Activity of the Hypothalamic-Pituitary-Adrenocortical System and Sleep-Wake Cycle in Rats with Acute Systemic Inflammation

M. I. Eliava, V. V. Grinevich*,, and G. A. Oganesyan**

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Activity of the hypothalamic-pituitary-adrenocortical system and EEG characteristics of the sleep-wake cycle were studied on adult male Wistar rats with acute inflammation produced by bacterial lipopolysaccharide in a dose of 250 μg/100 g body weight. Blood concentrations of adrenocorticotropic hormone and corticosterone increased by 6 and 10 times, respectively, 30 min after lipopolysaccharide administration and peaked 2 hours after challenge. In this period the sleep-wake cycle underwent the most pronounced changes that could be attributed to the stupor-like state observed in clinical practice. It was manifested in dissociation between locomotor activity of animals and EEG characteristics, suppression of EEG components in slow-wave sleep, increase in the number of β-waves, and decrease in the number of δ-waves in EEG. In the present work we consider possible mechanisms of temporal relationships between activity of the hypothalamic-pituitary-adrenocortical system and disorganization of the sleep-wake cycle during acute systemic inflammation.

Key Words: *sleep-wake cycle; adrenocorticotropic hormone; corticosterone; inflammation*

Dynamic characteristics and parameters of EEG in the sleep-wake cycle (SWC) of mammals correlate with activity of the hypothalamic-pituitary-adrenocortical system (HPAS) [12]. Glucocorticoids, the final products of HPAS, act as universal adaptogens for adverse environmental factors. Systemic inflammation is one of these critical states. Under these conditions glucocorticoids not only possess metabolic and behavioral activity, but also produce a direct immunosuppressive effect [7]. Treatment with endotoxin lipopolysaccharide (LPS) is a widely used model of systemic inflammation in animals [3,9]. LPS initiates the immune response via specific receptors on macrophages [4]. Changes in HPAS during inflammation produced by LPS were studied previously [1,11]. However, little is known about behavioral reactions caused by endotoxin in low doses [2,5,6,10]. Parameters of EEG objectively reflect the behavioral response. We found no published data on the state of HPAS and EEG characteristics of behavior during acute systemic inflammation. Here we studied changes in HPAS activity and behavioral reactions of rats (EEG parameters) produced by bacterial endotoxin.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g and having free access to water and food. The light cycle was 12 h. The animals were divided into 2 groups. Acute systemic inflammation in animals of both groups was produced by intraperitoneal injection of LPS from *E. coli* (serotype 0111:B4, Sigma) in a dose of 250 μg/100 g body weight in 300 μl physiological saline. In group 1 rats activity of HPAS and

I. M. Sechenov Institute of Evolutional Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg; *Russian State Medical University; **Research Center for Endocrinology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** m_eliava@hotmail.com. Eliava M. I.

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blood levels of adrenocorticotropic hormone (ACTH) and corticosterone were determined. In group 2 animals characteristics of SWC were evaluated by EEG.

In group 1 rats (n=4) a catheter (Intramedic PE 50, Parippany) was implanted into the caudal artery under pentobarbital anesthesia (40 mg/kg intraperitoneally). Another catheter was implanted into the abdominal cavity. LPS was administered via the abdominal catheter 1 week after surgery. The blood (1 ml) for hormone assay was collected via a catheter implanted into the caudal artery 5 min before and 30, 90, 120, and 180 min after LPS administration. Control rats (n=4) intraperitoneally received 300 μl physiological saline. ACTH concentration was measured using RMA kits (Euro-diagnostika). Corticosterone was determined by radioimmunoassay after dichloromethane extraction of 10 μl plasma [3]. The results were statistically processed using ANOVA software.

EEG was recorded in 4 rats of group 2. The animals were narcotized with nembutal (40 mg/kg intraperitoneally), chronic electrodes were stereotactically implanted above fields 3 (sensorimotor cortex) and 17 (visual cortex) and into the dorsal hippocampus. Electromyograms (EMG) were recorded using electrodes implanted into muscles of the neck. EEG and EMG were recorded on an EEG-80 eight-channel electroencephalograph (Medikor) coupled to an IBM PC AT-286 computer. Spectral analysis of EEG with rapid Fourier transform was performed in each state of the cycle at a sample rate of 128 Hz over 8 sec. Normalized powers of EEG signals were selected in 5 frequency ranges corresponding to δ-, θ-, α-, β_1-, and β_2-rhythms. For each animal 20-80 echos were recorded in various states of SWC and averaged. Experiments were performed at 10.00-16.00 for 3 days. Control recording were conducted on days 1 and 3. On day 2 parameters before and after intraperitoneal injection of LPS were recorded. The data for various groups were analyzed and summarized by standard mathematical methods using Student's t test.

RESULTS

Blood concentrations of ACTH and corticosterone increased 30 min after LPS administration by 6 and 10 times, respectively, and reached a maximum by the 2nd hour (p<0.001). Then these parameters progressively decreased (Fig. 1), but remained above the control to the end of observations (3 h, p<0.001). The structure of SWC was disorganized over the first 2 h after LPS administration. The behavioral component of wakefulness was absent in this period. Complete dissociation between EEG and behavioral characteristics of SWC was observed. The animals were motionless and did not react to environmental factors. The rats were alarmed only by vigorous stimulation (shaking, noise, and displacement within the cage), but then returned to the former state. The rapid-wave phase was completely reduced. Slow-wave sleep (SWS) with a typical pattern of electrical activity was observed only 2 h after administration of the preparation. The animals displayed locomotor activity over the next 2-h period. The total duration of SWS surpassed that observed during the first 2-h period (31.5±4.2%), but remained below the baseline level (44.0±1.5%). The duration of phases practically did not differ from the control. However, the rapid-wave phase was significantly reduced. It was manifested in shortening of the

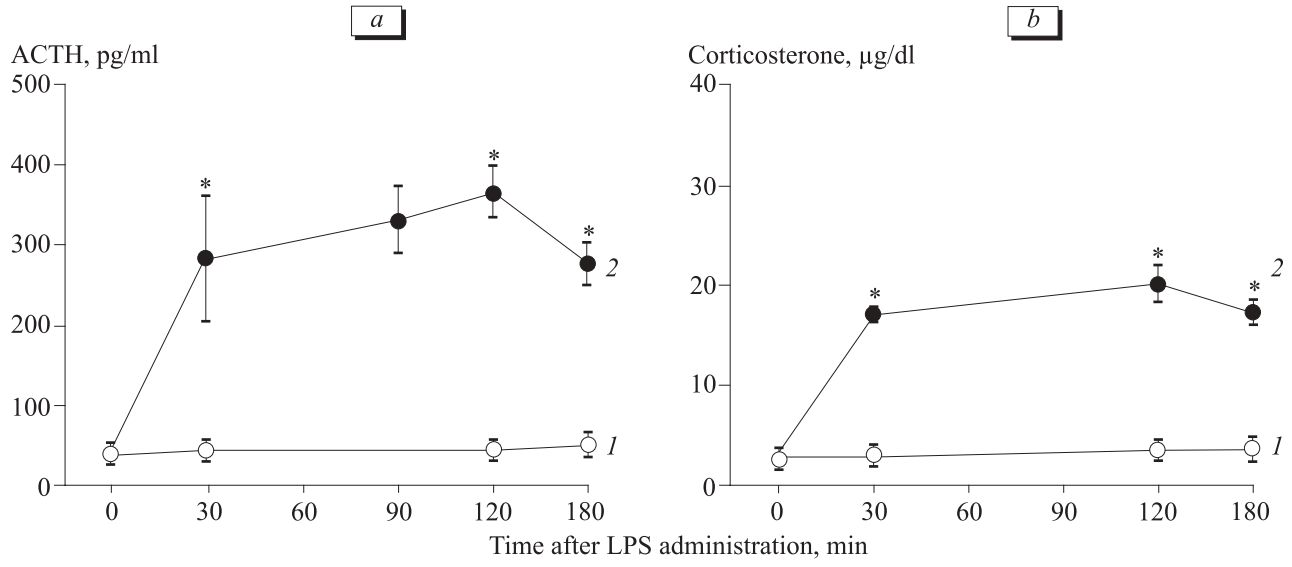

Fig. 1. Plasma levels of adrenocorticotropic hormone (*a*) and corticosterone (*b*) 30, 90, 120, and 180 min after administration of bacterial lipopolysaccharide (LPS, n=4). Control (concentration of hormones 5 min before LPS injection, *1*) and LPS (*2*). *p<0.001 compared to the control.

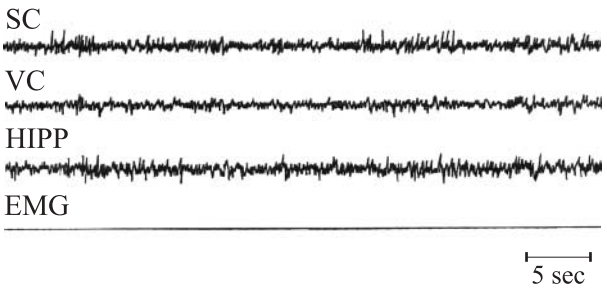

Fig. 2. EEG in rat 1 h after LPS administration. Leads: SM, sensorimotor cortex; VS, visual cortex; HIPP, hippocampus.

so-called rapid-wave phases fragmentation (0.30± 0.06 *vs*. 2.5±0.8 min in the control). Over the first 2 h after treatment EEG was presented by mixed electrical activity with predominant slow rhythms (Fig. 2). The ratio of δ-waves in SWS decreased ($p<0.05$), while the number of β_1- and β_2-waves increased compared to the control ($p<0.05$, Fig. 3). Spectral analysis showed that EEG characteristics of SWS approached the control level over the next 2 h of recording. The number of β-waves slightly increased only in the sensorimotor cortex. EEG characteristics of SWS returned to normal 4 h after LPS administration.

The effect of LPS on SWC was studied previously [2,6,10]. Here we used endotoxin in a high dose (250 μg/100 g). LPS in this concentration did not cause animal death, but produced a pronounced systemic reaction (pyloerection, diarrhea, and fewer) and activation of central and peripheral components of HPAS [3,9]. The main goal of our study was to determine parameters of SWC with respect to activity of HPAS in inflammation. The first 2-h period of SWC was of particular interest, since it coincided with significant changes in activity of HPAS and plasma levels of ACTH and corticosterone. Most authors believe that LPS markedly increases the total duration of SWS [2,6,10]. Other investigators reported that LPS suppresses slow-wave activity in EEG [5]. It should be emphasized that these experiments were performed with LPS in very low doses. Therefore, it was necessary to evaluate behavioral characteristics of rats under specified experimental conditions. EMG recording showed that the rats did not display locomotor activity, but retained

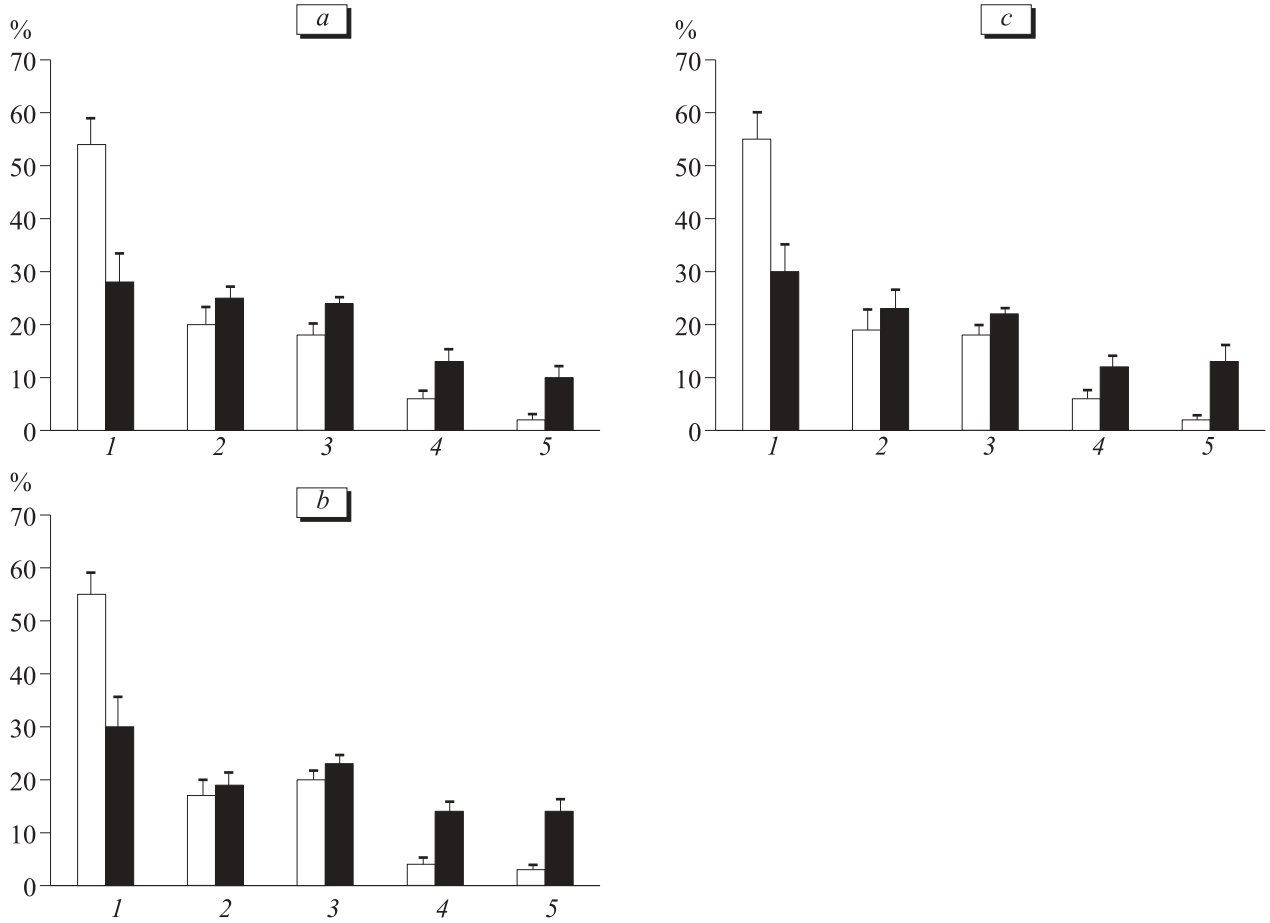

Fig. 3. Spectral characteristics of slow-wave sleep in rats under control conditions (light bars) and in the stuporous state after LPS administration (dark bars, *n*=4). Average parameters of δ- (*1*), θ- (*2*), α- (*3*), β_1- (*4*), and β_2-rhythms (*5*). Sensorimotor cortex (*a*), visual cortex (*b*), and hippocampus (*c*).

normal muscle tone over the first 2 h after treatment. Despite the predominance of slow-wave components in EEG, it was impossible to identify SWC as SWS. It should be noted that injection of LPS in a high dose was accompanied by a considerable increase in the ratio of β-waves. These changes reflect a negative correlation between 2 types of EEG activities. Probably, in animals LPS in high doses produces changes that are similar to the stuporous state in humans with severe intoxication and septic encephalopathy [8]. Fluctuations in EEG are associated with activation of brain processes. These changes are related to the influence of glucocorticoids. Recent studies showed [12] that administration of corticosterone in high doses led to similar changes in SWC [13].

These data confirm our hypothesis that corticosterone plays a major role in modulation of SWC during severe systemic inflammation. The predominance of SWS in SWC during inflammation produced by LPS in extremely low doses (~3 μg/100 g) is probably related to the absence of pronounced and long-term activation of HPAS [6]. Apart from glucocorticoids, some inflammatory factors (*e.g.*, proinflammatory cytokines) can affect SWC [1,3]. It cannot be excluded that cytokines activate the cerebral cortex via limbic structures of the brain. This problem requires further investigations. Our study revealed the temporal relationship between activation of HPAS and disorganization of SWC during acute systemic inflammation produced by endotoxin in a high dose.

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