PHYSIOLOGY

ATP Concentration in the Cingulum Bundle of Rats during Stimulation of the Ventromedial Hypothalamus

A. V. Gourine, A. Yu. Abramova, S. S. Pertsov, and K. V. Sudakov

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We measured the concentration of ATP in the posterior cingulum bundle of Sprague-Dawley rats during electrical stimulation of the negative emotiogenic zone in the ventromedial hypothalamus. Electrostimulation of the ventromedial hypothalamus in animals was accompanied by an increase in systolic and diastolic blood pressure, which illustrates the autonomic response to this treatment. Variations in BP of rats were followed by an increase in ATP content in the posterior cingulum bundle. After stimulation of the ventromedial hypothalamus, ATP concentration in the cingulum bundle reached the maximum levels of 1.3±0.3 μM (n=4) and 1.67±0.43 μM (n=10) and remained high for 42.6±7.5 sec. The enhanced release of ATP in the cingulate region of the brain is probably related to the involvement of this structure into emotional reactions. ATP plays a role of the major energy source and signal molecule, which provides the adequate biochemical and physiological processes and cell-to-cell interaction in CNS upon the exposure to negative emotiogenic factors.

Key Words: *ATP; emotions; cingulum bundle; ventromedial hypothalamus*

The development of emotional reactions in mammals is closely associated with functional activity of the limbic structures in the brain [7]. The ventromedial hypothalamus (VMH) plays a special role in these reactions. This structure includes centers for affective emotions of fear, fury, and defense [8]. VMH stimulation causes generalized changes in bioelectric activity in emotiogenic zones of the brain, including the limbic structures [3]. The cingulate cortex and cingulum bundle (CB) that play an important role in the emotional response also belong to the limbic structures. The presence of thalamic fibers in CB determines the distribution of excitation from the hypothalamic structures to the cingulate region of the brain. The cingulate area is involved in sensory, cognitive, emotional, and motor processes [6,13-15]. This structure plays a role in the mechanisms of nociception. Previous studies showed that electrical coagulation of the posterior CB is accompanied by significant changes in the nociceptive thresholds [2].

ATP is a direct source of energy for energy-dependent processes in living organisms. Nearly all cells in mammals, including neurons and glial cells, carry P2 purine receptors for ATP [9]. The data on high sensitivity of nociceptors to low concentrations of ATP illustrate the role of this biologically active compound in nociceptive signal transduction [4]. Strong evidence exists that ATP is involved in excitation transfer between various cells of CNS and peripheral nervous system. Moreover, ATP plays a special role in the neuroprotective effect of endogenous substances and regulation of blood circulation [1]. ATP increases activity of neurons

P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** nansy71@mail.ru. A. Yu. Abramova

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in various regions of the brain, which contributes to central regulation of physiological functions [10].

Much attention was paid to studying the role of ATP in a variety of essential processes. However, little is known about the involvement of this compound in the central mechanisms of emotional states [5]. Moreover, the role of ATP in systemic integration of brain structures mediating interrelated emotional and nociceptive reactions in mammals remains unknown.

Here we measured the concentration of ATP in the posterior CB of rats during stimulation of the negative emotiogenic zone in VMH.

MATERIALS AND METHODS

Experiments were performed on male Sprague-Dawley rats (n=14) weighing 270-320 g. The research was conducted according to the UK Animals Scientific Procedures Act (1986). The rats were intraperitoneally anesthetized with urethane (1 g/kg in 4 ml physiological saline) and ventilated with a special device for small laboratory animals (Harvard rodent ventilator, model 683, Harvard Apparatus). A TSE-Systems stereotaxic device was used for surgical manipulations on rat brain structures. A SNE100 bipolar electrode (Harvard Apparatus) was inserted into the VMH according to stereotaxic coordinates (AP=-2, L=+0.5, H=9.5) [12]. VMH was stimulated with direct current under stochastic conditions (pulse duration 1 msec, current strength 100-200 μA, frequency 100 Hz, 5-sec stimulation).

The femoral artery was catheterized for monitoring of BP variations in rats under various experimental conditions. ATP content in CB was measured with a special biosensor for ATP (Sarissa Biomedical). This biosensor consists of two enzymes (glycerol kinase and glycerol-3-phosphate oxidase) that form the matrix around a platinum wire (diameter 100 μ, length 1 mm) [9]. The "null" (control) biosensor is structurally similar to the ATP biosensor, but does not include specific enzymes. The sensors were *in vitro* calibrated before the start of experiments [10]. The ATP biosensor was inserted into VMH using a stereotaxic manipulator (AP=-4.5, L=±1, H=2) [12]. The "null" sensor was implanted into the corresponding contralateral structure. Both sensors were connected to a MicroC potentiostat (WPI). The maximum readings of the ATP biosensor and "null" biosensor were recorded (V). This procedure serves as a standard technique for evaluating the relative changes in ATP level [5,9,10]. ATP content was calculated with Spike2 software and expressed in micromoles. The level of BP and indications of the ATP biosensor and "null" sensor were recorded 30 min before VMH stimulation. The recordings were continued over the study period.

The results were analyzed with Spike2 software (Cambridge Electronic Design).

RESULTS

BP variations upon stimulation of VMH in mammals serve as a criterion for the stress response. Dysfunction of CNS cells is associated with the hypertensive reaction and increase in HR [11]. In our experiments, BP of rats was continuously monitored to verify the development of a negative emotional stress in animals during electrostimulation of VMH. Under basal conditions, the animals were characterized by systolic and diastolic BP (120±10 and 65±5 mm Hg, respectively; Fig. 1). VMH stimulation in rats was followed by an increase in BP up to 170±5 and 110±5 mm Hg, respectively (by 41.67 and 69.23%, respectively). The hypertensive reaction in animals was observed 2.0±0.3 sec after this treatment. Systolic and diastolic BP in rats were reduced (110±5 and 60±5 mm Hg, respectively) 5.8±1.2 sec after VMH stimulation. BP in animals returned to the baseline after 12.4±2.1 sec. Our results show that electrostimulation of VMH in rats is accompanied by a significant increase in systolic and diastolic BP. These changes are typical of the stress response.

The readings of the ATP biosensor and "null" sensor practically did not differ under basal conditions (Fig. 1). Therefore, the relative content of ATP in the posterior CB remains practically unchanged in intact animals.

During electrostimulation of VMH in rats, we observed a sharp rise of the curves that reflected reading of the ATP biosensor and "null" sensor. These parameters returned to the baseline immediately after stimulation of the brain structure. Short-term variations in the readings of the ATP biosensor and "null" sensor during VMH stimulation represent the response to electric current, but not a change in ATP content under these conditions.

When BP of rats returned to the baseline after VMH electrostimulation, we revealed a rise of the ATP biosensor curve (Fig. 1). ATP content in the posterior CB of rats progressively increased after stimulation of the negative emotiogenic zone in VMH. The maximum peaks in the ATP biosensor curve were shown to correspond to an ATP content of 1.3±0.3 μM (n=4) and 1.67±0.43 μM (n=10). ATP content in CB remained high for 42.6±7.5 sec. However, the "null" sensor readings remained unchanged under these conditions (no differences from the baseline).

Our results indicate that electrostimulation of VMH is accompanied by a strong autonomic response. This state is manifested in the increase in systolic and diastolic BP of experimental animals. BP variations

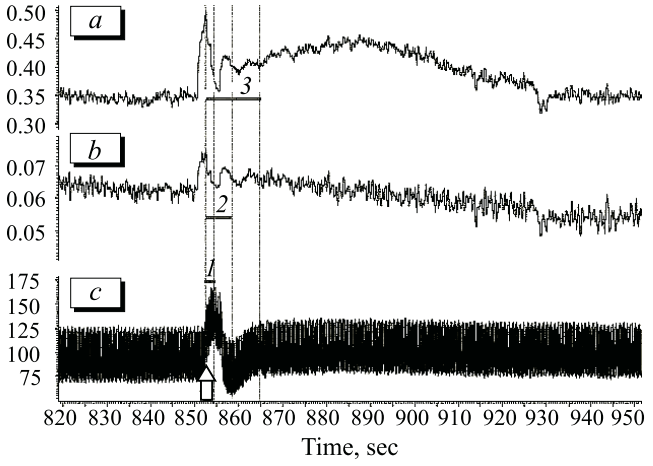

Fig. 1. BP and ATP content in the posterior CB of rats during electrostimulation of VMH. Readings of the ATP biosensor (V, *a*); readings of the "null" sensor (V, *b*); systolic (upper limit) and diastolic (lower limit) BP (mm Hg, *c*). Horizontal lines: latency of the increase in BP after VMH electrostimulation (*1*); period from VMH electrostimulation to the decrease in BP (*2*); latency of the increase in ATP content in CB after VMH electrostimulation (arrow, *3*).

under these conditions were followed by an increase in ATP content in the posterior CB. The enhanced release of ATP in the cingulate region of the brain during VMH stimulation is probably related to the involvement of this structure into emotional reactions. The increase in ATP content in CB during VMH electrostimulation can be associated with close anatomical relationships between these structures. For example, the fornix of the brain includes efferent fibers that extend from the hypothalamus to the limbic cortex.

The results of our study do not explain the cause of this phenomenon. Stress exposure is followed by a change in the metabolism of neurotransmitters, cytokines, hormones, and other biologically active substances in brain tissues. These variations are probably manifested in the increase in ATP content. ATP plays a role of the major energy source, which provides the adequate biochemical and physiological processes. Moreover, ATP is an important signal molecule that mediates the cell-to-cell interaction in CNS upon extreme conditions. The fine mechanisms for the involvement of ATP in systemic processes of emotional reactions in mammals require further investigations.

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