PHYSIOLOGY

Effects of Exogenous Application of Corticotropin-Releasing Hormone to Slices of the Olfactory Cortex from Rats with an Active Strategy of Adaptive Behavior on the Water-Immersion Model of Depression

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Experiments were performed on male Wistar rats. The specimens with an active strategy of behavior were exposed to unavoidable water-immersion stress. Surviving slices of the olfactory cortex were obtained 10 days after stress. The neurohormone had a strong inhibitory effect in 40% slices from active rats. The activity of glutamate receptors decreased, while the function of GABA receptors increased in 60% slices. Our results indicate that the depressive state of behaviorally active animals due to exposure to unavoidable stress is not necessarily mediated by the corticoliberinergic mechanisms in cortical structures.

Key Words: *active rats; depression; strategy of adaptive behavior; surviving slices of the olfactory cortex; a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; N-methyl-D-aspartate; excitatory postsynaptic potential; slow inhibitory postsynaptic potential*

Corticotropin-releasing hormone is a peculiar peptide regulator of adaptive behavior. This substance is an early transmitter of stress, which integrates all the components of the stress response [10]. Corticotropin-releasing hormone has a role of the neurohormone, neurotransmitter, and neuromodulator in stress signal transduction and stress response. This agent has a stimulatory or inhibitory effect on transsynaptic transduction, which involves standard neurotransmitters [9]. Corticotropin-releasing hormone has a variety of effects on adaptive behavior, which is manifested in the increase or decrease of behavioral activity (signs of freezing behavior and anxiety) [5]. These changes are particularly pronounced in animals with an active strategy of adaptive behavior. This form of behavior is increased in naive animals, but reduced in specimens exposed to mild stress. Active specimens are characterized by a more rapid development of the depressive state under unavoidable aversive conditions. Transient poststress psychopathy in these animals is followed by rapid recovery of functions. The de-

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gree of disturbances may increase in repeated exposure to stress--restress or administration of corticotropin-releasing hormone [3,4]. These data suggest that the animals with an active strategy of adaptive behavior constitute a specific phenotype, which is characterized by high sensitivity to unavoidable stress and corticotropin-releasing hormone. However, these specimens may be resistant to aversive factors. The corticoliberinergic mechanisms have an important role in the progression of these processes, since they primarily affect plasticity of brain structures.

This work was designed to evaluate the role of corticoliberinergic mechanisms in depression. We studied the effect of exogenous application of corticotropin-releasing hormone on glutamatergic and GABAergic synaptic transmission in surviving slices of the olfactory cortex. Brain slices were obtained from behaviorally active rats after unavoidable stress.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-280 g. They were tested in the T-maze to select the specimens with an active strategy of adaptive behavior [6,7]. The active and passive state of animals was evaluated from some behavioral parameters and expressed in the indexes of behavioral activity (IBA) and passivity (IBP). The average speed of movements was calculated. The rats with an active strategy of adaptive behavior were selected from these indexes (IBA=88.7±8.6; IBP=3.9±0.2; average speed of movements 5.84±0.17). Passive rats were excluded from the experiment. Ambivalent animals served as the control. The rats were exposed to unavoidable water-immersion stress as a model of posttraumatic stress disorder. The animals were fixed in iron boxes on a common platform. These specimens were immersed in water (16°C) in the supine position (for 1 h). The hair was dried after stress. The rats were placed in home cages and had free access to water and food. The animals were decapitated after 10 days. Surviving slices of the olfactory cortex were prepared [1].

Tangential sections (450-500 μ) were incubated in the medium of 124.0 mM NaCl, 5.0 mM KCl, 2.6 mM $CaCl_2$, 1.24 mM KH_2PO_4, 1.2 mM $MgSO_4$, 3.0 mM $NaHCO_3$, 10.0 mM glucose, and 23.0 mM Tris-HCl. The solution was saturated with oxygen. Temperature was maintained at 37°C. pH was 7.2-7.3. After 2-h incubation, the slices were transferred to a flow chamber (perfusion rate 2 ml/min). The air was saturated with oxygen.

Orthodromic stimulation of the proximal part in the lateral olfactory tract was performed with rectangular pulses (duration 0.1 msec, 1-5 V). They were delivered via platinum bipolar electrodes (interelectrode distance 0.5 mm) using an ESU-1 stimulator. Local potentials in slices were recorded using glass microelectrodes with 1 M NaCl (resistance 1-5 mΩ). The reference silver electrode was placed in a chamber.

Local potentials were digitized on a MD 32 analog-digital device (sample rate 20 kHz) and analyzed with special software. We estimated a change in the amplitude of postsynaptic excitatory components of local potentials, including the a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) components of the excitatory postsynaptic potential (EPSP). The induction of these components was inhibited by specific blockers (CNQX, DNQX, and APV) [11,12]. A quantitative study was performed to evaluate the amplitude of the slow inhibitory postsynaptic potential (IPSPs). Potential generation is mediated by the GABAergic mechanisms and reflects the activation of a recurrent inhibition system [13]. The amplitude of components was measured from the isoline to the peak.

Corticotropin-releasing hormone (Sigma) at a concentration of 10^{-10} M was dissolved in the incubation medium immediately before the experiment. Local potentials in brain slices were recorded over 15 min under control conditions. These slices were perfused with a medium containing corticotropin-releasing hormone for 20 min. They were washed for 15 min. The study was conducted on 20 slices from active rats.

The results were analyzed by nonparametric Mann—Whitney *U* test and Excel 7 software. The differences were significant at $p<0.05$.

RESULTS

The amplitude of local potentials in postsynaptic structures of brain slices from active rats was different after water-immersion stress. These data illustrate differences in the excitability. Brain slices were divided into the following two groups: group 1, low baseline excitability; and group 2, normal baseline excitability. The amplitude of postsynaptic components in group 1 local potentials under control conditions and treatment with corticotropin-releasing hormone was much lower than that in local potentials of slices from ambivalent rats. The amplitude of group 2 local potentials was similar to that of local potentials in ambivalent rats.

Variations in the amplitude of group 1 and 2 local potentials were different after treatment with

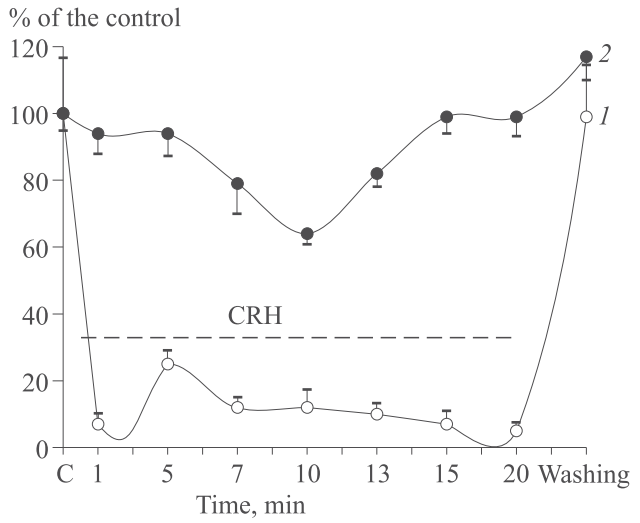

Fig. 1. Amplitude of AMPA EPSP in slices of active rats with low (*1*) and normal baseline excitability (*2*) after exogenous application of corticotropin-releasing hormone (10^{-10} M). Here and in Figs. 2 and 3: C, control. Ordinate: amplitude of AMPA EPSP. Dotted line: application of corticotropin-releasing hormone. *n*, number of slices: n=8 (group 1) and n=12 (group 2). CRH, corticotropin-releasing hormone.

corticotropin-releasing hormone. The amplitude of AMPA EPSP in group 1 decreased to 95% of the control level 1 min after treatment with the neurohormone and remained unchanged in the follow-up period (n=10, U=21, p<0.05). The amplitude of AMPA EPSP returned to normal after washing (Fig. 1).

In group 2, the effect of corticotropin-releasing hormone was manifested in a decrease in the activity of AMPA receptors. These changes were revealed 5 min after treatment and became most pronounced by the 10th minute (35% decrease; n=10, U=19, p<0.05; Fig. 1). The amplitude of AMPA EPSP spontaneously returned to the baseline level in the follow-up period (despite the influence of corticotropin-releasing hormone). After washing the amplitude of AMPA EPSP not only returned to normal, but exceeded the baseline by 10% (Fig. 1).

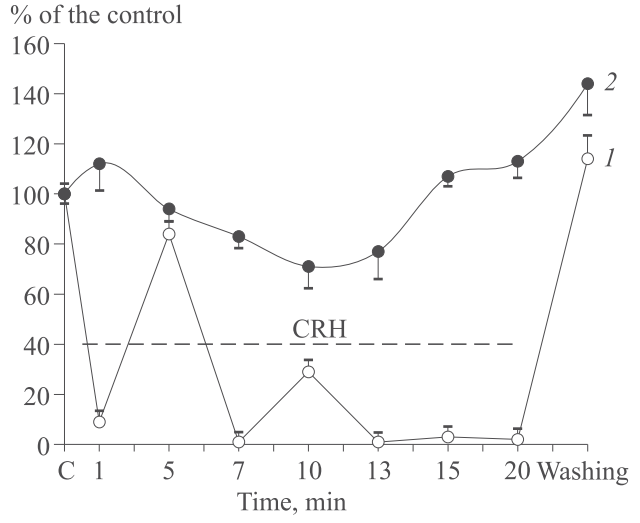

Fig. 2. Amplitude of NMDA EPSP in slices of active rats with low (*1*) and normal baseline excitability (*2*) after exogenous application of corticotropin-releasing hormone (10^{-10} M).

Variations in the amplitude of AMPA EPSP in slices of various groups were different only during exposure to corticotropin-releasing hormone, but returned to the baseline after washing (Fig. 1). The data illustrate a reversible effect of this hormone on the AMPA-mediated mechanisms of glutamatergic transmission.

Corticotropin-releasing hormone-induced variations in the amplitude of NMDA EPSP in groups 1 and 2 coincided with those of AMPA EPSP. For example, the amplitude of NMDA EPSP in group 1 was reduced by 7% on the 1st minute of exposure to corticotropin-releasing hormone. Activity of NMDA receptors increased in the initial period, but then underwent wavelike changes. These receptors were completely blocked until the end of treatment with the neurohormone (Fig. 2). Activity of NMDA receptors was not only restored, but even increased after washing (Fig. 2).

Corticotropin-releasing hormone had a short-term potentiating effect on the activity of group 2 NMDA receptors. This effect of neurohormone was observed 1 min after treatment. The activity of NMDA receptors decreased by 30% over the next 10 min (n=10, U=17, p<0.05). Despite the action of neurohormone, activity of NMDA receptors returned to normal by the 15th minute (Fig. 2). After washing, the amplitude of NMDA EPSP was restored and exceeded the baseline level (Fig. 2).

These data indicate that corticotropin-releasing hormone has an inhibitory effect on NMDA-mediated processes. The inhibition was particularly pronounced in slices with low baseline excitability. The recovery of activity of NMDA receptors after washing reflects a reversible inhibitory effect of this hormone.

Exogenous application of corticotropin-releasing hormone produced the opposite changes in activity of GABA receptors (amplitude of IPSPs; Fig. 3). The amplitude of IPSPs in group 1 slices decreased in a wavelike manner over 13 min after treatment with the neurohormone. Blockade of the GABAergic mechanisms was observed from the 13th to the 20th minute. After washing, the amplitude of EPSPs returned to the baseline level (Fig. 3).

The amplitude of IPSPs in group 2 slices increased by 50% after application of corticotropin-releasing hormone (n=10, U=20, p<0.05). The amplitude of IPSPs was above normal after washing (n=10, U=15, p<0.05; Fig. 3).

The exogenous hormone produced different changes in the GABA-mediated mechanisms in slices of various groups. The activity decreased in slices with low excitability, but increased in slices with normal excitability. These data show that stress has the opposite modulatory effects on GABAergic processes.

Hyperactivation of corticoliberinergic mechanisms in the brain cortex was induced by exogenous application of corticotropin-releasing hormone to brain slices of stress-exposed active rats. A strong inhibitory effect was observed in 40% slices. Corticotropin-releasing hormone had the opposite effect on glutamatergic and GABAergic synaptic transmission in 60% slices from active rats. We revealed a slight decrease in the activity of glutamatergic receptors and activation of GABA receptors.

To explain the observed changes, it is necessary to analyze the effects of corticotropin-releasing hormone on surviving slices of the brain from nonstressed animals. Application of corticotropin-releasing hormone to surviving slices of the olfactory cortex from Wistar rats was accompanied by an increase in neuronal excitability. It was manifested in an increase in the amplitude and duration of some components of glutamatergic excitatory synaptic transmission (AMPA EPSP and NMDA EPSP). By contrast, the inhibitory GABAergic mechanisms were suppressed under these conditions. Increasing the concentration of exogenous neurohormone in the incubation medium causes an imbalance between excitation and inhibition in slices, which results in epileptiform activity [2]. The data indicate that this neurohormone increases the excitability of nervous tissue and, therefore, prevents the development of depression. These properties are typical of corticotropin-releasing hormone as an immediate stress hormone. Moreover, pretreatment of surviving brain slices with corticotropin-releasing hormone contributes to normal synaptic transmission upon exposure to "dysfunction-inducing agents" from the cerebrospinal fluid of drug abusers [7].

These changes should abolish the depressive state of nerve cells after application of exogenous neurohormone. However, it was true only for the function of GABAergic transmission in slices with normal excitability. Severe stress was followed by significant changes in the glutamatergic and GABAergic mechanisms in other slices. The initial potentiating action of corticotropin-releasing hormone did not normalize these processes.

Our results suggest that the effect of exogenous corticotropin-releasing hormone in cortical structures depends on baseline excitability. Severe stress decreases the activity of cortical structures. Hence, application of corticotropin-releasing hormone is insufficient to normalize the function of cortical structures. This goal may be achieved by treatment with substances (*e.g.*, endogenous compounds) that have an optimizing effect on the excitatory and inhibitory mechanisms.

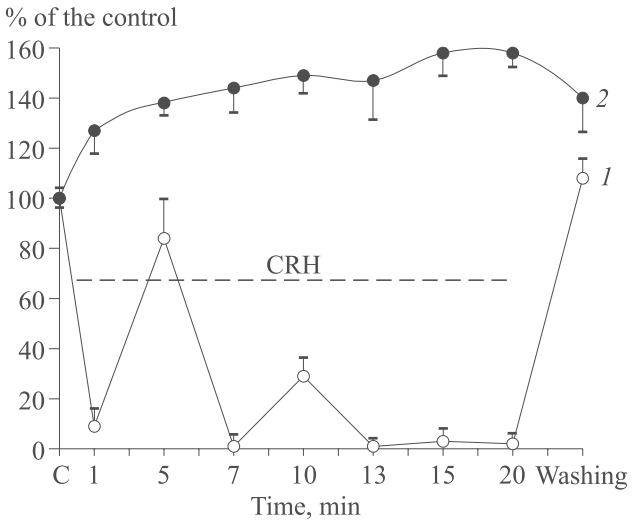

Fig. 3. Amplitude of IPSPs in slices of active rats with low (*1*) and normal baseline excitability (*2*) after exogenous application of corticotropin-releasing hormone (10^{-10} M).

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