PHYSIOLOGY

Changes in β-Endorphin Level in the Cingulate Cortex in Rats after Peripheral Loperamide and Methylnaloxone Administration at Rest and during Emotional Stress

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β-Endorphin content in the extracellular space of rat cingulate cortex was measured using intravital microdialysis followed by ELISA. Intragastric administration of μ-opioid ligands loperamide and methylnaloxone not crossing the blood-brain barrier produced different effects on β-endorphin level: loperamide reduced and methylnaloxone significantly increased the release of β-endorphin into the extracellular space of rat cingulate cortex. Emotional stress caused by immobilization resulted in slight increase in β-endorphin level in the cingulate cortex. Peripheral administration of loperamide (but not methylnaloxone) significantly increased the release of the neuropeptide during stress. These findings support our hypothesis of reciprocal interaction between the central and peripheral compartments of the endogenous opioid system and provide explanations for the anti-stress effects of loperamide.

Key Words: *β-endorphin; blood-brain barrier; microdialysis; loperamide; methylnaloxone*

Opioid receptors and their ligands are widely distributed not only in brain structures, but also in peripheral organs and tissues, particularly in the gastric mucosa. We previously proposed a hypothesis on reciprocal interactions between the central and peripheral compartments of the endogenous opioid system [3]. Intragastric administration of loperamide and methylnaloxone produced different effects on central functions [2,3], despite the fact, that these opioid receptor ligands do not cross the blood-brain barrier [6,7]. It was hypothesized that loperamide activation of peripheral opioid receptors suppresses, while methylnaloxone inhibition of peripheral opioid receptors activates the central opioid compartment. Peripheral administration of loperamide increases the density of μ-opioid receptors in the cortex and hypothalamus, whereas methylnaloxone decreased it [1].

Here we studied possible changes in β-endorphin release in one of the central structures associated with the endogenous opioid system, in the anterior cingulate cortex. Since the level of β-endorphin significantly increases in rat cortex during emotional stress [5,8], we measured β-endorphin content in perineuronal space of the anterior cingulate cortex at rest and during immobilization stress.

MATERIALS AND METHODS

Experiments were carried out on 27 Wistar rats weighting 180-200 g. The rats had free access to food and water and were kept under 12-h artificial illumination

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regimen. The rats were divided into 3 groups, 9 animals per group. The experiments were performed in accordance with requirements of order No. 267 Ministry of Health of the Russian Federation (19.06.2003), and "Regulations for experiments with experimental animals" (P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Protocol No. 1 from 3.09.2005).

The content of β-endorphin in the area of anterior frontal cortex was determined using microdialysis followed by ELISA of the obtained dialysis samples. Three successive samples (1 h each) were collected: from freely moving rats over 1 h before stress exposure, during 1-h immobilization on a platform [4], and from freely moving rats over 1 h after the end of stress exposure.

A guide cannula for microdialysis sampler (CMA Microdialysis) was stereotaxically implanted under ketamine anesthesia into the area of the anterior cingulate cortex according to Stereotaxic Rat Brain Atlas coordinates [9]: A=1.6 mm, L=-1.8 mm, H=-2.2 mm, 48 h before microdialysis. On the day of the experiment, the microdialysis sampler (CMA Microdialysis) with membrane length of 2 mm and membrane pore size 20 kDa was inserted through the cannula. The membrane part of the sampler was positioned in the area of the anterior cingulate cortex. Accuracy was assessed morphologically on serial brain sections prepared on a freezing microtome and stained with cresyl violet as described previously [10]. The samplers were perfused with artificial cerebrospinal fluid at a rate of 1 μl/min. Dialysis samples obtained within the first two hours after insertion were excluded from the analysis. After implantation, the rats were kept in individual cages under freely moving conditions.

Twenty minutes before collection of the first dialysis sample, one of the test solutions (volume 0.3 mm) was intragastrically administered through a metal probe: distilled water (group 1), 5 mg/kg body weight naloxone (Sigma-Aldrich) in distilled water (group 2), and 5 mg/kg body weight loperamide (Sigma-Aldrich) in distilled water (group 3). Eighty minutes after administration, the rats were exposed to stress by fixing their paws on the platform for 1 h.

Endorphin content in the collected dialysis samples was measured by ELISA using Rat Endorphin ELISA kit (Peninsula Laboratories, LLC, S-1264) with measurement range 0-25 ng/ml. Optical density values for standard endorphin dilutions measured by ELISA were used for construction of optical density–endorphin concentration curve. Endorphin concentration in the test dialysis samples was calculated by the equation corresponding to this curve.

The data were represented as mean±standard error of the mean. Statistical analysis was performed using multiple ANOVA and post-hoc-analysis (Student–Newman–Keuls).

RESULTS

Background dialysate from control animals contained 0.6±0.3 ng/ml β-endorphin. Immobilization stress induced a transient increase in the content of β-endorphin followed by its return to the initial value (Fig. 1). Intragastric administration of methylnaloxone led to a 3-fold increase in β-endorphin concentration in the cingulate cortex. However, loperamide administration only slightly decreased β-endorphin release (Fig. 1). Immobilization stress against the background of loperamide administration produced massive β-endorphin release from nerve endings in the anterior cingulate cortex. Methylnaloxone had almost no effect on changes in β-endorphin concentration during the stress.

Thus, these results support the hypothesis about reciprocal interactions between the central and peripheral compartments of the endogenous opioid system. Inactivation of peripheral opioid receptors probably located in the gastrointestinal mucosa results in intensification of β-endorphin release in rat cingulate cortex, which attests to activation of the central compartment of the endogenous opioid system. Activation of peripheral opioid receptors by loperamide led to inhibition of the central compartment, presumably, due to significant decrease in opioid receptor density in the cortex [1], previously demonstrated by us, rather than due to a decrease in β-endorphin release from nerve endings. We recently found that intragastric administration of loperamide significantly reduces anxiety in rats and produces a stress-protective effect. The results of this study attest to possible mechanism for this effect: the effects of stress factors can be attenuated by intensive

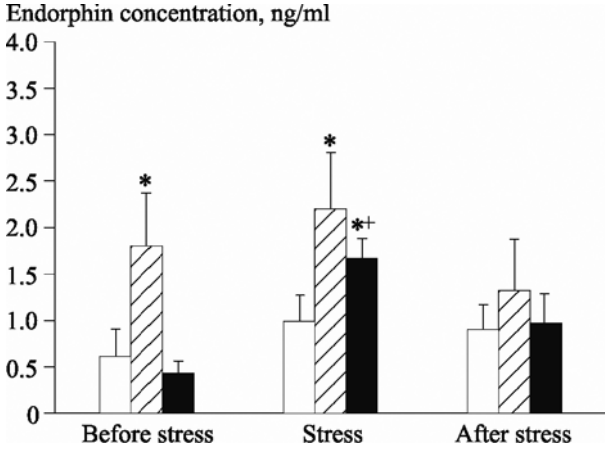

Fig. 1. Endorphin content in extracellular space of rat cingulate cortex. Light bars: intragastric administration water; shaded bars: naloxone administration; dark bars: loperamide administration. $p<0.05$ compared to: *control group, +value before stress.

β-endorphin release into the perineuronal space, which can result in GABA-ergic neuron disinhibition due to μ-opioid receptor activation.

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