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Does central IL-1β affect GnRH secretion in the hypothalamus of anoestrous ewes via different regulatory pathways?

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ABSTRACT. The study was designed to identify the central pathways through which central interleukin-1β (IL-1β) affects gonadotropin-releasing hormone (GnRH) release in anoestrous ewes. Our results show that intracerebroventricular (icv.) injection of IL-1β (50 μg) decreases the GnRH concentration in the perfusates collected from the medial basal hypothalamus/median eminence (MBH/ME), increases its type I receptor gene expression in the preoptic area, anterior hypothalamus (AHA) and medial basal hypothalamus, and significantly decreases the neuropeptide Y (NPY) mRNA level in the AHA and MBH. No effect of IL-1β treatment was found on plasma cortisol concentration, catecholamine (norepinephrine and dopamine) levels in perfusates, as well as cyclooxygenase-1 (*COX-1*) and cyclooxygenase-2 (*COX-2*) gene expression in the hypothalamus. Our data demonstrate that central IL-1β suppresses GnRH release mainly by acting directly through its own hypothalamic receptors, and its effect could be, at least partially, caused by changes in the NPY level, which is known as an important modulator of GnRH biosynthesis and release.

Introduction

Gonadotropin-releasing hormone (GnRH) is a hypothalamic neuropeptide that plays a key role in controlling reproduction at the level of the brain. GnRH must be released in a *pulsatile manner* to ensure maintenance of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion from the pituitary. GnRH-containing neurons are distributed diffusely throughout the hypothalamus and project to the median eminence where they release GnRH from their axon terminals into the hypophysiotropic portal system (Silverman, 1994). In sheep, GnRH neurons are spread from the brain septum and the diagonal band of Broca, through the preoptic area (POA) to the anterior hypothalamus AHA) and the medial basal hypothalamus (MBH). Nonetheless, the most GnRH-immunoreactive structure in the hypothalamus is the POA, where more than 50% of GnRH neurons are located (Caldani et al., 1988). The secretory activity of GnRH neurons is regulated by multiple neural systems that involve neurotransmitters, neurohormones, and peptides. The most important are: prostaglandins, norepinephrine (NE), dopamine, γ-aminobutyric acid (GABA), β-endorphin, neuropeptide Y (NPY), and corticotropin-releasing hormone (CRH). Our previous studies have shown that peripheral LPS treatment causes suppression of the hypothalamic–pituitary–gonadal (HPG) axis of anoestrous ewes largely due to changes in GnRH biosynthesis (Herman and Tomaszewska-Zaremba, 2010).

It is well established that an immune/inflammatory challenge increases the concentration of proinflammatory cytokines, such as tumor necrosis factor (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6), in the peripheral blood (Givalois et al., 1994).

These cytokines are also present in the cerebrospinal fluid (CSF) of rats and other mammals during inflammation. It is suggested that these mediators modulate the neuroendocrine system at the level of the brain. (Watanobe and Hayakawa, 2003). Central IL-1β is considered to be an important negative regulator of HPG axis activity at the level of the hypothalamus in the rat (Kalra et al., 1990; Kang et al., 2000). The origin of the IL-1β present in the CSF seems to be varied: peripheral cytokines can cross the blood-brain barrier (BBB), another source of central IL-1β could be local synthesis in such CNS cells as microglia and astrocytes (Banks et al., 1995). The thesis about the important role of central IL-1β in the transmission of inflammatory signals into the CNS is supported by the widespread presence of IL-1R1 in the brain, where its expression has been found even in neurons (McMahan et al.,1991; Herman et al., 2010).

The presented study was designed to identify the central pathways through which intracerebroventricular (icv.) injection of IL-1β affects GnRH release.

Material and methods

Animals

The studies were performed on adult, 3-year-old Blackhead ewes in the anoestrous season (May). All animals were in good condition, with a body condition score of 3 points (on a five-point scale). The animals were maintained indoors in individual pens and exposed to natural daylight. The ewes were well adapted to the experimental conditions; they always had visual contact with their neighbors, even during the experimental period, to prevent the stress of social isolation. The animals were fed a constant diet of commercial concentrates with hay and water available *ad libitum*.

All procedures on animals were performed with the consent of the Local Ethics Committee of the Warsaw University of Life Sciences.

Surgical procedures

One month before starting the experiment, stainless steel guide cannulas (1.2 mm OD) were implanted under stereotaxic control into the third brain ventricle and into the MBH/ME region of all ewes (n = 12) through a drill hole in the skull (Traczyk and Przekop, 1963). The guide cannulas were fixed to the skull with stainless steel screws and dental cement. The external opening to the canal was closed with a stainless steel cup. The placement of the guide cannula in the MBH/ME was confirmed after slaughtering by the infusion of a small volume of blue ink.

Experiment I. The effect of icv. IL-1β injection on the release of GnRH, catecholamines, and cortisol

Venous catheters were implanted into the jugular vein on the day before the experiment. The ewes (n = 12) were randomly divided into two experimental groups: the control group (n = 6) and the IL-1β-treated group (50 μg/animal, icv., n = 6). The dose of IL-1β was chosen according to our earlier works (Herman et al., 2012). The treated animals received a single icv. injection of IL-1β (Bachem, Switzerland) dissolved in 50 μl of Ringer's solution at a dose of 50 μg per animal through stainless steel guide cannulas. Control animals received an equivalent volume of Ringer's solution. Jugular blood samples were taken for measurement of cortisol at 15-min intervals, beginning 2 h before icv. injection of IL-1β or an equivalent volume of Ringer's solution, and continuing 4 h after administration. The perfusates for assaying the GnRH and catecholoamine levels were concomitantly collected from the MBH/ME using the push-pull method (Misztal et al., 2010) at 30-min intervals beginning 2 h before and continuing 4 h after IL-1β/Ringer's solution injection. Perfusates (~ 0.3 ml) were taken using an microinjection pump (CMA/Microdialysis, Stockholm, Sweden). The samples were kept in an ice bath during sampling and stored at –80°C until GnRH and catecholamine assays were performed.

Experiment II. The effect of icv. IL-1β injection on hypothalamic gene expression

The same animals (n = 12) as in experiment I were used after 2 weeks of convalescence. The hypothalamic structures, i.e., the preoptic area (POA), anterior hypothalamus (AHA), and medial basal hypothalamus (MBH), were collected 2 h after icv. injection of IL-1β (50 μg, icv., n = 6) or an equivalent volume of Ringer's solution (icv., n = 6). The brains were rapidly removed from the animals and the hypothalamic structures were dissected. All tissues were frozen immediately after collection in liquid nitrogen and were stored at –80°C.

Assays

Radioimmunoassay for cortisol. Cortisol concentrations were determined by radioimmunoassay (RIA) according to Kokot and Stupnicki (1985), using rabbit anti-cortisol antisera (R/75) and HPLC-grade cortisol standard (SIGMA, USA). The assay sensitivity was 0.95 ng · ml^{-1} and the intra- and inter-assay coefficients of variation for cortisol were 10% and 12%, respectively.

Determination of GnRH concentration in the perfusates. The level of gonadoliberin in perfusates from the MBH/ME was determined with a commercial GnRH ELISA kit (CUSABIO BIOTECH Co.,Ltd; China). All steps in the assays were performed according to the kit manual guide. A*bsorbance* was measured at *450 nm* using a Lab System Multiscan *RC* 96-well plate *reader* (*Labsystem*; Finland). The detection limit was 1.25 pg · ml^{-1}.

Determination of catecholamines and their metabolite concentrations in perfusates. Catecholaminergic system activity was evaluated on the basis of the extracellular concentration of norepinephrine (NE), dopamine (DA), and their main metabolites: 4-hydroxy-3-methoxy-phenylglycol (MHPG), 3,4-dihydroxy-phenylacetic acid (DOPAC), and homovanilic acid (HVA). The concentrations of these compounds were analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection, as described previously (Tomaszewska-Zremba et al., 2002). The limit of detection was 4 pg · 50 µl^{-1} for NE, 5 pg · 50 µl^{-1} for DA, 3 pg · 50 µl^{-1} for MHPG, DOPAC, and HVA.

Determination of the relative gene expression level. Total RNA from the hypothalamic areas (POA, AHA, MBH) was isolated using the NucleoSpin® RNA II kit (MACHEREY-NAGEL GmbH & Co; Germany) according to the manufacturer's instructions. The amount and purity of total RNA was quantified photospectrometrically by measuring the optical density at 260 and 280 nm in a NanoDrop 1000 instrument (Thermo Fisher Scientific Inc., Waltham, USA) and RNA integrity was checked by 1% agarose gel electrophoresis. cDNA was synthesized using the components of the DyNAmo™ SYBR® Green 2-Step qRT-PCR Kit (Finnzymes; Finland) according to the manufacturer's instructions. For the PCR-assay, 900 ng total RNA were used as the starting material for cDNA preparation in a reaction volume of 20 µl.

Real-Time PCR was carried out using SYBR® Green 2-Step qRT-PCR Kit (Finnzymes; Finland) components and HPLC-grade oligonucleotide primers synthesized by Genomed (Poland). Specific primers for determining the expression of housekeeping genes and the genes of interest were designed using Primer 3 software. The primer sequences were: IL-1R1: 5'-TGGAGGCTGATAAATGTGAGG-3' (forward) and 5'-TAGATACAGGCGTCGTGCTG-3' (reverse) (GenBank accession no. NM_001206735), genera ted product size 150 bp; NPY: 5'-ATCACCAGGCAGAGATACGG-3' (forward) and 5'-TTTCATTTCCCATCACCACA-3', (reverse) (GenBank accession no. NM_001009452.1), generated product size 132-bp; cyclooxygenase (*COX*)-1: 5'-TACTATCCATGCCAGCACCA-3' (foward) and 5'-GGAGCCAGGTCCATATCTCA-3'(reverse) (GenBank accession no. BC134517.1), generated product size 123-bp; *COX-2*: 5'-TGTTGACGTCGAGATCACATT-3'(forward) and 5'-GGAGCCAGGTCCATATCTCA-3' (reverse) (GenBank accession no. AF031698.1), generated product size 124-bp; β-actin (ACTB): 5'-CTTCCTTCCTGGGCATGG-3'(forward) and 5'-GGGCAGTGATCTCTTTCTGC-3' (reverse) (GenBank accession no. U39357), generated product size 168-bp; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5'-AGAAGGCTGGGGCTCACT-3' (forward) and 5'-GGCATTGCTGACAATCTTGA-3' (reverse) (GenBank accession no. NM-001034034), generated product size 134-bp; cyclophilin C (PPIC): 5'-ACGGCCAAGGTCTTCTTTG-3'(forward) and 5'-TATCCTTTCTCTCCCGTTGC-3' (reverse) (GenBank accession no. NM-001076910), generated product size 131-bp. Each PCR reaction contained 10 µl PCR Master Mix (2 x), 7 µl RNase-free water, 2 µl primers (1 µl each, working concentration was 0.5 µM) and 1 µl cDNA template. The reactions were run on a Rotor-Gene 6000 (Qiagen, Dusseldorf, Germany) using the following protocol: 95°C for 15 min to activate Hot Star DNA polymerase, followed by 30 cycles of 94°C for 5 s for denaturation, 56°C for 20 sec for annealing, and 72°C for 15 sec for extension. After the cycles, a final melting curve analysis with continuous fluorescence measurements was performed to confirm the specificity of the amplification.

Data analysis

Catecholoamine, GnRH, and plasma cortisol concentration data analysis. The results are presented as hormone/catecholoamine concentration expressed as a mean ± SEM. All experiments consisted of a baseline period when no treatment was given (–2 to 0 h before) and period when the treatments were applied (+1 to +4 h after IL-1β or icv. Ringer's solution injection). Two-way ANOVA was used, followed the Mann-Whitney U test.

Relative gene expression data analysis. All data were analyzed using Rotor Gene 6000 v.1.7. software. Relative gene expression was determined using the Relative Expression Software Tool 2008,

first published by Pfaffl et al. (2002) and based on the PCR efficiency correction algorithm published previously by Pfaffl (2001). To compensate for variation in cDNA concentrations and PCR efficiency between tubes, an endogenous control gene was amplified in each sample and used for normalization. Initially, three housekeeping genes: *GAPDH*, *β-actin*, *PPIC* were tested. BestKeeper software was used to determine the most stable housekeeping gene for normalizing expression of genes of interest. BestKeeper is based on pair-wise correlation analysis of all pairs of candidate genes (Plaffl et al., 2004) and calculates variations of all reference genes (SD (± Ct)). The best endogenous control gene was *ACTB*, but in the hypothalamus, it was *GAPDH*. These genes had the lowest SD (± Ct) values of all the genes tested and a good correlation coefficient with the remaining analyzed housekeeping genes.

The results are presented as the relative gene expression of the target gene vs. endogenous control gene, in relative expression value as mean ± SEM. The significance of differences between the experimental groups was assessed using the Mann-Whitney U test.

Results

Effect of icv. IL-1β injection on the plasma cortisol concentration

We found no effect of IL-1β injection into the third ventricle on the plasma cortisol level (data not shown).

Effect of icv. IL-1β administration on GnRH release

The concentration of GnRH in perfusates from control animals did not differ significantly during any period of collection. In ewes treated with IL-1β, GnRH concentrations in perfusates from the MBH/ME decreased significantly ($P \leq 0.05$) compared with those in perfusates from control ewes treated with Ringer solution (Figure 1).

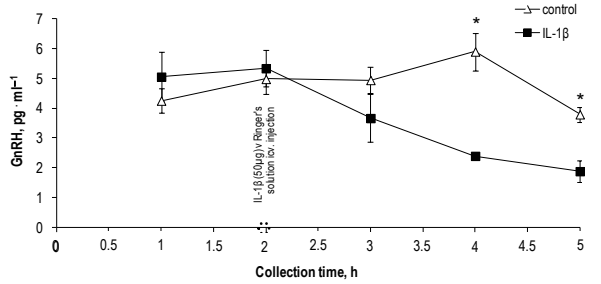

Figure 1. Concentration of GnRH (± SEM) in perfusates collected from MBH/ME of anoestrous ewes injected with Ringer's solution (triangle) and IL-1β (squares). All reagents were injected into the third ventricle. * $P \leq 0.05$

Effect of icv. IL-1β injection on *IL-1R1*, *NPY*, *COX-1*, and *COX-2* genes expression in hypothalamic structures

It was found that icv. IL-1β injection significantly increased IL-1R1 gene expression in the POA (mean exp. 1.90 ± 0.21; $P < 0.01$), AHA (mean exp. 1.73 ± 0.28 $P < 0.05$), and MBH (mean exp. 1.76 ± 0.31; $P < 0.01$) compared with the control group (mean exp.: 1 ± 0.21; 1 ± 0.17; 1 ± 0.20, respectively) but decreased the NPY mRNA level in the AHA (mean exp. 0.20 ± 0.25; $P < 0.01$) and MBH (mean exp. 0.52 ± 0.28; $P < 0.05$) compared with the control (mean exp.: 1 ± 0.20; 1 ± 0.14, respectively) while it did not affect NPY gene expression in the POA (Table 1). It must be stressed that the absolute level of NPY mRNA in the control animals was strongly differentiated among hypothalamic structures. The highest *NPY* expression was found in the MBH, and the lowest, in the POA. The NPY mRNA level was about 25-fold higher in the MBH compared with the POA, and approximately two-fold higher than determined in both the AHA and ME. There were no effects of icv. IL-1β injection on *COX-1* and *COX-2* expression in the hypothalamus (data not shown).

Table 1. The effect of icv. IL-1β injection on *IL-1R1* and *NPY* gene expression in the structures of hypothalamus in anoestrous ewes

Structure	IL-1R1 vs. GAPDH	
	control	IL-1β
POA	1 ± 0.21	1.90 ± 0.15**
AHA	1 ± 0.17	1.73 ± 0.28*
MBH	1 ± 0.20	1.76 ± 0.31*
	NPY vs. GAPDH	
	control	IL-1β
POA	1 ± 0.21	1.20 ± 0.16
AHA	1 ± 0.20	0.20 ± 0.25**
MBH	1 ± 0.14	0.52 ± 0.28*

POA – the preoptic area; AHA – the anterior hypothalamus; MBH – the medial basal hypothalamus; control – group (n = 6) injected icv. with Ringer's solution; IL-1β – group (n = 6) injected icv. with IL-1β in a dose 50 μg. Data are presented as a mean value ± SEM; * – $P \leq 0.05$ (asterisks indicate values that differ significantly from the control group). ** $P \leq 0.01$ (asterisks indicate values that differ significantly from the control group)

Effect of icv. IL-1β injection on the concentrations of catecholamines and their metabolites

In the control treatment, NE was found in the range from 46 to 204 pg · 50 μl^{-1} perfusate, with visible differences in concentrations among the ewes. The concentration of DA was below the limits of detection in both groups. MHPG, in the range of 60 to 650 pg · 50 μl^{-1}, DOPAC, in the range of 2000 to 4500 pg · 50 μl^{-1}, HVA in the range of

780 to 1440 pg · 50 μl^{-1} were detected in the control samples. IL-1β infusion had no visible effect on NA and DOPAC levels in the MBH-ME of anoestrous ewes. The central administration of IL-1β significantly reduced the extracellular MHPG and HVA concentrations in perfusates from this structure of the hypothalamus (Table 2).

Table 2. The mean (± SEM) concentration of catecholamines and their metabolites in perfusates from MBH/ME in control and IL-1β treated anoestrous ewes

Compound	Control pg/50 μl	IL-1β pg/50 μl
NE	190.17 ± 67.41	202.57 ± 63.06
MHPG	469.49 ± 64.04	155.61 ± 23.61**
DOPAC	2993.33 ± 133.23	3153.46 ± 295.39
HVA	1228.61 ± 140.21	202.60 ± 48.30**

Control – group (n = 6) injected with Ringer's solution; IL-1β group (n = 6) received the icv. IL-1β injection (50 μg); Data are presented as a mean value ± SEM; ** P < 0.01 (asterisks indicate values that differ significantly from the control group). NE – norepinephrine, MHP HG – 4-hydroxy-3-methoxy-phenylglycol 3,4, DOPAC – dihydroxyphenylacetic acid and HVA – homovanilic acid

Discussion

Our results show that icv. IL-1β injection caused a decrease of GnRH concentrations in perfusates from the MBH/ME. This phenomenon is not surprising because in our previous studies on anoestrous ewes we showed that central injection of IL-1β resulted in a decrease of *GnRH* expression in the POA and in a decrease of the GnRH concentration in the CSF (Herman et al., 2012). There is little data concerning the effect of central IL-1β on *GnRH* gene expression and release in ewes. In a study performed on rats, a significant decrease in the level of *GnRH* expression in the POA after a 4-day infusion of IL-1β into the third ventricle was found (Rivest et al., 1993). In another study, the central injection of IL-1β did not result in changes in the GnRH mRNA levels in the POA and MBH of male rats (Kang et al., 2000). The data obtained in earlier studies during analysis of *GnRH* expression in the ME of anoestrous ewes show that central injection of IL-1β dramatically decreases the amount of GnRH mRNA in this structure. The large reduction in the level of GnRH mRNA in the ME, where only GnRH nerve terminals are located, suggests that IL-1β induces changes in GnRH mRNA levels not connected directly with its gene expression. It is possible that IL-1β affects GnRH mRNA transport from pericarions to nerve terminals located in the ME (Herman and Tomaszewska, 2010).

In the present experiment, we observed that icv. injection of IL-1β caused an increase in *IL-R* gene expression in the POA, AHA, and MBH. This suggests that the ability of IL-1β to inhibit GnRH release could be an effect of its direct action in the hypothalamus IL-1 type I receptors. The presence of IL-1 receptor type I in the CNS neurons suggests possible direct regulation of the neuroendocrine system by IL-1β (French et al., 1999). Moreover, *in vitro* studies showed that the IL-1 receptor is present in the membrane of immortalized GnRH neurons, the Gnv-4 cell line, which synthesize and release gonadoliberin (Igaz et al., 2006). This strongly supports the thesis that the ligand of this receptor may directly regulate these neurons' activity and play an important role in the modulation of GnRH secretion. The results of our previous studies performed on ewes also suggest that IL-1R1 plays an important role in the transmitting of inflammatory signals from the periphery to the brain during immune/inflammatory challenges. We showed that the immune stress caused by peripheral LPS injection increased the synthesis of IL-1β in the hypothalamus, as well as the gene expression of its corresponding type I receptor (Herman et al., 2010).

The pleiotropic character of IL-1β suggests that this cytokine can promote other central processes that inhibit *GnRH* gene expression and secretion. According to literature reports, catecholamines have an important role in the regulation of GnRH secretion. In ewes during the anoestrous season, dopamine has a predominant inhibitory effect, while the role of norepinephrine has not been unequivocally defined. Based on these observations it can be assumed that IL-1β could suppress GnRH secretion indirectly, inducing changes in the level of catecholamines. One of the indirect mechanisms modulating GnRH secretion *via* central IL-1β could be NE release. This neurotransmitter is known as a stimulator of GnRH neurons; all factors decreasing NE secretion cause profound reduction of *GnRH* expression (Kang et al., 1998). Notwithstanding, the results of the present study show that infusion of IL-1β into the third ventricle did not influence catecholamine levels in the MBH/ME. Despite the changes in MHPG and HVA (metabolites of noraderenaline and dopamine) levels, it seems that in anoestrous ewes, IL-1β did not influence GnRH release through interaction with catecholamines. It is worth mentioning that conclusions about transmitter utilization based on metabolite levels should be made cautiously because transmitter and metabolite levels appear to change independently of one another (Wong et al., 1993). On the other hand, in a study performed on cyclic rats, intraperitoneal IL-1β treatment blocked the LH surge and the rise in NE release in the MPOA, but no changes were observed in dopamine release (Sirivelu et al., 2009). The dif-

ferent effects of IL-1β on NE levels determined in our study and by other research groups could be due to the animal models used in these studies. In contrast to rats, the role of NE in the regulation of GnRH/LH release in ewes in the nonbreeding season is less well known. While reports implicating the noradrenergic system in the MBH/ME of ewes in regulation of GnRH release indicate a stimulatory role of NE on GnRH secretion before a preovulatory LH surge (Clarke et al., 1999), the mode of action of NE in the control of GnRH release in this region of anoestrous ewes is not well recognized. In light of numerous results implicating NE in the control of GnRH release, the importance of the noradrenergic system in the inhibition of secretion of this hormone from the hypothalamus of anoestrous ewes is limited to the POA (Tomaszewska-Zaremba and Przekop, 2005).

The other pathway through which IL-1β may affect GnRH secretion is its influence on NPY synthesis in hypothalamus. In our experiment we demonstrated that in anoestrous ewes, IL-1β injected icv. decreased *NPY* expression in the MBH and AHA without affecting this parameter in the POA. This suggests that, at least partially, central IL-1β could modulate GnRH release through influencing the synthesis of this neuropeptide. In the hypothalamus, NPY is produced primarily in the arcuate nucleus (ARC), but there is morphological evidence indicating colocalization of NPY and GnRH neurons in the POA and ME (Li et al., 1999). It is thought that NPY participates in the regulation of the reproductive functions mainly by controlling gonadotropic hormone secretion (Kaynard et al., 1990; Mc Donald et al., 1989). The stimulatory effect of icv. NPY on LH release was demonstrated in ovariectomized (OVX) rats treated with ovarian steroids and during the preovulatory period (Kalra and Crowley, 1984). In contrast, icv. infusions of NPY in OVX rats (McDonald et al.,1989) or monkeys (Kaynard et al., 1990) resulted in suppression of the episodic release of LH, presumably through the inhibition of GnRH. Experiments on female mice with NPY agonists indicated that the mode of action of NPY on GnRH secretion depends on the receptor type (Roa and Herbison, 2012); so Y_1 receptors were responsible for the suppressive effect of NPY on GnRH neuron activity, whereas post-synaptic Y_4 receptors were responsible for stimulatory effects. The results obtained in sheep are more contradictory and depend on the experimental model. NPY suppressed LH release in both OVX and OVX estrogen-treated sheep (McShane et al., 1992), while Porter et al. (1993) failed to observe any effects of icv. NPY infusions on LH concentration and pulse parameters in cycling and OVX or OVX estrogen-treated ewes. In a study performed on ewes in two phases of the early anoestrous period, Wójcik-Gładysz et al. (2003) showed that NPY was able to affect GnRH/LH secretion in early anoestrous, but only during a short period. NPY infused into the third ventricle stimulated GnRH release from nerve terminals in the ME. Based on these results, it was proposed that the sensitivity of the GnRH/LH system to the modulatory action of NPY was decreased in females with diminished sexual activity and reduced ovarian steroid blood concentrations.

Prostaglandins (PG) seem to be other important regulators in the mechanism of suppression of GnRH/LH concentrations during immune challenge (Harris et al., 2000). *In vivo* experiments on OVX rats and *in vitro* experiments on MBH fragments showed that IL-1β suppressed LH release, but not FSH release. This LH suppression was caused by inhibition of prostaglandin E_2 (PGE_2)–mediated release of GnRH (Rettori et al., 1991). Growing evidence suggests that a number of cytokines mediate this inhibition *via* PG-dependent pathways. For example, blockade of PG synthesis by indomethacin, a COX inhibitor, can prevent the IL-1β- and TNF-α-induced inhibition of LH secretion in gonadectomized rats (Yoo et al., 1997). There are also reports about the abolition of the suppressive effect of immune stress on GnRH/LH secretion in sheep after treatment with flurbiprofen, an inhibitor of PG synthesis. These results suggest a crucial role of PGs in mediating between the immune and reproductive systems (Harris et al., 2000). In our experiment, however, we did not observe any changes in *COX-1* and *COX-2* expression in the studied hypothalamic structures after IL-1β treatment. This lack of effect on *COX-1* expression was not surprising because it is very stable and constitutive gene in most mammalian cells (Hla et al., 1986). On the other hand, the fact that IL-1β did not elevate *COX-2* expression is interesting because IL-1β is known to be an important stimulator of both *COX-2* gene expression and activity (Hla and Nielsen, 1992). Seeing that the regulation of *COX-2* expression is a key to the control of PG synthesis (Huang et al. 1998) and that icv. injection of IL-1β did not cause any changes in *COX-1* and *COX-2* expression in the hypothalamus, it can be speculated that in anoestrous ewes, IL-1β inhibits GnRH release independently from a prostaglandin-dependent pathway or, at least, it is not one of the most important pathways of the central effects of this cytokine. It should be stressed that our results confirm another work car-

ried out on sheep with a PG synthesis inhibitor, flurbiprofen, which successfully abolished fever caused by endotoxin treatment, which was evidence for inhibition of PG synthesis in the hypothalamus, but it did not abolish the inhibitory effect of inflammatory challenge on LH secretion (Breen et al., 2004). All of these observations suggest that a PG-dependent pathway is not essential in the mediation between the immune and reproductive systems at the level of the hypothalamus.

The inhibition of GnRH release during an immune/inflammatory challenge could be an effect of stress, which is known to be a potent modulator of *GnRH* expression in the hypothalamus (Łapot et al., 2007). Still, the fact that in our study we did not observe any changes in plasma cortisol concentrations after central IL-1β injection suggests that reduced GnRH release was not a result of stress axis activation. Nonetheless, in a study performed on sheep (Vellucci et al. 1995) it was shown that central injection of human recombinant IL-1β increased cortisol levels. These authors also found that the changes in the blood cortisol concentration in sheep were dose-dependent and were induced only by the highest dose of IL-1β. All together, these observations suggest that although the activation of the stress axis by IL-1β could certainly lead to GnRH suppression, its activation is not essential for transmitting the inhibitory effect of IL-1β on GnRH secretion.

Conclusions

The results of this study show that central IL-1β inhibits GnRH release mainly directly through its corresponding type I receptor. Nonetheless, the indirect action of this cytokine on GnRH secretion through other central pathways involving neurotransmitters and neuropeptides cannot be excluded. The obtained results suggest that an important role in this indirect action of IL-1β may be played by the NPY system, however, this requires future detailed research.

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