Effect of Bacterial Endotoxin on Secretion and Synthesis of Vasopressin during Saline Load in Rats

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Secretion and synthesis of vasopressin was studied in adult male Wistar rats receiving lipopolysaccharide in a dose of 250 μg/100 g body weight and subjected to moderate osmotic stimulation (2% NaCl perorally for 6 days). Lipopolysaccharide stimulated secretion of vasopressin into the blood. It should be emphasized that the content of vasopressin mRNA in gigantocellular nuclei of the hypothalamus decreased, which probably reflected intensification of its translation. The observed changes and slight increase in transcription of the vasopressin gene (determined by the content of heterogeneous nuclear RNA) provide intensive secretion of this neurohormone into the blood.

Key Words: *vasopressin; gigantocellular nuclei; hypothalamus; saline load; lipopolysaccharide*

Vasopressin (VP) is a nonapeptide hypothalamic neurohormone involved in the regulation of water and electrolyte balance and adaptive reactions. VP plays an important role under critical conditions, *e.g.* acute systemic inflammation produced by administration of endotoxins from gram-negative bacteria (lipopolysaccharide, LPS) [9]. Expression of VP under these conditions was studied in recent years [3-8]. These investigations produced different results, which can be explained by different doses of endotoxin, routes of administration, and species of experimental animals (rats, sheep, and pigs). The general conclusion is that LPS has no effect on the synthesis and secretion of VP. Our previous studies showed that administration of LPS stimulates synthesis and secretion of VP under conditions of severe osmotic shifts (*e.g.*, injection of hypertonic NaCl [5] or complete water deprivation [6]). Here we studied the effects of LPS on gigantocellular vasopressinergic neurons of the hypothalamus during moderate osmotic stimulation, which included saline load with 2% NaCl given perorally for 6 days.

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MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats weighing 250-300 g maintained at 12 h:12 h light-dark regimen. The animals were divided into 4 groups. The control group included 5 rats. Group 1 animals (n=17) were killed 3 (n=6) and 6 h (n=11) after intraperitoneal injection of LPS in a dose of 250 μg/100 g (*E. coli*, serotype 0111:B4, Sigma). Six-day saline load in group 2 rats (n=6) was produced by substitution of water in drinking bowls with 2% NaCl. Group 3 animals (n=14) were subjected to saline load with 2% NaCl for 7 days and killed 3 (n=7) and 6 h (n=7) after LPS administration. After decapitation the plasma and brain were frozen on dry ice and stored at -70°C until radioimmunoassay and *in situ* hybridization.

Plasma Na^+ concentration was measured using a ion-specific electrode analyzer (Beckman Elise Na/K/Cl Analyzer). After extraction with acetone and chloroform the content of arginine-VP was estimated by radioimmunoassay [10]. Plasma levels of VP and Na^+ were measured at the Department of Endocrinology (Georgetown University, Washington). The sensitivity threshold was 0.5 pg/ml. Antibodies to arginine-VP were characterized by 100% cross-reactivity with ly-

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TABLE 1. Plasma Levels of VP and Na^+ 3 and 6 h after Administration of Bacterial Endotoxin LPS during Saline Load (*M*±*m*)

Group	Before injection		After injection			
			3 h		6 h	
	VP, pg/ml	Na^+, mmol/liter	VP, pg/ml	Na^+, mmol/liter	VP, pg/ml	Na^+, mmol/liter
Control	1.85±0.44	144.52±3.32	2.25±1.30	138.36±3.34	1.48±0.35	145.42±2.79
Saline load	2.16±0.83	149.78±4.08	7.60±3.56*	148.34±4.55	4.87±0.95	142.1±3.7

Note. *$p<0.05$ compared to parameter before treatment.

sin-VP and practically did not interact with oxytocin, arginine-vasotocin, and desmopressin (<0.1%).

Experiments with *in situ* hybridization were performed by V. V. Grinevich at the Department of Physiology of Endocrine System (National Institute of Health, USA). Two molecular probes were used. Probe I for heterogeneous nuclear RNA (hnRNA) corresponded to a 735-nucleotide fragment in exon 1 of the VP gene cloned into plasmid pGEM-3mRNA [5]. Probe II for matrix RNA (mRNA) of VP was a 230-nucleotide fragment in exon 3 of the VP gene cloned into plasmid pGEM-4Z [5]. The probes were synthesized using Riboprobe reagents for *in vivo* transcription (Promega) with ^{35}S-labeled uracil triphosphate and cytosine triphosphate [4]. Hybridization was performed by the standard method [4], which included fixation of defrosted brain sections in 4% paraformaldehyde, acetylation in 0.25% acetic anhydride, dehydration, delipidation, and incubation with probes in the hybridization buffer (2×10^6 Ci/μl). After incubation nonspecific binding was stopped by repeated washing in citrate buffered saline of decreasing concentrations containing formamide and ribonuclease A. The preparations were exposed with Kodak BioMax MR X-ray films. The density and area of signals were assayed using a computer-assisted system for image analysis (Imaging Research) equipped with NIH Image 1.0 software [4].

RESULTS

The contents of VP and Na^+ in the blood remained unchanged after 6-day saline load (Table 1). LPS had no effect on VP and Na^+ concentrations. Plasma VP level in animals subjected to saline load increased by 4 times 3 h after LPS administration ($p<0.05$) and 2-fold surpassed the control over the next 3 h (Table 1). However, we revealed no changes in plasma osmolarity.

In the hypothalamic gigantocellular nuclei saline load increased optical density of VP hnRNA in the supraoptic (SON) and paraventricular nucleus (PVN) of the hypothalamus by 32 and 46%, respectively, compared to the control (Figs. 1 and 2). LPS alone did not change optical density of the VP hnRNA signal. Treatment with endotoxin after saline load slightly increased the signal in SON (by 10%, Figs. 1 and 2), but not in PVN.

Study of labeling density for VP mRNA showed that saline load markedly increased the intensity of signals in SON and PVN of the hypothalamus by 42 and 27%, respectively ($p<0.01$, Figs. 1 and 2). LPS had no effect on labeling density of VP mRNA in rats with normal water supply. By contrast, the content of VP mRNA slightly decreased 3 h after administration of LPS to animals subjected to saline load. Significant changes were observed 6 h after treatment ($p<0.05$).

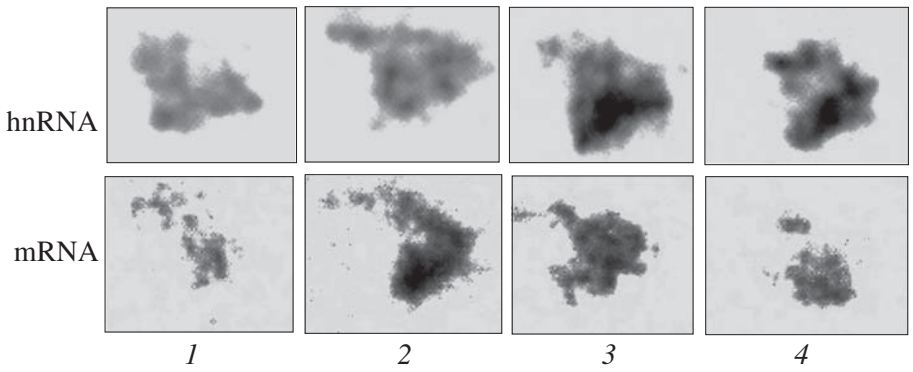

Fig. 1. Transcripts of heterogeneous nuclear RNA (hnRNA) and matrix RNA (mRNA) for vasopressin in the supraoptic hypothalamic nucleus after injection of bacterial endotoxin LPS to rats subjected to 6-day saline load (X-ray films). Here and in Fig. 2: control (*1*), saline load (*2*), and 3 (*3*) and 6 h after LPS administration to rats with saline load (*4*).

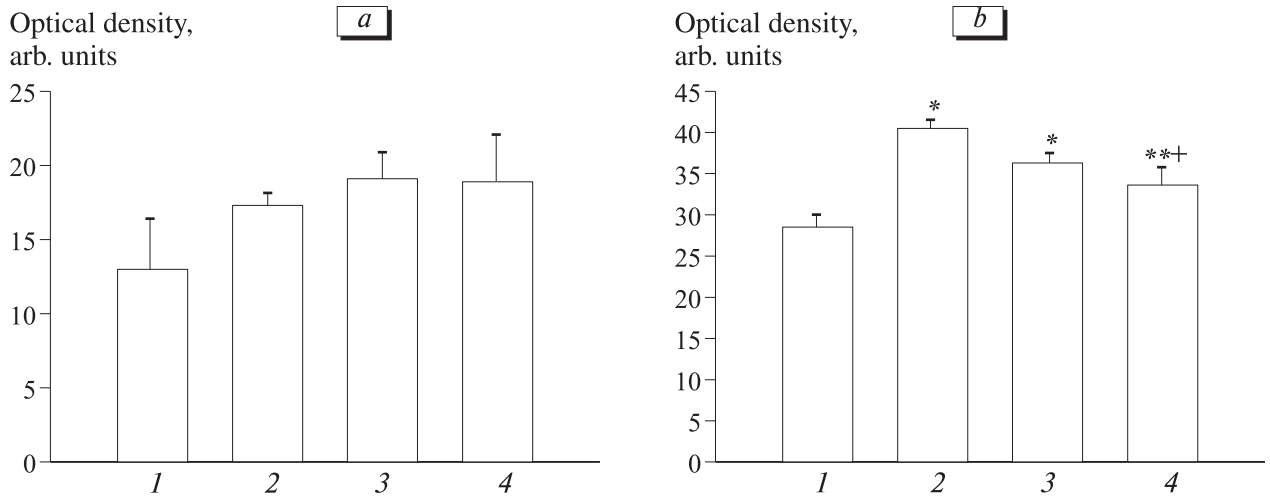

Fig. 2. Effects of bacterial endotoxin LPS on the contents of hnRNA (*a*) and mRNA for vasopressin (*b*) in the supraoptic nucleus of rat hypothalamus after saline load. *$p<0.01$ and **$p<0.05$ compared to the control; $^{+}p<0.05$ compared to saline load.

The content of VP mRNA in SON and PVN decreased by 17 and 19%, respectively (Figs. 1 and 2).

Our results indicate that LPS potentiates VP secretion in the blood under conditions of osmotic stimulation, which is consistent with published data [5,6] and reflects general reaction of gigantocellular neurons in the hypothalamus. In our study the increase in blood VP concentration was less pronounced than in other experiments (treatment with hypertonic solution [5] or water deprivation [6]). It was probably related to moderate osmotic stimulation with saline load. Potentiation of VP secretion was accompanied by a considerable decrease in the contents of VP mRNA in the hypothalamic gigantocellular nuclei. These changes reflect intensification of VP mRNA translation. In parallel, the content of VP hnRNA in SON also increased, which attested to enhanced transcription of the VP gene. The increase in VP hnRNA content was not statistically significant, which can be explained by insufficient sensitivity of *in situ* hybridization for evaluation of minor changes. It cannot be excluded that even a slight increase in transcription and translation of the VP gene (hnRNA and mRNA, respectively) was sufficient to stimulate secretion of this neurohormone in the blood. SON is characterized by higher reactivity than PVN and plays a major role in this process [2].

It should be emphasized that the reaction of hypothalamic vasopressinergic neurons does not depend on variations in plasma osmolarity and hemodynamic parameters [5] and reflects a new mechanism underlying the regulation of gigantocellular neurons during inflammation. Proinflammatory cytokines are locally expressed in the hypothalamus and hypophysis under the influence of LPS and stimulate the synthesis and secretion of VP [1,10]. This problem requires further investigations.

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