Antipyretic Effect of Neuropeptide Galanin in Endotoxin-Induced Fever

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 1, pp. 73-77, January, 2001
Original article submitted April 2, 1999

Neuropeptide galanin produces an antipyretic effect in experimental pyrogenic reaction induced by intraperitoneal injection of lipopolysaccharide. Central intracerebroventricular injection of 100 ng galanin significantly attenuated, but did not completely abolish fever. Central galanin injection potentiated endotoxin-induced activation of the noradrenergic system and blocked activation of the serotoninergic system of the anterior hypothalamus.

Key Words: *galanin; thermoregulation; pyrogenesis; neurotransmitters*

Studies of the central mechanisms regulating pyrogenic reaction demonstrated an important role of neuropeptides in the inhibition of fever response. In particular, fever can be suppressed by arginine-vasopressin and α-melanocyte stimulating hormone [3].

Injection of galanin, a neuropeptide of the central and peripheral nervous systems in humans and animals [9,13], into the paraventricular hypothalamic nuclei causes a short-term inhibition of heat production under conditions of normothermia [12]. Our previous studies showed that blockade of galanin receptors in the brain with specific antagonists potentiates fever response caused by systemic administration of lipopolysaccharide (LPS) [2]. These facts point to an important role of galanin in the mechanisms of hyperthermia inhibition. Our aim was to study of thermoregulatory effects of galanin after its central administration and LPS-induced changes in the neurotransmitter system in brain structures.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats kept under standard conditions with free access to food and water. *E. coli* LPS (serotype 055:B5, Sigma; 25 mg/kg) was injected intraperitoneally; control animals were injected with apyrogenic physiological saline. Galanin synthesized at the Institute of Extrapure Biopreparations was injected intracerebroventricularlly (i.c.v.) via a cannula chronically implanted (stereotaxic coordinates AP +0.5 mm, DS ±1.5 mm, H 3.5 mm) 12-15 days before the experiment. Control animals received apyrogenic physiological saline under the same conditions. Rectal temperature was measured with a TPEM-1 electronic thermometer.

For modulation of pyrogenic reaction galanin was administered in doses of 2 μg and 100 ng per rat.

The content of biogenic amines and their metabolites in the brain was measured by high-performance liquid chromatography with electrochemical detection. The animals were decapitated, and anterior and posterior hypothalamus were immediately frozen in liquid nitrogen and stored at -70°C. The contents of dopamine (DA), norepinephrine (NE), serotonin (5-HT), and their metabolites, DOPA, homovanillic acid (HVA), 3-methoxy-4-hydroxyphenyl glycol (MHPG) and 5-hydroxyindoleacetic acid (HIAA) were measured. Chromatography was carried out as described previously [2]. The concentration of biogenic amines and their metabolites was presented in ng/mg tissue.

The content of corticosterone in rat serum was determined by competitive binding assay [4] with cold (Sigma) and ^{3}H-corticosterone (St.-Petersburg). Antiserum against corticosterone was obtained from rabbits immunized with albumin-conjugated corticosterone-21-semisuccinate [4]. The calibration curve

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0007-4888/01/1311-0060$25.00

(0.125-10 ng) was built using a corticosterone standard. Radioactivity was measured on an LQ Betta counter and the content of corticosterone was calculated in ng/ml serum.

The data were processed statistically using Student *t* test.

RESULTS

Systemic administration of LPS increased body temperature, the type of fever reaction depended on the dose of LPS. The mean increase in core temperature induced by 25 μg/kg LPS was 1.0-1.8°C, the reaction peaked 2 h postinjection.

Galanin (2 μg, i.c.v.) produced no hypothermic effect (Fig. 1, *a*, 2, *a*). Injection of 100 ng galanin 5-10 min before or 1 h after intraperitoneal administration of LPS significantly attenuated pyrogenic response (Fig. 1, *b*, 2, *b*). Intracerebroventricular injection of galanin did not completely inhibit pyrogenic reaction: core temperature increased 2 h after LPS injection. Control systemic injection of 100 ng galanin did not attenuate fever, which confirmed the assumption on central mechanisms of hypothermic effect of galanin.

Attenuation of pyrogenic reaction by endogenous galanin [2] and our findings allow us to conclude that galanin plays an important role in the modulation of the pyrogenic effect of endogenous cytokines. Since galanin was shown to inhibit phosphoinositol metabolism [15], its hypothermic effect is probably realized via inhibition of prostaglandin synthesis as mediators of the pyrogenic action of cytokines.

LPS causes changes in NE, DA, and 5-HT metabolism in some brain structures, in particular, in the hypothalamus [1,6]. Galanin is colocalized with many neurotransmitters and modulates their action [11]. To estimate the effect of galaninergic system on LPS-induced changes in the DA, NE, and 5-HT systems we analyzed the content of these neurotransmitters and their metabolites in hypothalamic structures.

Galanin modulates LPS-induced changes in the content of neurotransmitters in the anterior hypothalamus. Systemic administration of LPS induces the reaction of the hypothalamic neurotransmitter systems, especially NE-ergic system [1,6]. In our experiments the content of NE decreased, while the content of its metabolite MHPG and MHPG/NE ratio increase 2 h after endotoxin injection, which suggests activation of NE metabolism induced by systemic LPS administration. Intracerebroventricular injection of galanin potentiated activation of the NE-ergic system (Table 1). In control animals (receiving no LPS) galanin also activated NE metabolism. This effect was similar to that induced by LPS (Table 1).

It was previously demonstrated that NE produced a complex effect on thermoregulation. According to the concept of I. Bligh [5], NE acts as an inhibitory transmitter for heat- and cold-activated neurons. M. T. Lin *et al.* [10] showed that injections of NE and sympathomimetics to rats cause hypothermia in cold environment (8°C) and hyperthermia at 22-30°C. It is also known that fever is associated with a shift in the initial point of thermoregulation and enhancement of heat production (as during adaptation to cold), which suggests a hypothermic effect of NE under these conditions. These facts and our findings allow us to conclude that the NE-ergic system of the anterior hypothalamus is involved in the regulation of thermal re-

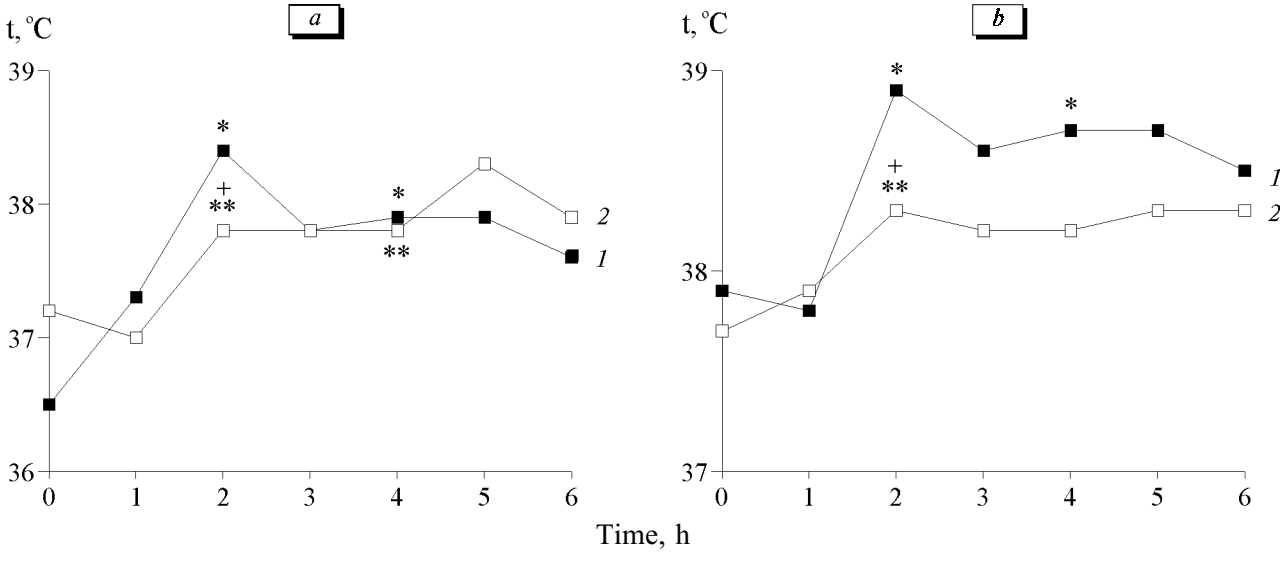

Fig. 1. Dynamics of core temperature in rats after systemic administration of bacterial lipopolysaccharide against the background of 2 μg (*a*) and 100 ng (*b*) galanin. *1*) control; *2*) galanin. **p*<0.01, ***p*<0.05 compared to baseline, ^{+}p<0.05 compared to the control.

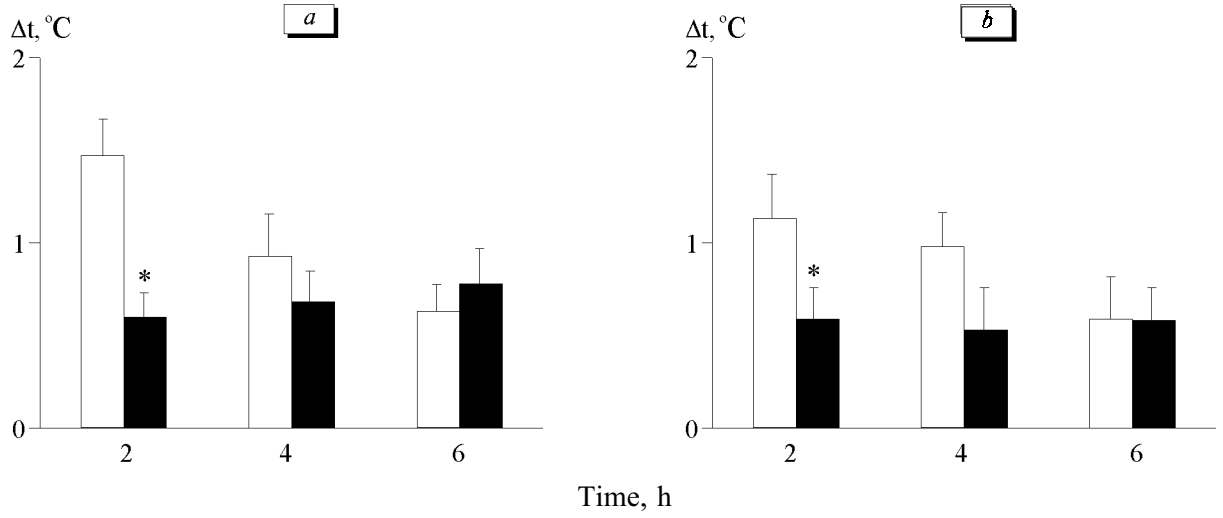

Fig. 2. Effect of galanin (filled bars) in doses 2 μg (*a*) and 100 ng (*b*) on fever response (D of baseline core temperature). **p*<0.05 compared to the control (open bars).

action and that this activation triggers the mechanisms limiting fever reaction in rats. Exogenous galanin can potentiate or promote this activation. This assumption is confirmed by the absence of attenuation of LPS-induced pyrogenic reaction under conditions of galanin receptor blockade.

Metabolism of 5-HT and DA was markedly activated 4 h after LPS injection [1]. In our experiments endotoxin injection decreased 5-HT content in the anterior hypothalamus 2 h 20 min postinjection, but HIAA/5-HT ratio at this stage increased insignificantly. However, intracerebroventricular injection of galanin abolished this reaction (Table 1). The effect of galanin on LPS-induced activation of the DA-ergic system was minor (Table 1). Thus, the interaction between the galanin and NE-ergic systems plays a key role in the modulation of the early phase of pyrogenic reaction.

This assumption is confirmed by the fact that hypothermic effect was manifested when galanin was injected before LPS or during the development of pyrogenic reaction, *i.e.* after activation of prostaglandin synthesis.

Mechanisms involving corticotropin-releasing hormone (CRH) participate in the cytokine-induced fever reaction. It was shown that pyrogenic effect of interleukin-1β, 6, and 8 (but not interleukin-1α and TNF-α) can be blocked by antagonists or antibodies to CRH receptors [16]. This implies an important role

TABLE 1. Content of Neurotransmitters and Their Metabolites in Rat Anterior Hypothalamus 2 h after Intracerebroventricular Injection of Galanin during Pyrogenic Reaction Induced by Intraperitoneal Injection of LPS (*M*±*m*)

Parameter	Physiological saline, i/p and i.c.v. (control 1)	LPS i/p and physiological saline i.c.v. (control 2)	LPS i/p and galanin i.c.v.	Physiological saline i/p/ and galanin i.c.v.
NE	1.724±0.070	1.648±0.050	1.617±0.087	1.438±0.078**
MHPG	0.232±0.010	0.303±0.020**	0.325±0.027	0.323±0.008*
MHPG/NE	0.139±0.010	0.184±0.010**	0.203±0.026	0.227±0.017*
5-HT	0.354±0.036	0.189±0.013**	0.465±0.019^{+}	0.432±0.030
HIAA	0.353±0.049	0.339±0.070	0.420±0.019	0.425±0.018
HIAA/5-HT	1.013±0.092	1.878±0.495	0.951±0.055	0.998±0.106
DA, ng/mg	0.646±0.080	0.285±0.018*	0.329±0.003^{+}	0.203±0.099*
DOPA, ng/ml	0.195±0.026	0.120±0.014**	0.120±0.014	0.166±0.069
DOPA/DA	0.348±0.037	0.341±0.026	0.365±0.047	0.469±0.146
HVA, ng/mg	0.043±0.004	0.030±0.005	0.041±0.009	0.046±0.023
HVA/DA	0.059±0.004	0.089±0.008**	0.115±0.022	0.120±0.036

Note. **p*<0.01, ***p*<0.05 compared to control 1; $^{+}$*p*<0.05 compared to control 2.

of interaction between galanin and CRH in galanin modulation of LPS-induced pyrogenic reaction. M. Niimi *et al.* [14] demonstrated colocalization CRH and galanin in neurons of the paraventricular hypothalamic nuclei. S. C. Hooi *et al*. [8] showed that galanin injected in the paraventricular hypothalamic nuclei attenuated stress-stimulated secretion of ACTH, while immune blockade of galanin increased plasma content of ACTH, which pointed to hypothalamic regulation of CRH activity by galanin. In our study, attenuation of fever after intracerebral injection of galanin was not associated with altered reaction of the hypothalamus-pituitary-adrenal system in response to LPS injection: corticosterone concentration after i.c.v injection of physiological saline and galanin attained 266.7±10.5 and 264.0±11.2 ng/ml, respectively, against the background of intraperitoneal LPS administration and 187.5±31.9 and 138.3±30.6 ng/ml, respectively, after inraperitoneal injection of physiological saline. Therefore, activation of this system is probably not involved in the mechanisms of antipyretic action of galanin.

Thus, galanin is involved in the mechanisms of negative feedback regulation during CNS reaction to activation of the immune system, it exhibits central antipyretic activity, and modulates neurotransmitter reactions induced by systemic administration of endotoxin.

This study was supported by the Russian Foundation for Basic Research (grant No. 99-04-49479).

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