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EFFECT OF THYROTROPHIN RELEASING HORMONE ON OPIATE RECEPTORS OF THE RAT BRAIN

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An important role in the regulation of neurotransmitter processes is played by biologically active substances known as neuromodulators, which affect the release and uptake of transmitters and the sensitivity of receptors to them. The most important contribution to modulation of the "classical" monoamine mediator systems is made by neuropeptides, including endogenous opioids [2]. Meanwhile, much less attention was been paid to the study of regulation of the functions of opiate and other peptidergic systems of the CNS, which possess modulator activity. Information on this problem in the literature is not yet sufficient to allow definite conclusions to be drawn with respect either to the mechanisms of this regulation or to the nature of the factors responsible for it.

Investigations have shown the mutual influence of peptidergic and neurohumoral systems: Close connections have been found between β-endorphin, on the one hand, and blood plasma levels of various hormones, on the other hand [7, 12]. It has been demonstrated that disturbances of the functional relations between the neuroendocrine and neuromodulator systems may lie at the basis of the development of psychotic states [6].

It is understood that modulator systems can be regulated at any level and, in particular, at the level of reception of peptide ligands. Salsolinol has been shown to cause changes in binding activity of opiate receptors *in vitro* relative to enkephalins, and to exhibit, under these circumstances, ability to interact antagonistically with receptor structures [5]. It has also been shown that different types of opiate receptors can be regulated by guanyl nucleotides and by certain metallic cations [11].

It has recently been shown that the hypothalamic hormone thyrotrophin releasing hormone (TRH) has the properties of a morphine antagonist, blocking its inhibitory action on respiration and, to a lesser degree, its analgesic action [3]. This suggests that the antagonistic effects of TRH are mediated through its interaction with opiate receptors.

The aim of this investigation was to test this hypothesis experimentally.

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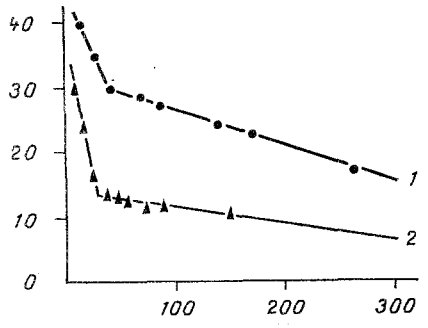

Fig. 1. Analysis of ^{3}H-DADL saturation of opiate receptors of rat hypothalamus and midbrain in presence of TRH. Abscissa, B; ordinate, B/F × 10^3/mg protein. B) Concentration of specifically bound label (in pmole/liter/mg protein); F) concentration of label in reaction mixture (in nM). 1) Without TRH; 2) in presence of 1 μM TRH. Results of one of three similar experiments are shown.

EXPERIMENTAL METHOD

Noninbred male rats weighing 200-220 g were used. The animals were decapitated, the brain was quickly removed in the cold, and parts of it corresponding to the midbrain and hypothalamus excised. The membrane fraction of the brain cells, containing opiate receptors, was obtained by a modified incubation method [1]. The binding characteristics of the opiate receptors were determined in the presence of bacitracin, a nonspecific protease inhibitor, using the filtration method [14]. Radioactivity was counted on an SL-3120 scintillation spectrometer (Roche-Bioélectronique, France), after preliminary immersion of the filters in 1 M Hyamine solution in methanol. Specific interaction between label and receptors was determined as the difference between binding by membrane in the presence and absence of 2.5 μM D-ala^2-met-enkephalinamide (DALA). The results were subjected to statistical analysis by Student's t test.

The following compounds were used in the experiments: ^{3}H-D-ala^2-enkephalin (5-L-leu)-(^{3}H-DADL, 40 Ci/mmole), ^{3}H-leu-enkephalin (37 Ci/mmole), and ^{3}H-DALA (27 Ci/mmole), all from Amersham Corporation, England; DALA (from Serva, West Germany); bacitracin and Tris (from Sigma, USA), Hyamine-10X, PPO, and POPOP (from Koch-Light, England). The TRH was generously provided by M. I. Titov (All-Union Cardiologic Science Center, Academy of Medical Sciences of the USSR). The remaining reagents were of Soviet origin.

EXPERIMENTAL RESULTS

Determination of the ability of THR to interact with opiate receptors of the rat hypothalamus and midbrain by competitive displacement analysis showed that TRH, in concentrations up to 10^{-5} M, caused no replacement of tritiated leu-enkephalin, DADL, or DALA in the receptor binding sites. A very small decrease in specific binding of the labels was observed only when the TRH concentration was increased to 10^{-4} M. These results agree with data in the literature on the absence of activity of TRH in relation to binding sites of opiate receptors [4, 8].

Meanwhile in concentrations of 10^{-8}-10^{-6} M TRH activated receptor binding of all the labeled ligands used by 10-20% (results not shown), evidence of a possible change in the affinity of the opiate receptors. To test this hypothesis the parameters of interaction of the opiate receptors with ^{3}H-DADL were determined; justification for the choice of this compound for investigation was described previously [1].

The results of saturation of opiate receptors by the enkephalin analog in the presence of 10^{-6} M TRH and in its absence are shown graphically on a Scatchard plot in Fig. 1. TRH had a marked effect on the affinity of the binding sites of both types discovered for the label: The equilibrium dissociation constant (K_d) for high-affinity receptors was reduced, whereas that for low-affinity receptors was increased. Similar results were obtained by the use of TRH in concentrations of 10^{-7} and $5 \cdot 10^{-6}$ M (Table 1), but in these cases changes in reception were less marked.

There is no doubt that the triggering stage in the formation of pharmacologic effects of narcotics, including inhibition of respiration and analgesia, is their interaction with specific receptor structures located in the CNS. Despite the fact that the fine mechanisms of inhibition of ventilation of the lungs by therapeutic doses of morphine is not yet clear, it is suggested that this effect is determined by a decrease in sensitivity of brain-stem neurons to CO_2 [9]. It has been shown that naloxone restores the normal inhibition of respiration by morphine [10], and that TRH has a similar action [3]. It would therefore be natural to suggest that the mechanisms of the antagonistic effect of these two substances are similar.

TABLE 1. Effect of TRH on Affinity of Opiate Receptors of Rat Hypothalamus and Midbrain to ^{3}H-DADL (M ± m, n = 3)

TRH concentration, μM	K_d for high-affinity binding sites, nM	K_d for low-affinity binding sites, nM
0	2,3±0,6	17,8±0,9
0,1	1,7±0,5	23,4±2,0
1	0,9±0,3	28,1±1,1*
5	1,4±0,3	19,8±1,3

Legend. *P < 0.01.

We know that naloxone can block the pharmacologic effects of morphine by its interaction with binding sites of opiate receptors, and can thereby prevent their reaction with molecules of the narcotic. Conversely, data in the literature [4, 8] and our own results are evidence that the action of TRH is realized by a different mechanism. Over a wide range of concentrations (10^{-8}-10^{-5} M) TRH does not cause replacement of opioids in binding sites, i.e., it does not interact with the active center of the receptor. Meanwhile, in the region of low concentrations, it may exhibit a tendency toward an increase in receptor binding of ligands. It must be pointed out that the concentration at which TRH affects interaction of opioids with receptors corresponds in order of magnitude to the doses of it used in experimental pharmacology [3].

The results of direct determination of the binding characteristics of opiate receptors in the presence of TRH led to the conclusion that the tendency for the level of specific binding of opioids to increase, which was recorded, probably reflects an increase in affinity of high-affinity receptors for opiate ligands. At the same time it has been shown that TRH has a modulating effect on the binding characteristics of both high- and low-affinity opiate receptors, and reduces the affinity of the latter. The naloxone-like action of TRH [3], discovered in the course of pharmacologic research [3], may evidently be due to a decrease in the affinity of low-affinity opioid binding sites. Consequently, opiate-dependent mechanisms of the reversal of the effects of morphine by naloxone and TRH (at least at the reception level) are different.

Since TRH, while modifying the characteristics of opiate reception, does not bind with the active center of the receptors, it can be postulated that interaction of the hormone with them takes place in a different locus. The problem of the arrangement of these hypothetical allosteric "modulation centers" on the receptor complex, and their specificity and number remains a matter for debate, but there are grounds for assuming the existence of several types of these sites. This hypothesis is confirmed by the fact that TRH modulates the affinity of different types of opiate receptors, detectable biochemically, in different directions. Meanwhile, there is an equally probable point of view, namely that modulation by TRH of opiate reception may be the result of its effect on the microenvironment of the receptor.

These results suggest the existence of interconnections between the opiate systems and TRH, and this is confirmed by data in the literature on the effect of opioids on TRH release from the hypothalamus [13]. Although it is too early to reach definite conclusions on the mechanisms of this mutual influence and its physiological significance, it can be tentatively suggested that TRH abolishes the pharmacologic effects of morphine by modulating the functional state of opiate reception.

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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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