Contribution of Galaninergic Structures of the Brain to Reaction to Lipopolysaccharide

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Relationship between blocking of galanine receptors in the brain and lipopolysaccharide-induced two-wave reaction and changes in neurotransmitter metabolism in the anterior and posterior hypothalamus, caused by provision of this reaction, are studied in rats. Intraventricular administration of the galanine receptor antagonist M-15 prevented the decrease in rectal temperature 3 h after intraperitoneal injection of lipopolysaccharide, thus rendering the pyrogenic reaction a prolonged single-wave pattern. Neurotransmitter changes, specifically, a decrease in the dopamine content in the anterior and increase in the posterior hypothalamus during reaction to endotoxin, are blocked by specific antagonist of the galanine receptor. These data indicate the participation of galaninergic mechanisms of the brain in central reactions to lipopolysaccharide.

Key Words: *galanine; brain; pyrogenesis; neurotransmitters*

Bacterial lipopolysaccharide (LPS) activates immune cells, inducing production of inflammation cytokines which mediate central effects of the endotoxin: fever, abnormal behavior, and increased plasma corticosteroid level. Recent studies demonstrate that these effects are paralleled by changes in the content of biogenic amines in the hypothalamus, hippocampus, and frontal cortex [4]. The role of biogenic amines of different brain structures in pyrogenic, hormonal, and behavioral response to systemic LPS is not quite clear. It is obvious that, besides classical neurotransmitters, neuropeptides play a modulating role in these processes. We investigated galanine, because this neuropeptide, highly prevalent in the peripheral and central nervous system and regarded mainly as an inhibitory modulator, is often concurrent with catecholamines [10]. It inhibits noradrenaline release [3], dopamine release [13], and metabolism in the median eminence of the hypothalamus [6]. Galanine distribution in the central nervous system, namely, a high density of galanine receptors and galanine-positive neurons in hypothalamic nuclei, neuroendocrine lobe of the pituitary and macula cerulea [1,16], i. e., in brain areas directly participating in neuroimmune reactions, suggests a modulating action of this neuropeptide in immunogenesis, specifically, at early stages of infectious process.

Neuronal and other than neuronal galanine or a galanine-like peptide is involved in inflammation of the rat limb caused by local injection of carraginane [7]. We observed an increase in galanine mRNA level in the cells of inflamed rat limb derma and in neurons of the spinal dorsal horn median plate, ipsilateral to focus of inflammation, while in the corresponding spinal root ganglia of the same segments the level of galanine mRNA was decreased.

Our purpose was to verify the hypothesis about brain galanine involvement in processes associated with response to endotoxin, to detect its possible role in modulation of centrally mediated thermal reaction, and in neurotransmitter changes in the activity of hypothalamic monoaminergic systems, concomitant with LPS administration.

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MATERIALS AND METHODS

Experiments were carried out with male Wistar rats kept under standard conditions. Light regimen was as follows: day from 8:00 to 20:00 and night from 20:00 to 8:00. LPS (Sigma, *E. coli,* serotype 055:B5) was injected intraperitoneally in a dose of 15 μg/kg. Apyrogenic normal saline was injected to controls. The galanine receptor antagonist M-15 synthesized at the Neurochemistry Department of Stockholm University (3 nmol in 10 μl normal saline) was injected in brain ventricles through cannulas. Control rats were injected with apyrogenic normal saline. Cannulas were implanted 10 days before experiment by means of stereotaxic device by the following coordinates: AP 0.5 mm, DS 1.5 mm, H 3.5 mm.

Rectal temperature was measured by a TPEM-1 electric thermometer (Kazan) for 4.5 h after injection of endotoxin, after which the animals were decapitated, and anterior and posterior hypothalamus structures were isolated to measure biogenic amines and their metabolites. After isolation, brain structures were weighed, frozen in liquid nitrogen, and stored at -70°C.

The content of biogenic amines in the brain was measured by high-performance liquid chromatography with electrochemical detection. Dopamine, noradrenaline, serotonin, and their metabolites 3,4-dioxyphenylacetic acid (DOPAA), homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol, and 5-hydroxyindolacetic acid were determined. Chromatographic system consisted of a Gilson 305 pump, injector (Rheodyne), metal column (4.0×250 mm) packed with Spherisorb ODS 2 reverse-phase adsorbent (Pharmacia), precolumn, and LC-4B amperometric detector (BAS). Biogenic amines were measured at a fiber glass carbon electrode potential of 0.75 V against silver chloride reference electrode. Mobile phase consisted of 0.02 M citrate-phosphate buffer, pH 3.5, 0.2 mM EDTA disodium salt, 0.3 mM sodium octylsulfonate, and 15% methanol, flow rate 0.7 ml/min, duration of analysis 35 min. Standard monoamine solutions were prepared by consecutive dilutions. Qualitative analysis of the studied compounds was carried out by retention parameters, quantitative by the absolute calibration method. Concentrations of biogenic amines and their metabolites were calculated in ng/mg tissue.

Results were statistically processed using Student's *t* test.

RESULTS

Intraperitoneal injection of LPS in a dose of 15 μg/kg led to the development of pyrogenic reaction in experimental animals (rectal temperature increased by 1°C in comparison with the controls). The temperature curve had two waves, the first peak was observed 2 h and the second 4.5 h after LPS injection. Three hours postinjection body temperature normalized (Fig. 1, *a*). In one experimental group, the animals were injected with the galanine receptor antagonist 80 min after intraperitoneal LPS, during the development of inflammatory reaction. Blocking of galanine receptors in the brain altered the pattern of temperature reaction: it became single-wave, i. e., there was no normalization of temperature 3 h after LPS injection (Fig. 1, *b, c*). A similar temperature reaction was observed after injection of higher doses of LPS (25 mg/kg), when increased temperature did not decrease for 4-5 h after endotoxin administration. We suppose that temperature drop 3 h after LPS injection in a dose of 15 μg/kg is mediated at least partially by the inhibitory effect of galanine. Previously we demonstrated the capacity of galanine to produce hypothermal effect after a single injection in the hypothalamic paraventricular nuclei [11]. This effect is similar to that of noradrenaline [15] con-

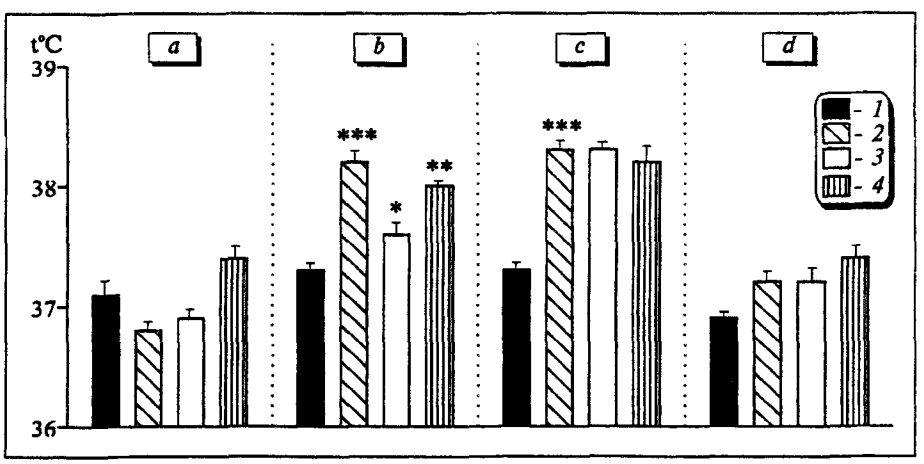

Fig. 1. Temperature reactions ofrats before injections (*1*), 2 (*2*), 3 (*3*), and 4.5 h (*4*) after injection of lipopolysaccharide (LPS). Here and in Figures 2 and 3: *a*) control group (normal saline intraperitoneally and into the third brain ventricle); *b*) LPS intraperitoneally, normal saline into the third brain ventricle; *c*) LPS intraperitoneally, M-15 into the third brain ventricle; *d*) normal saline intraperitoneally, M-15 into the third brain ventricle; n=6-8. *p<0.05, **p<0.01, ***p<0.001 *vs.* the control.

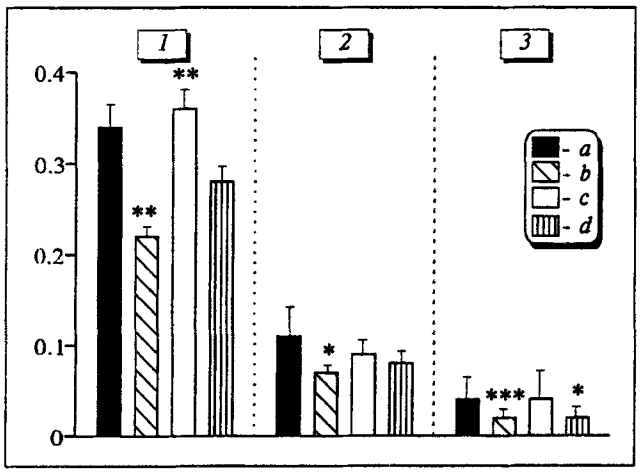

Fig. 2. Activity of the dopaminergic systems of anterior hypothalamus. Here and in Fig. 3: *1*) dopamine; *2*) 3,4-dioxyphenylacetic acid; *3*) homovanillic acid. Ordinate: dopamine content, ng/mg tissue.

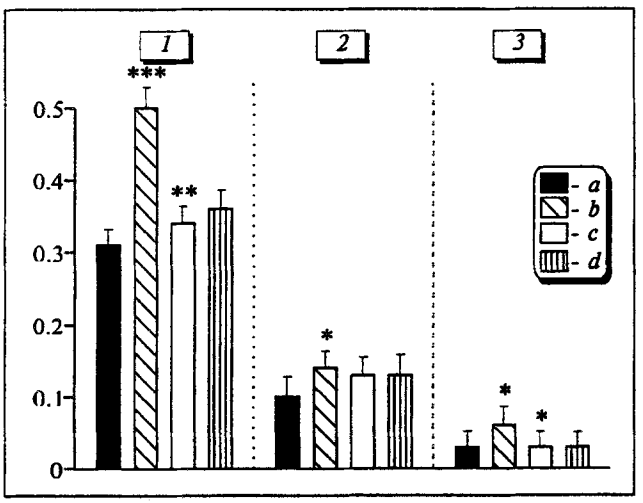

Fig. 3. Activity of dopaminergic systems of the posterior hypothalamus.

current with galanine in paraventricular nuclei neurons [9] and confirms the role of galanine as an anabolic neuropeptide. Galanine may affect different components of the pyrogenic reaction; pyrogenic effects of the majority of cytokines mediating response to LPS were shown to be related to prostaglandin production [2]. Galanine capable of decreasing the activity of the phosphoinositol pathway [8] can thus lead to suppression of prostaglandin production. In addition, fever and thermogenesis caused by central injections of interleukins-1β, -6, and -8 are caused by release of corticotrophin releasing hormone [14], and galanine concurrent with this hormone in hypothalamic paraventricular nuclear neurons [12] can decrease the content of the corresponding hormones in the plasma [5].

To ensure that the blocking of antipyrogenic effect of galanine is mediated by its receptor antagonist (a chimeric peptide galanine-[1-12]-Pro-substance P-[5-11]-amide) but not by pyrogenic effect of the antagonist molecule or its fragment, we injected normal saline intraperitoneally and M-15 into the brain ventricle in one group of animals. The temperature in these rats did not differ from that in intact controls (Fig. 1, *d*).

Measurements of monoamines in the hypothalamic structures 5 h after LPS injection showed decreased content of dopamine and its major metabolites DOPAA and HVA in the anterior hypothalamus (Fig. 2), which agrees with published data [4]. Injection of the galanine antagonist M-15 blocked this effect. An opposite reaction was observed in the posterior hypothalamus: increased content of dopamine and its metabolites in animals treated with LPS and no such effect in animals treated with M-15 (Fig. 3).

Thus, specific blocking of intracerebral galanine receptors cancels the reactions of dopaminergic systems of the anterior and posterior hypothalamus to LPS, which proves the participation of galanine in endotoxin-induced activation of these systems. We observed no changes in the concentrations of noradrenaline, serotonin, and their metabolites in the anterior and posterior hypothalamus 5 h after LPS injection in any of the examined groups, which can be explained by relatively late terms of investigation.

Changes in the pattern of pyrogenic reaction and concomitant changes in dopamine metabolism in the anterior and posterior hypothalamic structures caused by specific blocking of galanine receptors in the brain indicate the involvement of galaninergic mechanisms of the central nervous system in the reaction induced by systemic LPS administration.

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