with these antibodies in a concentration of 5-20 μg/ml (0.02 M Na-phosphate buffer, pH 7.5, with 0.15 M NaCl, 0.2% NaN_3, and 0.2% Tween-20) for 16-20 h at 4°C the antigen-antibody complexes were demonstrated with the aid of horseradish peroxidase-labeled donkey antibodies against rabbit immunoglobulins (concentration 70 μg/ml, incubation for 1 h at 20°C). Peroxidase activity was demonstrated by staining with diaminobenzidine (0.5 mg/ml, 0.05 M Tris-HCl, pH 7.5, in the presence of 0.0045% H_2O_2 for 30 min at 20°C).

To prove the specificity of staining, control sections were incubated with high-affinity antibodies against peptides, exhausted beforehand with an excess of the corresponding antigen, or with neutral rabbit serum.

EXPERIMENTAL RESULTS

The method of immunohistochemical analysis used enabled peptide fragments of POMC to be identified in sections through the adrenals of rats and mice (Fig. 1: a, c, e, g). The specificity of this identification was confirmed by the complete absence of staining in control sections preincubated with exhausted antibodies (Fig. 1: b, d, f, h).

Microscopic analysis of sections through the rat and mouse adrenals, treated with high-affinity antibodies to β-endorphin showed that immunoreactive β-endorphin was present in both medulla and cortex of the adrenals (Fig. 1: a, c). Tissue of the adrenal medulla was stained approximately uniformly, whereas in the adrenal cortex β-endorphin was located mainly on the boundary between the zona fasciculata and zona reticularis, and also in cells of the zona reticularis of the cortex.

In sections treated with high affinity antibodies to ACTH, immunoreactive ACTH also was identified in both the medulla and cortex of the adrenals (Fig. 1: e, g). Just as in the case of antibodies to β-endorphin, the medulla was stained relatively uniformly, whereas in the adrenal cortex immunoreactive ACTH was located mainly in the zona reticularis and on the boundary between it and the zona fasciculata.

When the patterns of distribution of immunoreactive β-endorphin and ACTH were compared, they were found to coincide virtually completely in the tissues. In addition, on immunohistochemical identification of ACTH the intensity of staining in sections through the mouse adrenals was rather higher than in sections through rat adrenals.

The following conclusions can thus be drawn from the data described above: 1) immunoreactive peptide fragments of POMC (β-endorphin and ACTH) are present in the adrenals of rats and mice; 2) these peptides are found in the medulla and in the zone reticularis and on the boundary between it and the zona fasciculata in the cortex of the adrenal; 3) the topography of distribution of immunoreactive β-endorphin and ACTH in the adrenal tissues coincides.

This complete coincidence of the topography of distribution of immunoreactive β-endorphin and ACTH, which are two independent fragments of the POMC molecule, in tissues of the rat and mouse adrenals can be taken as evidence of their coupled synthesis in the composition of the

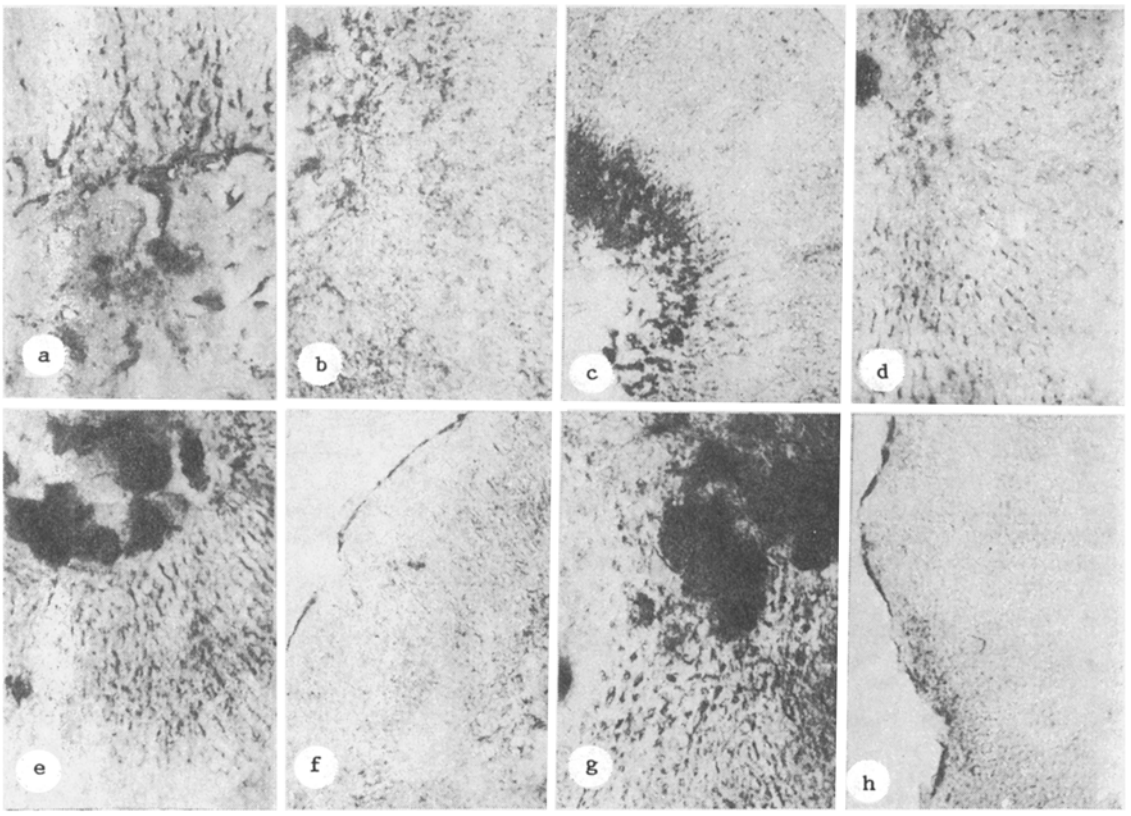

Fig. 1. Immunohistochemical identification of POMC peptide fragments in sections through rat and mouse adrenals: a) demonstration of β-endorphin in sections through rat adrenal, b) control with exhausted affinity antibodies; c) demonstration of β-endorphin in section through mouse adrenal, d) control with exhausted affinity antibodies; e) demonstration of ACTH in section through rat adrenal, f) control with exhausted affinity antibodies, g) demonstration of ACTH in section through mouse adrenal, h) control with exhausted affinity antibodies. Magnification: ocular 10, objective 7.

POMC molecule in the corresponding adrenal cells. It is very unlikely that their presence in the adrenal tissues may be entirely hypophyseal in origin, for in that case the pattern of distribution of these peptides could hardly be identical. Consequently, the most likely explanation is their "endogenous" origin in the tissues of the adrenal gland. This conclusion is in agreement with the fact that high-molecular-weight POMC is present in extracts of the bovine adrenal, which has recently been demonstrated by immunoblotting [1].

The results indicate that mechanisms of regulation of corticosteroid formation in the adrenals, independent of pituitary, and with the participation of peptide fragments of POMC formed in the adrenals, may exist. It is possible that this mechanism of regulation of adrenal function is widespread among mammals of different species and is responsible for the rapid response of the body to extremal factors, such as stress, whereas the "pituitary" neuropeptides, transported with the blood stream, serve to maintain the tone of corticosteroid production under ordinary conditions.

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DEFICIENCY OF GABA-ERGIC INHIBITION DUE TO EARLY POSTNATAL CYCLOHEXIMIDE TREATMENT CORRECTED BY PYRACETAM

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Important indications for the use of pyracetam* are disturbances of memory functions in children exposed in the antenatal or early postnatal period to toxic influences. Since we know that proteins and nucleic acids play an important role in learning processes, and that pyracetam has a favorable effect on these processes, to analyze the mechanism of action of this drug early postnatal interference with protein metabolism is particularly interesting. In young rats the period between the 6th and 8th days after birth is characterized by a rapid increase in the number of neurons and synapses in the cerebral cortex [5, 7], and by maximal rates of protein, RNA, and DNA synthesis [9, 10]. The degree of development of GABA receptors toward the end of the first week is 25% of that in adults [6]. However, the increase in activity of the GABA shunt enzymes, glutamate decarboxylase and succinic semialdehyde dehydrogenase [13], which takes place at the 6th-8th days of ontogeny, creates conditions for maturation of the GABA system.

In this investigation we studied the effect of inhibition of protein synthesis during this period on the learning capacity of adult animals and electrophysiological parameters of GABA-ergic inhibition and we analyze the effects of pyracetam on these processes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats. From the progeny of 10 females, on reaching the age of 7 days, 30 young rats were selected and divided into three equal groups randomly. Animals of group 1 received isotonic NaCl solution from the 7th through the 14th days after birth (control). The remaining rats were given cycloheximide (CHX), an inhibitor of protein synthesis, in a dose of 0.6 mg/kg on the 7th day [12, 13], after which some of the animals received isotonic NaCl solution from the 8th through the 14th days (group 2), and the other rats received pyracetam on the same days in a dose of 200 mg/kg (group 3). All substances were injected subcutaneously. The effect of CHX and pyracetam was assessed on the basis of several tests. From the 7th through the 14th days of life and at the end of the experiments the increase in body weight was noted. The remaining experiments were conducted on grown animals, starting from the age of 3 months. The motor activity was first estimated with the "Opto-Varimex" apparatus (USA), and 7 days later the animals' behavior was studied in an open field test on five successive days. Another 14 days later the ability of the rats to learn a conditioned active avoidance reflex (CAAR) was studied in a shuttle box, the animals receiving 60 combinations daily for 3 days. The conditioned acoustic stimulus was presented for 5 sec alone, and later for 5 sec accompanied by reinforcement by painful electrical stimulation (interval between combinations 5-60 sec). The number of avoidances and the number of interstimulus responses for every 10 consecutive presentations was recorded. Another 7 days later the rats were tested by a modified conditioned passive avoidance reflex (CPAR) method in a chamber consisting of two compartments: a lit compartment, initially avoided by the rat, and a dark compartment, initially preferred, in which the rat received painful stimulation. The degree of

*A Soviet GABA analog.

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