to restore its endogenous opiate systems and to exhibit their maximal antinociceptive effect under conditions of exposure to damaging radiation of this particular intensity. It is also evident that gamma quanta in a dose of 150 Gy selectively "injure" the opioidergic mechanisms regulating pain of electrical nature. Otherwise it is difficult to explain preservation of the analgesic activity of morphine in thermal pain tests and, at the same time, the development of hyperalgesia against the background of morphine during painful electrical stimulation in the same irradiated animals. Thus a change in activity of the opioidergic systems largely determines the direction and the phasic character of changes in nociceptive sensitivity during radiation injury. From the practical point of view it is important to note that opiates completely exhibit their analgesic action at different periods of time during the first 24-h period after irradiation. Meanwhile the need for a differential approach to their use, allowing for the genesis of the stimuli evoking the pain syndrome, during exposure to ionizing radiation will be evident.

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EFFECT OF ETHANOL ON NEUROPEPTIDE, ACTH, AND CORTICOSTERONE CONCENTRATIONS IN IMMOBILIZATION STRESS

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KEY WORDS: rats; brain; adrenals; Met-enkephalin; Leuenkephalin; ACTH; β-endorphin; corticosterone; immobilization stress.

Ethanol consumption is largely dependent on the character of response of animals to stress [4]. The widespread use of alcohol, it is suggested, is due to its stress-protective action, to its ability to abolish emotional strain [2, 4, 7]. However, animal experiments have shown that ethanol consumption in animals rejecting ethanol under free choice conditions initially increases in response to stress, whereas conversely, animals more sensitive to stress consume significantly less ethanol than those resistant to stress [4, 12]. To elucidate the details of the action of ethanol during stress we studied the effect of ethanol on plasma concentrations of corticosterone, ACTH, and β-endorphin, used as quantitative parameters of the level of response to stress [10, 11]. Considering that the level of ethanol consumption and the formation of dependence to it may be controlled by the endogenous opiate system and delta sleep-inducing peptide (DSIP) [3, 5, 7, 8], we also determined concentrations of enkephalins and DSIP in different parts of the brain of animals exposed to stress and receiving ethanol.

EXPERIMENTAL METHOD

Experiments were carried out on male laboratory albino rats weighing 200-250 g, kept on a standard diet with the natural alternation of light and darkness (November-December). Before the experiments the animals were divided into three groups: 1) intact rats kept

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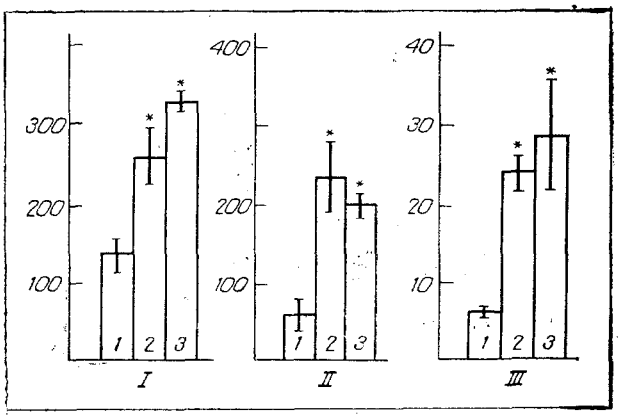

Fig. 1. Plasma levels of β-endorphin, ACTH, and corticosterone in rats. I) β-Endorphin (in fmoles/ml); II) ACTH (in pg/ml); III) corticosterone (in μg/ml). Here and in remaining figures: 1, 2, 3) rats of groups 1, 2, and 3, respectively. *$p < 0.05$ compared with control.

in a common cage; 2 and 3) rats immobilized in the prone position for 6 h. Before immobilization, the rats of group 3 were given an intraperitoneal injection of 20% ethanol solution in a dose of 1 g/kg body weight, and the rats of group 2 received the equivalent volume of physiological saline. All animals were decapitated at the same time, the brain (after division into regions: cerebral cortex, striatum, thalamus, medulla with pons) and adrenals were quickly frozen in liquid nitrogen and weighed, after which the specimens were extracted with acetic acid by the method described previously [7]. Blood plasma was obtained by centrifugation after addition of EDTA. The corticosterone concentration was determined by radioimmunoassay (RIA), using antiserum generously provided by G. V. Katsiya (Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR), using ^{3}H-corticosterone from "Amersham" as the labeled ligand. The DSIP concentration was determined by RIA, using a technique developed by the present writers [6]. The ACTH concentration was recorded by RIA, using a standard kit of reagent from "Amersham." Met- and Leu-enkephalin and β-endorphin levels were measured by RIA, using antiserum generously provided by A. A. Dmitriev (All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR), by a method described previously [7, 8]. The log-logit transformation method was used to calculate concentrations of neuropeptides and hormones. The significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

The immobilized animals were distinguished by their higher than normal excitability. Gentle tapping on the tables was accompanied by attempts to get free and by vocalization. Meanwhile animals receiving ethanol did not respond to external stimulation of this kind throughout the period of the experiment. The presence of a stress reaction was judged also by elevation of plasma corticosterone, ACTH, and β-endorphin levels. As Fig. 1 shows, these parameters remained high during immobilization stress when exposure to it was preceded by injection both of physiological saline and of ethanol.

The Leu-enkephalin concentration in different parts of the rat's brain was unchanged both by exposure to stress and by injection of ethanol (Fig. 2). Meanwhile stress was accompanied by a marked lowering of the Met-enkephalin concentration in the striatum and medulla + pons. Preliminary injection of ethanol completely prevented the fall in the Met-enkephalin concentration in the striatum but did not affect the stress-induced fall of the Met-enkephalin level in the medulla. A somewhat different pattern was found with enkephalins in the adrenals: during immobilization stress the Met-enkephalin concentration rose significantly, but preliminary injection of ethanol, just as in the striatum, blocked this effect (Fig. 3).

The DSIP concentration did not change significantly during immobilization stress. After preliminary injection of ethanol the DSIP concentration in the thalamus showed a marked increase in the period of the stress reaction, and a tendency for the DSIP level in the medulla to increase also was recorded. Injection of DSIP has been shown to have

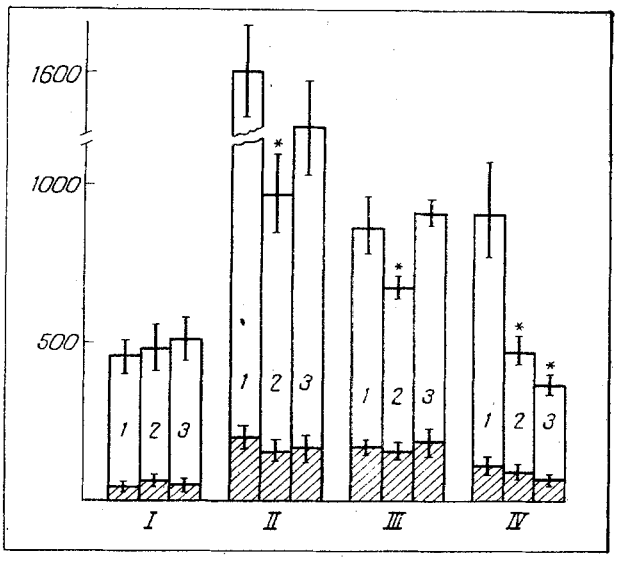

Fig. 2. Effect of immobilization stress on enkephalin concentrations in rat brain after injection of ethanol. Unshaded columns – Met-enkephalin, shaded – Leu-enkephalin (in fmoles/mg tissue). I) Cerebral cortex, II) striatum, III) thalamus, IV) medulla with pons.

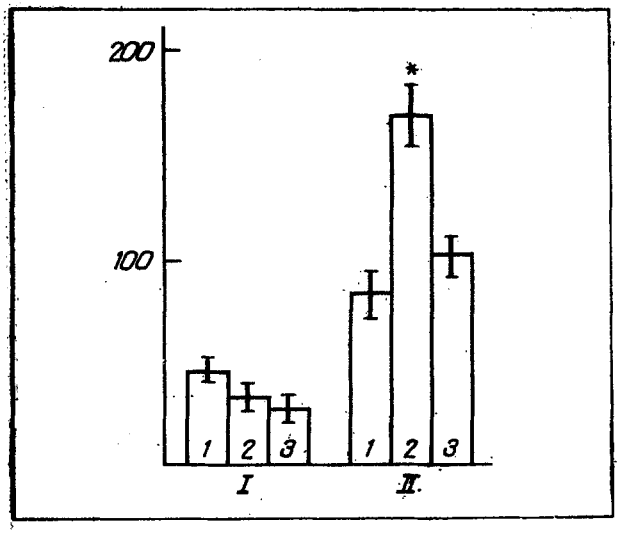

Fig. 3. Effect of immobilization stress and ethanol on enkephalin concentrations in adrenals (in fmoles/mg tissue). I) Leu-enkephalin, II) Met-enkephalin.

a protective action in various kinds of stress [1, 9]. Hence it can be concluded that the antistress action of ethanol can be realized not only through the enkephalinergic system, but also through changes in the DSIP concentration.

Ethanol can thus potentiate the stress-induced activation of the pituitary-adrenal system, raising β-endorphin and corticosterone levels, but at the same time it may block changes in the enkephalinergic system in the CNS and adrenals against the background of stress. Further, under the influence of ethanol the compensatory powers of the animal not directly involved in the response to stress may also be mobilized, as shown by elevation of the DSIP concentration.

It can be tentatively suggested that ethanol modifies the response of the body to stress, strengthening some and weakening other components of the stress reaction. This may probably be why ethanol weakens the manifestations of some types of stress but strengthens those of others.

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OPIOID PEPTIDES AS REGULATORS OF ACETYLCHOLINESTERASE ACTIVITY

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KEY WORDS: acetylcholinesterase; endorphins; enkephalins; regulation of enzyme activity.

Because peptides are present in synaptic endings of neurons along with "classical" neurotransmitters [9] and since these are released together during depolarization of the axon membrane, it has been claimed that the modulating role of peptides can be reduced either to modification of the characteristics of excitable membranes or to their action on the release, reception, and metabolism of a mediator [5]. On the basis of a certain analogy between receptor and enzyme, we postulated that peptide hormones can act directly on the enzymes of neurotransmitter metabolism.

The aim of this investigation was to study the direct action of opioid peptides, including enkephalins and their fragments and short endorphins, on activity of acetylcholinesterase (AChE; EC 3.1.1.7), the enzyme hydrolyzing the cholinergic neurotransmitter acetylcholine.

EXPERIMENTAL METHOD

A water-soluble preparation of AChE from human blood erythrocytes [1] (Research Institute of Vaccines and Sera, Perm'), identical to the brain enzyme for substrate-inhibitor specificity [7], was used as the model with which to study peptide interaction. Preparations of peptide hormones were generously provided by M. I. Titov (All-Union Cardiologic Scientific Center, Ministry of Health of the USSR, Moscow). AChE activity was determined by a modified Ellman's method [8] at 37°C (pH 7.5), using acetylthiocholine bromide as the

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