Effect of Intraperitoneal Administration of Bacterial Lipopolysaccharide on Synthesis of Pro-Opiomelanocortin mRNA in Rat Tanycytes

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Intensity of pro-opiomelanocortin mRNA synthesis in tanycytes of adrenalectomied and sham-operated rats was studied by *in situ* hybridization 4 h after intraperitoneal injection of bacterial LPS. The results were compared with pro-opiomelanocortin mRNA expression in rats subjected to immobilization stress. Immobilization and LPS injection to adrenalectomied and sham-operated animals sharply reduced pro-opiomelanocortin mRNA expression in tanycytes. Injection of LPS to sham-operated animals and immobilization stress caused a significant increase in blood corticosterone level. On the other hand, LPS injection did not change low blood level of corticosterone in adrenalectomied rats. The data indicate a synergic inhibitory effect of glucocorticoids and LPS on synthetic activity of tanycytes.

Key Words: *tanycytes; lipopolysaccharides; pro-opiomelanocortin*

Interactions between the nervous, endocrine, and immune systems provide coordinated regulation of dynamic homeostasis. Despite the progress in studies of integration of these regulatory systems, the mechanisms of inter-systems communications remain not quite clear. For example, possible effects of immune stimulation and peripheral inflammation on the specialized lining of the bottom of the third cerebral ventricle (the so-called tanycytic ependyma) involved in the neuroendocrine regulation processes [1,5] was not studied.

The apical pole of these cells is washed by liquor of the third cerebral ventricle, while the basal pole directly contacts with capillaries of the portal system of the median eminence. The tanycytic glia transports substances captured from the liquor to the portal vessels [4]. Tanycytes express numerous hormone and neuropeptide receptors, synthesize prostaglandin E_2 and transforming growth factor [2], and are involved in regulation of the thyroid gland, gonads, and adrenal cortex [1,5]. It was found that metabolic activity of tanycytes inversely correlates with adrenocorticotropic function of the pituitary [1]. Since inflammation developing in response to bacterial infection is paralleled by activation of the hypothalamic-pituitary-adrenal system [5], it is logical to hypothesize that functional activity of the tanycytic glia decreases under these conditions.

In order to verify this hypothesis, we studied synthetic activity of tanycytes in intact animals and after intraperitoneal injection of bacterial LPS to adrenalectomied rats and compared it with tanycyte reaction of control animals to immobilization stress. Using this experimental model it is possible to identify the nature of the factor modulating tanycytes (glucocorticoids or endotoxin proper). The expression of pro-opiomelanocortin (POMC) mRNA served as the marker of synthetic activity (this molecule is the precursor of β-endorphine, adrenocorticotropic hormone, and α-melanocyte-stimulating hormone).

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MATERIALS AND METHODS

Experiment was carried out on 24 male outbred albino rats (200-250 g) kept under standard conditions in accordance with regulations on handling laboratory animals, determined by the local ethic committee of the Udmurt State University. The adrenals were removed in 10 animals one week before the experiment. Control group consisted of 10 sham-operated rats. Animals of an additional group (4 rats) were daily exposed to 2-h fixation by four limbs in the supine posture during one week. On the day of experiment, 5 adrenalectomied and 5 sham-operated rats were intraperitoneally injected with 250 μg/kg *Salmonella abortus equi* LPS (Sigma Aldrich) in 1 ml saline. The remaining 5 adrenalectomied and 5 sham-operated rats were injected with 1 ml sterile saline.

Four hours after injection, the rats of experimental and control groups were decapitated and the blood was collected for corticosterone measurement by EIA using commercial kit (Cayman Chemical, MI) according to manufacturer's instruction. The brain was removed and frozen in carbon dioxide for studies by *in situ* hybridization. Cryostat sections of the brain (14 μ) were mounted on Probe slides and incubated for 16 h at 42°C with a radiolabeled probe complementary to POMC mRNA sequences 266-319. The sections were washed in citrate buffer, dehydrated in alcohol battery, dried on air, and coated with emulsion. After exposure in darkness, the preparations were developed, fixed, washed in water, stained with toluidine blue, and examined in a Nikon Eclipse 200F microscope in the bright and dark fields. Silver granules above labeled cells were counted using ImageProPlus 6.0 software. The cell was considered labeled, if the number of silver granules above it 5-fold surpassed the background values. The significance of differences between the means was evaluated by Student's *t* test using Statistica 6.0 software.

RESULTS

Autoradiographic label corresponding to location of POMC mRNA was detected in ependymal gliocytes of the median eminence of the third cerebral ventricle (Fig. 1, *a*, *b*). Injection of bacterial endotoxin caused a drastic reduction of POMC mRNA synthesis in tanycytes of sham-operated and adrenalectomied rats in comparison with the control (by 75.9 and 70.6%, respectively; Fig. 1, *c*; Table 1). A significant reduction (by 77.7%) of autoradiographic label was also found in animals exposed to immobilization stress.

Rats exposed to immobilization had significantly higher levels of blood corticosterone in comparison with the controls. A similar increase was observed in

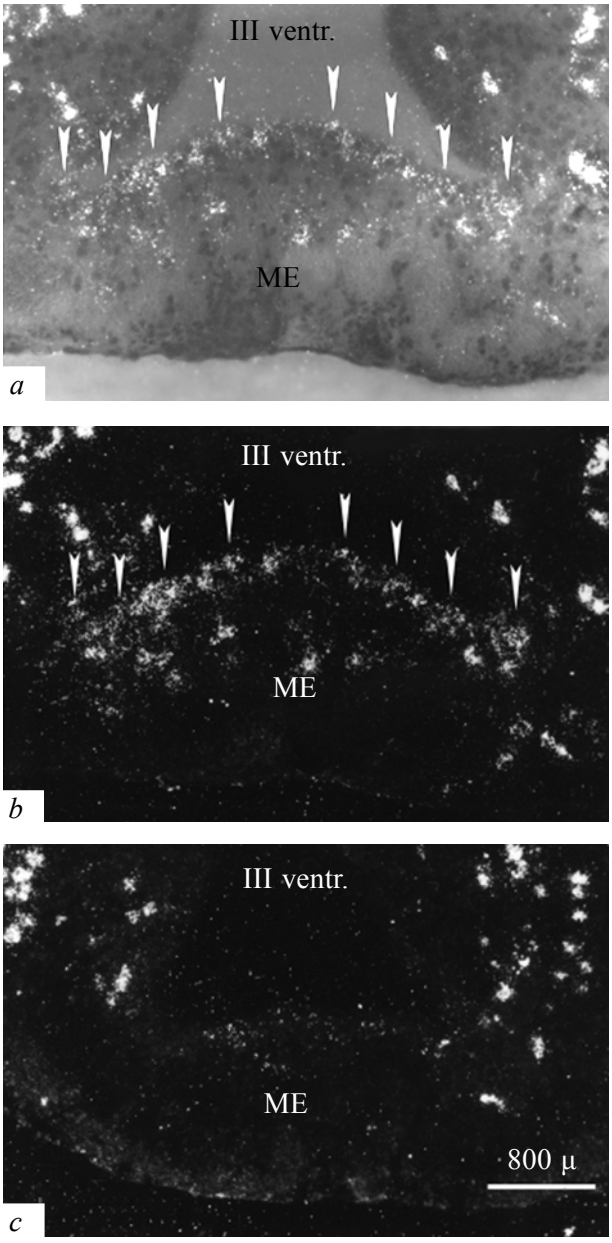

Fig. 1. Location of POMC mRNA autoradiographic label in tanycytes of the third brain ventricle of sham-operated rats in bright (*a*) and dark (*b*) field (arrows). Injection of LPS to sham-operated animals leads to significant reduction of autoradiographic label in tanycytes (*c*). III ventr.: third cerebral ventricle; ME: median eminence.

sham-operated rats 4 h after LPS injection (Table 1). On the other hand, corticosterone levels in adrenalectomied rats were significantly lower than in sham-operated ones and virtually did not change after LPS injection.

Hence, immobilization stress and intraperitoneal injection of LPS to sham-operated rats caused an increase of the blood corticosterone level and reduced the intensity of POMC mRNA synthesis in tanycytes. The fact that similar reduction of POMC mRNA synthesis intensity was observed after LPS injection to adrenalectomied animals indicates that the inhibitory

TABLE 1. Intensity of POMC mRNA Expression in Tanycytes and Blood Corticosterone Level in Control and Experimental Rats (*M*±*m*)

Group	POMC mRNA, % of control	Blood corticosterone, ng/ml
Sham-operated (control)	100.0±11.5	140.4±20.3
Sham-operated+LPS	24.1±15.6*	382.6±27.3*
Adrenalectomied	106.8±15.6	57.1±26.6
Adrenalectomied+LPS	29.4±18.4^{+}	68.8±34.1
Immobilization stress	22.3±16.4*	368.4±28.2*

Note. $p<0.001$ compared to: *control, $^{+}$adrenalectomied animals.

effect was due to high corticosterone levels and to bacterial endotoxin (or cytokines induced by it). Presumably, these factors synergically inhibit POMC mRNA synthesis in tanycytic ependymocytes under conditions of bacterial infection. Inflammatory processes stimulate the hypothalamo-pituitary-adrenal system [5], in turn, glucocorticoids produced by the adrenal cortex cause a potent anti-inflammatory effect [3]. Presumably, tanycytic glia serves as a negative feedback component in the neuroendocrine system regulating the blood glucocorticoid level, which is inhibited during bacterial infection.

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