Abstract

Inflammation which is caused by bacterial or viral infections affects the female reproductive system, causing disturbances in the hypothalamic-pituitary-gonadal (HPG) axis secretion. Bacterial endotoxin (lipopolysaccharide, LPS) is commonly used to induce immune stress in experiments. LPS is an integral part of the cell wall of Gram-negative bacteria, and acts on target cells through Toll-like receptor 4 (TLR4). The results of previous studies, demonstrating the presence of TLR4 in the hypothalamus, may indicate the involvement of endotoxin in the inhibition of reproductive process at the level of the central nervous system (CNS). The aim of this doctoral thesis was to verify the research hypothesis, that LPS administered intravenously acts directly through TLR4 receptors at the level of hypothalamus and pituitary, inhibiting the activity of the HPG axis in adult ewes. The in vitro study was performed on pituitary explants collected from 12 Blackhead ewes in follicular phase of the estrous cycle. In this experiment the effect of LPS on the luteinizing hormone (LH) secretion from the anterior pituitary explants collected from control ewes and from animals during acute inflammation induced by intravenous endotoxin injection was examined. The explants were incubated in the appropriate media (control medium; medium with gonadotropin-releasing hormone – GnRH; medium with GnRH and LPS; medium with GnRH, LPS and LPS-binding protein – LBP). After the incubation, gene expression of luteinizing hormone β subunit (LHβ), GnRH receptor (GnRH-R) and TLR4 was determined by real-time PCR method. The level of LH in collected media samples was determined by radioimmunoassay method (RIA). There was observed reduced LH release in explants from both control and LPS-treated ewes incubated in media supplemented with LPS, as well as with LPS and LBP, in comparison to explants incubated in medium with GnRH. The addition

of LPS alone (without LBP) to the media, wherein explants obtained from control animals were incubated, did not affect LHβ gene expression, hovever the addition of LBP has proved necessary to reduce the LHβ mRNA level in these explants. In explants from sheep which were injected intravenously with endotoxin, the response to LPS was independent of presence of LBP in medium. The in vivo experiment was conducted on 20 Polish Longwool ewes in the anestrous season. The animals were assigned to four experimental groups. Sheep from control group and from LPS group received intracerebroventricularly (icv) Ringer-Locke solution, ewes from anti-LPS group received icv anti-LPS antibody, while animals from anti-TLR4 group received icv antibodies binding TLR4 receptor complex (anti-LBP and anti-MD-2 antibodies) to block this receptor. Then, saline was intravenously administered to the control group, and LPS to the animals from other groups. Blood samples were collected from each ewe. The levels of LH and cortisol were determined in this samples by RIA method. Gene expression of, GnRH, GnRH-R, LHβ and TLR4 were determined by real-time PCR method in selected hypothalamic structures (the preoptic area – POA, the anterior hypothalamus – AHA, the medial basal hypothalamus – MBH, the median eminence – ME) and in the anterior pituitary (AP). Injection of LPS caused a decrease in the level of GnRH gene expression both in POA and in the ME. Central administration of anti-LPS antibody to the third ventricle, as well as blocking of TLR4 receptor components, significantly increased, reduced after endotoxin injection, the GnRH gene expression, but only in the ME. Moreover, in the AP, TLR4 blockade (in anti-TLR4 group) restored LHβ gene transcription to the control level. As far as peripheral LH concentration is concerned, this effect was not observed. To sum up, the obtained results suggest, that bacterial endotoxin may directly modulate the activity of the HPG axis in ewe at the CNS level. The effect of LPS on LH secretion from the pituitary may be dependent on the physiological state of animal / immunological status of animal. TLR4 receptor participates, at least partially, in the mechanism of inhibiting the GnRH/LH secretion during immune stress at the CNS level.