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Photoperiod-dependent effect of inflammation on nocturnal gene expression of proinflammatory cytokines and their receptors in *pars tuberalis* of ewe

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ABSTRACT. The study was designed to determine the influence of photoperiod on the nocturnal gene expression of proinflammatory cytokines such as interleukin (IL)-1β (*IL1B*), IL-6 (*IL6*), tumor necrosis factor α (*TNF*) and their receptors: IL-1 type I receptor (*IL1R1*), IL-6 receptor (*IL6R*), glycoprotein 130 (*IL6ST*), TNF type I and II receptors (*TNFRSF1A* and *TNFRSF1B*, respectively) in the *pars tuberalis* (*PT*) of ewe with the endotoxin-induced acute inflammation. The studies were performed on adult ewe (n = 24) in two photoperiods: long night (LN; 16:8; October) and short night (SN; 8:16; June). The animals from each photoperiod were divided into two groups: control (n = 6) and lipopolysaccharide (LPS)-treated (n = 6). All experiment steps were performed in the darkness. Two hours after the sunset the ewe received the intravenous injection of LPS or an appropriate volume of saline. Three hours after the injections all animals were slaughtered. It was found that melatonin concentration in control ovine serum was higher ($P < 0.05$) during LN than SN period. Endotoxin decreased ($P < 0.05$) the melatonin release only in ewe kept under SN condition. The transcripts encoding all examined proinflammatory cytokines and their receptors were expressed in the *PT*. Moreover, the *PT* collected from LPS-treated ewe during LN were characterized with higher ($P < 0.05$) expression of *IL6*, *TNF*, *IL6ST*, *TNFRSF1A* and *TNFRSF1B* genes compared with the tissues collected during SN. Similar influence of photoperiod was also observed in the case of *TNF* gene expression in the control sheep. Obtained results suggest that the *PT* may be one of the gateways for immune-endocrine interactions and these interactions may be affected by the photoperiod.

Introduction

Pituitary gland plays an important role in the maintaining homeostasis since it is involved in the regulation of numerous physiological processes including reproduction (Perez-Castro et al., 2012). The pituitary gland is functionally and anatomically connected with the hypothalamus by the median eminence (ME), therefore the pituitary gland is an intermediary organ for physiological signal exchanges between the hypothalamus and peripheral organs (Perez-Castro et al., 2012). The adenohypophysis is composed of hormone-secreting epithelial cells and divided into three discrete parts such as: *pars distalis* (*PD*), *pars intermedia* (*PI*) and *pars tuberalis* (*PT*). The *PT* develops from the lateral lobes of the Rathke's pouch (Lafarque et al., 2004; Yasuo and Korf, 2011). The *PT* plays an important role in the hypothalamic-pituitary interaction because this pituitary region surrounds the infundibular

stalk and is the exclusive pituitary structure in close anatomical contact with the medial-basal hypothalamus, ME and the third ventricle (Guerra et al., 2010). The *PT* is bathed in the cerebrospinal fluid (Guerra et al., 2010; Yasuo and Korf, 2011) and possessed by intercellular channels needed for direct communication with the subarachnoid space (Morgan and Williams, 1996; Lafarque et al., 2004; Dupré, 2011). The *PT* is composed of the follicular cells – small cells without secretory features, *PT*-specific secretory cells and *PD*-like cells (Dupré, 2011). The *PT*-specific secretory cells contain a small number of secretory granules and aggregate or scatter glycogen particles (Morgan and Williams, 1996; Yasuo and Korf, 2011). It was established that gonadotrophs from the *PT* may contain and secrete some amount of the luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Mignot and Skinner, 2005; Perez-Castro et al., 2012). Colocalization of LH and other hormones like prolactine, growth hormone (GH) and thyroid-stimulating hormone (TSH) was immunocytochemically demonstrated by Mignot and Skinner (2005). Additionally, some studies showed that the *PT* can release factors which can modulate the activity of the *PD* lactotrophs (Lafarque et al., 2004). For that reasons, it is suggested that the *PT* supports the *PD* functions (Lafarque et al., 2004). However, some studies on melatonin receptors in the pituitary gland showed that melatonin-binding sites of high density are present in the *PT*, whereas they are absent in the *PD* (Morgan and Williams, 1996). Therefore, it is suggested that the *PT* may mediate the melatonin effect on the neuroendocrine function and may be involved in the photoperiodic regulation of pituitary hormones secretion (Morgan and Williams, 1996; Lafarque et al., 2004). Photoperiod influences the reproductive function of seasonal breeders including sheep, however the exact mechanism of which photoperiod affects reproductive system is not completely understood (Goodman et al., 2010). Moreover, both seasonal (Vázquez et al., 2007) and diurnal rhythms (Mattern et al., 1993) occur in the gonadotropins secretion even in non-seasonal breeders.

Many studies showed that both acute and prolonged inflammation affect endocrine system functioning in numerous animal species, including sheep (Herman and Tomaszewska-Zaremba, 2010; Fergani et al., 2012; Danek and Żurek, 2014; Herman et al., 2014a). Circulating inflammatory mediators acting both at the hypothalamic (Herman et al., 2014b) and pituitary level (Tsagarakis et al., 1998) play an important role in the induction of endocrine disorders during inflammation. Due to rich vascularization of the *PT,* the cells located in this pituitary region are an easy target for blood born inflammatory mediators such as interleukin-1 (IL-1β, *IL1B* for gene), interleukin-6 (IL-6, *IL6* for gene) and tumor necrosis factor (TNF, *TNF* for gene) α which may affect the activity of cells located in the *PT* acting *via* their corresponding receptors. This local action of the inflammatory mediators in the *PT* could have a profound effect on the secretory activity of the whole pituitary gland. It is worth mentioning that photoperiod influences immune system affecting the synthesis of proinflammatory cytokines (Haldar and Ahmad, 2010). It was described that there is diurnal fluctuation in both pro- and anti-inflammatory cytokines synthesis (Lange et al., 2010).

The aim of the study was to determine the effect of acute inflammation induced by intravenous lipopolysaccharide (LPS) injection on the nocturnal mRNA expression of proinflammatory cytokines and their corresponding receptors in the *PT* of ewe under different photoperiodic condition.

Material and methods

Animals and experimental design

The experiments were carried out on Blackhead ewe (n = 24) during long night (16:8, October; n = 12) and short night (8:16, June; n = 12) periods. The animals were maintained indoors in individual pens and were exposed to natural daylight present at 52°N latitude and 21°E longitude. The ewe were maintained in good conditions, i.e. their body condition was estimated at 3 in a five-point scale (Russel, 1991) and they were adapted to the experimental conditions for one month. The ewe had constant visual contact with each other in order to avoid isolation stress. The animals were fed constant diet of commercial concentrates with hay and water available *ad libitum*, according to the recommendations proposed by the National Research Institute of Animal Production for adult ewe (IZ PIB-INRA, 2009).

The study included two analogical experiments. The animals (n = 12) in each photoperiod were divided into two subgroups: control (n = 6) and LPS-treated (n = 6). Two hours after the sunset an appropriate volume of LPS from *Escherichia coli* 055:B5 (400 ng · kg^{-1}) (Sigma-Aldrich, St Louis, MO, USA) dissolved in saline (0.9% w/v NaCl; Baxter, Deerfield, IL, USA) was injected intravenously (i.v.) into the jugular vein. The maximum volume of injected LPS solution (10 mg · l^{-1}) has never exceeded 2.5 ml. The control group received the same volume of NaCl (based on their body weight). The efficiency of the LPS treatment to induce an

inflammatory response in the animal was estimated basing on the measurement of the body temperature. All procedures were conducted in the darkness with the use of red light. Three hours after the LPS or saline injection blood samples were collected by intrajugular catheter and then all animals were euthanized by decapitation. The brain was immediately removed from the skulls, and the *pars tuberalis* (*PT*) was dissected, immediately frozen in liquid nitrogen and stored at –80 °C until further assay.

All procedures were performed in agreement with the Local Ethics Committee of Warsaw University of Life Sciences - SGGW.

Assays

Radioimmunoassay for melatonin. Melatonin concentration in plasma was analysed by radioimmunoassay double-antibody method according to the method of Fraser et al. (1983), and modified in our laboratory, using anti-ovine melatonin serum (AB/S/01, Stockgrand Ltd., Surrey, UK). Synthetic melatonin as a standard (Sigma-Aldrich, St. Louis, MO, USA), and [O-methyl-3H]-melatonin (Amersham PLC, Amersham, UK) as a tracer, were used. The sensitivity of the assay was 16.8 ± 8.0 pg · ml^{-1} and the intra- and interassay coefficients of variation were 10.5 and 13.2%, respectively.

Determination of the relative gene expression. The NucleoSpin® RNA kit (MACHEREY-NAGEL GmbH and Co, Düren, Germany) was used to isolate the total RNA from the *PT* fragments.All isolation steps were conducted in accordance with the manufacturer instruction. The purity and concentration of the isolated RNA was quantified spectrophotometrically with the use of NanoDrop 1000 instrument (Thermo Fisher Scientific Inc.; Waltham, MA, USA). The integrity of isolated RNA was confirmed by electrophoresis with the use of 1% agarose gel stained with ethidium bromide. The Maxima™ First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific Inc.; Waltham, MA, USA) was used to perform cDNA synthesis. As a starting material for cDNA reversed transcription reaction (RT) synthesis 2 µg of total RNA was used. Real-Time RT-PCR was carried out with the use of the HOT FIREPol EvaGreen® qPCR Mix Plus (Solis BioDyne; Tartu, Estonia) and HPLC-grade oligonucleotide primers (Genomed; Warszawa, Poland). The primer sequences were designed using Primer 3 software (Herman et al., 2014b) (Table 1).

Table 1. All genes analysed by real-time PCR are listed with their full names and abbreviations

GenBank Acc. No.	Gene	Amplicon size, bp	Forward/ reverse	Sequence 5'→ 3'
NM_001034034	*GAPDH* glyceraldehyde - 3 - phosphate dehydrogenase	134	forward	AGAAGGCTGGGGCTCACT
			reverse	GGCATTGCTGACAATCTTGA
U39357	*ACTB* beta actin	168	forward	CTTCCTTCCTGGGCATGG
			reverse	GGGCAGTGATCTCTTTCTGC
NM_001076910	*PPIC* cyclophilin C	145	forward	TGGCACTGGTGGTATAAGCA
			reverse	GGGCTTGGTCAAGGTGATAA
X54796.1	*IL1B* interleukin 1 beta	137	forward	CAGCCGTGCAGTCAGTAAAA
			reverse	GAAGCTCATGCAGAACACCA
NM_001206735.1	*IL1R1* interleukin 1 receptor, type I	124	forward	GGGAAGGGTCCACCTGTAAC
			reverse	ACAATGCTTTCCCCAACGTA
NM_001009392.1	*IL6* interleukin 6	165	forward	GTTCAATCAGGCGATTTGCT
			reverse	CCTGCGATCTTTTCCTTCAG
NM_001110785	*IL6R* interleukin 6 receptor	149	forward	TCAGCGACTCCGGAAACTAT
			reverse	CCGAGGACTCCACTCACAAT
XM_004016974	*IL6ST* glycoprotein 130	139	forward	GGCTTGCCTCCTGAAAAACC
			reverse	ACTTCTCTGTTGCCCACTCAG
NM_001024860	*TNF* tumor necrosis factor	153	forward	CAAATAACAAGCCGGTAGCC
			reverse	AGATGAGGTAAAGCCCGTCA
NM_174674	*TNFRSF1A* tumor necrosis factor receptor, type 1	137	forward	AGGTGCCGGGATGAAATGTT
			reverse	CAGAGGCTGCAGTTCAGACA
NM_001040490	*TNFRSF1B* tumor necrosis factor receptor, type 2	122	forward	ACCTTCTTCCTCCTCCCAAA
			reverse	AGAAGCAGACCCAATGCTGT

One reaction mixture of total volume amounting 20 µl contained: 4 µl of PCR Master Mix (5×), 14 µl of RNase-free water, 1 µl of primers (0.5 µl each primer, 0.25 µM working concentration) and 1 µl of the cDNA template. The reactions were conducted on Rotor-Gene 6000 instrument (Qiagen; Dusseldorf, Germany) with the following protocol: 95 °C for 15 min and 30 cycles of 95 °C for 10 s for denaturation, 60 °C for 20 s for annealing and 72 °C for 10 s for extension. The specificity of the amplification was confirmed by a final melting curve analysis. The relative gene expression was calculated using the comparative quantification option (Rasmussen, 2001) of the Rotor Gene 6000 software 1.7. (Qiagen; Dusseldorf, Germany). Three housekeeping genes were examined: glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β-actin (*ACTB*) and cyclophilin C (*PPIC*). *GAPDH* were chosen for further analysis based on the result analysis performed on BestKeeper software (Pfaffl et al., 2004). The average relative quantity of gene expression in the control group of the *PT* collected during SN was set to 1.0.

Statistical analysis

The statistical analysis was performed using the STATISTICA 10 software, 2010 (Stat Soft. Inc.; Tulsa, OK, USA). The results of gene expression were analysed using two-way analyses of variances (ANOVA) to identify significant influence of two parameters (photoperiod and LPS-treatment) and followed by a post-hoc Tukey's test. The results are presented as mean ± SEM. Statistical significance was set at $P < 0.05$.

Results

Nocturnal melatonin concentration in control ovine serum was higher ($P < 0.05$) during long night (LN) than short night (SN) period. Endotoxin injection decreased ($P < 0.05$) the melatonin concentration only in ewe kept under SN condition (Figure 1).

Intravenous injections of endotoxin increased ($P < 0.05$) the relative gene expression of *IL1B* in the *pars tuberalis (PT)* collected during SN (9-fold) and LN (16-fold) when compared with this gene expression in the corresponding control groups (Figure 2A). lipopolysaccharide (LPS) did not affect the interleukin-1 receptor (IL-1R, *IL1R* for gene) of mRNA expression in the *PT* (Figure 2B). Moreover, no photoperiod effect on the expression of both these genes was found.

Endotoxin injection elevated ($P < 0.05$) gene expression of *IL6* during SN (170-fold,) and LN (496-fold) compared with the proper control group (Figure 3A). It was found that LPS more strongly

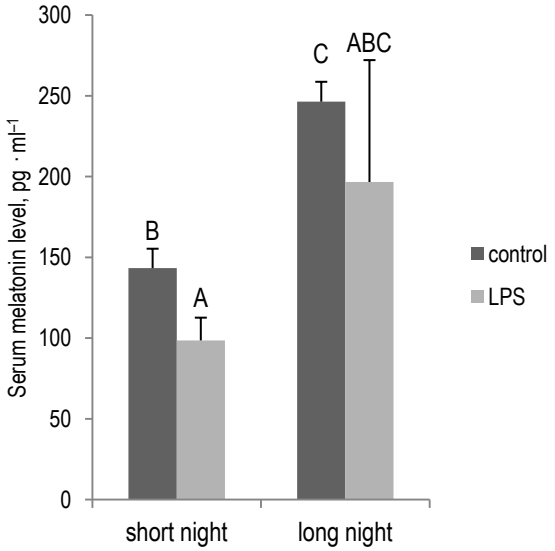

Figure 1. Effects of photoperiod and lipopolysaccharide (LPS) treatment on melatonin concentration in ovine serum collected from control and LPS-treated ewe. Data is presented as mean value ± SEM; ^{ABC} – bars with different letters differ significantly according to Tuckey's post-hoc test ($P < 0.05$)

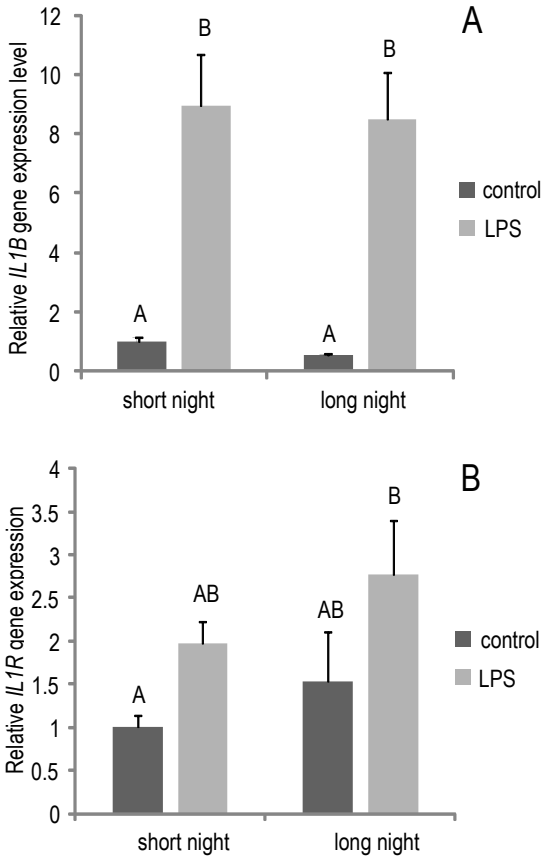

Figure 2. Effects of photoperiod and lipopolysaccharide (LPS) treatment on relative interleukin-1β (*IL1B*; (A) and its receptor (*IL1R*; (B) mRNA levels in *pars tuberalis* (*PT*), normalized to endogenous control glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Data is presented as mean value ± SEM; ^{AB} – bars with different letters differ significantly according to Tuckey's post-hoc test ($P < 0.05$)

($P < 0.05$) stimulated *IL6* gene expression during LN than SN period. Administration of endotoxin did not influence the gene expression of interleukin-6 receptor (IL-6R, *IL6R* for gene) regardless the photoperiod (Figure 3B) but elevated ($P < 0.05$) the level of glycoprotein (gp) 130 (*IL6ST* for gene) mRNA in the *PT* during LN (2-fold) compared with the control group (Figure 3C). It is worth noting that the influence of LPS on the level of *IL6* and *IL6ST* mRNA in the *PT* was stronger ($P < 0.05$) during LN than SN.

Endotoxin injection did not affect *TNF* gene expression but the level of *TNF* transcript determined

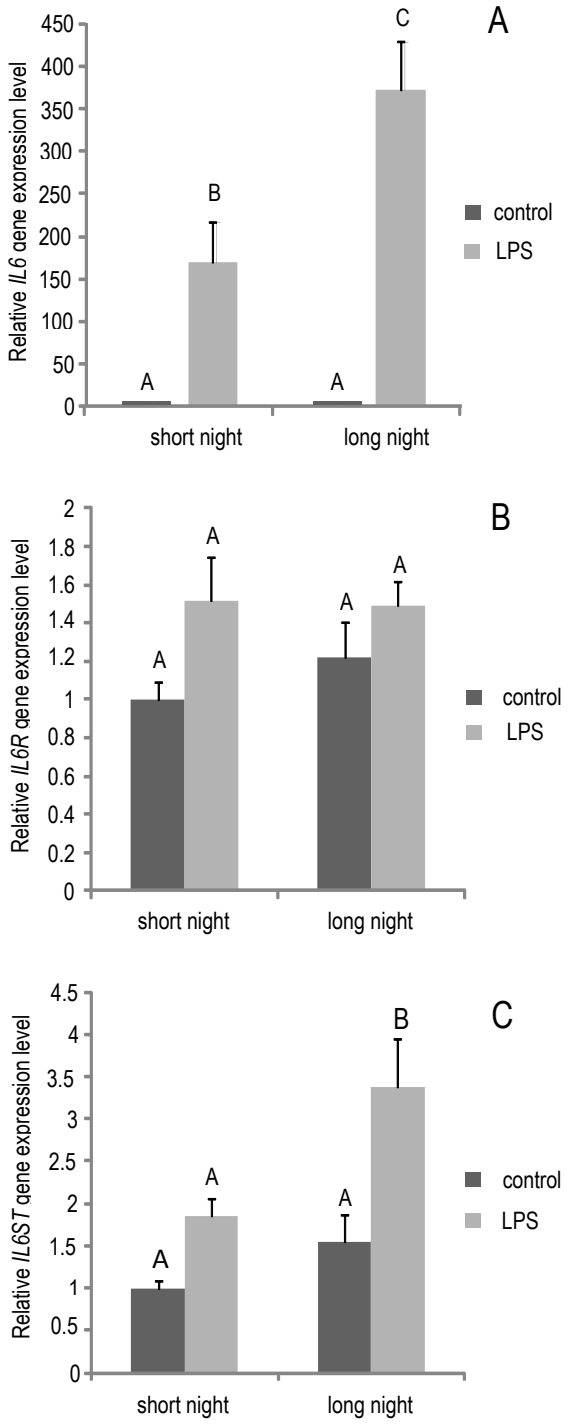

Figure 3. Effects of photoperiod and lipopolysaccharide (LPS) treatment on relative interleukin 6 (*IL6*; (A)) and its receptors: *IL6R* (B) and *IL6ST* (C) mRNA levels in *pars tuberalis* (*PT*), normalized to endogenous control glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Data is presented as mean value ± SEM; ^{ABC} – bars with different letters differ significantly according to Tuckey's post-hoc test ($P < 0.05$)

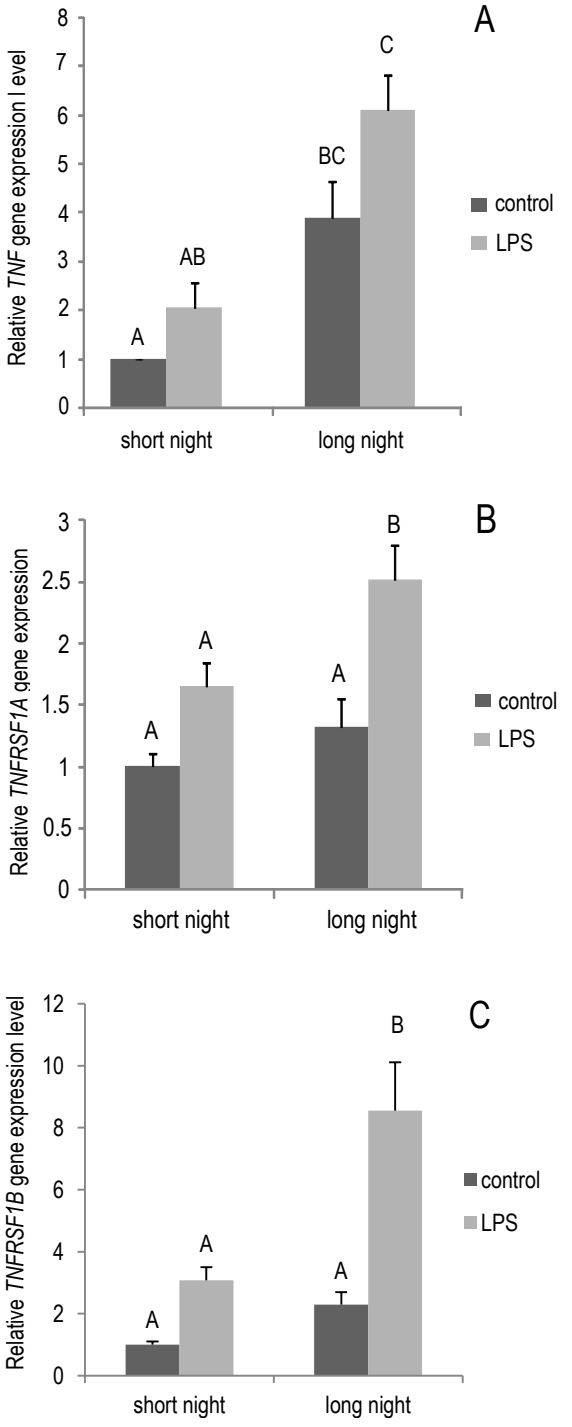

Figure 4. Effects of photoperiod and lipopolysaccharide (LPS) treatment on relative tumour necrosis factor α (*TNF*; (A)) and its receptors: *TNFRSF1A* (B) and *TNFRSF1B* (C) mRNA levels in *pars tuberalis* (*PT*), normalized to endogenous control glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data is presented as mean value ± SEM; ^{ABC} – bars with different letters differ significantly according to Tuckey's post-hoc test ($P < 0.05$)

in control group in LN period was higher ($P < 0.05$) when compared to the control group from SN photoperiod (Figure 4A). No LPS-induced changes in the gene expression of both tumour necrosis factor receptor (TNFR) type 1 (*TNFSF1A* for gene) and type 2 (*TNFSF1B* for gene) were observed during SN (Figures 4B and C). However, endotoxin stimulated ($P < 0.05$) both *TNFRSF1A* (2-fold) and *TNFRSF1B* (4-fold) mRNA expression during LN period in comparison with the corresponding control group.

Discussion

Our study showed that cells located in the *pars tuberalis (PT)* expressed mRNA encoding receptors for proinflammatory cytokines such as IL-1β, IL-6 and TNFα. As the existence of cytokine receptors on the membrane is essential for the transduction of the cytokine signal into the cell, obtained data suggests that proinflammatory cytokines may influence the *PT* cells activity. It is worth mentioning that this is the first scientific report describing the occurrence of transcript encoding these interleukins and their corresponding receptors in the *PT*. However, based on Real-Time PCR assays it is impossible to judge in which type of the *PT* cells those mRNAs were expressed. The expression of IL-1R in the mouse pituitary gland had been previously shown by Takao et al. (1993) who found that LPS treatment enhanced IL-1β synthesis but down-regulated IL-1R expression. Although the IL-6 signalling pathway includes IL-6R and signal transducer gp130, only *IL6R* transcript was detected in the rat anterior pituitary (Tsagarakis et al., 1998). In our study, LPS enhanced *IL6ST* gene expression during LN but did not affect *IL6R* mRNA level. Our results are partially different from the studies performed on rats. It was demonstrated that LPS injection increased both *IL6ST* and *IL6R* transcription in the rat pituitary (Vallières and Rivest, 1997). On the other hand, the *in vitro* study on the human hepatocytes performed by Bauer et al. (1989) showed that LPS down-regulates *IL6R* mRNA synthesis in these cells. Probably, the way in which LPS regulates *IL6R* transcription may be dependent upon the kind of cells expressing this receptor as well as used animal model species. Our study showed that inflammation induced by bacterial endotoxin stimulated the TNF receptors gene expression in the *PT* only during LN period. It is the first scientific report showing the stimulatory influence of LPS on the TNF receptors mRNA expression in the pituitary tissue. However, increased expression of *TNFRSF1A* and *TNFRSF1B* may not result from direct effect of LPS on the *PT* cells but may be induced by the ligand of these receptors, which circulating concentration is significantly increased during LPS-induced inflammation (Danek and Żurek, 2014). It was showed that TNFα stimulated *TNFRSF1B* mRNA and its protein expression in human pancreatic cancer cells (Kalthoff et al., 1993). We also found that the basal gene expression of proinflammatory cytokines receptors in the *PT* was similar during LN and SN. The fact that LPS promotes both TNF receptors gene expression and *IL6ST* gene expression in LN but not in SN period may result from the different level of melatonin during these two photoperiods, because the *PT* is exclusive pituitary structure expressing melatonin binding sites (Cogé et al., 2009).

It is well known that immune function, like other common physiological and behavioural process, undergoes daily variation. Enhanced immune functions are generally observed in short days (Haldar and Ahmad, 2010). All laboratory studies of photoperiodic changes in immune parameters of mammals demonstrated enhanced immune function in short winter-like photoperiod, for example in mice significantly increased lymphocyte, neutrophil and white blood cell counts occur in short photoperiods (Blom et al., 1994). Moreover, the increased activity of lymphoproliferative immune cells and changes in spleen morphology occur in hamsters under short day (Nelson, 2004). Photoperiodic information from the environment is conveyed to the organism by a circadian rhythm of melatonin production by the pineal gland. The circulating level of melatonin depends on both daytime and season. Our studies showed that circulating level of melatonin is higher during LN than SN. Such result is fully consistent with previousely published data, which also showed higher secretion of melatonin in sheep during winter months when night period is longer (Todini et al., 2011).

Because time of melatonin secretion is longer during LN, its proinflammatory action can be stronger than during SN period (Mauriz et al., 2013). It is well established that melatonin takes part in immune response stimulation or regulation of immunodeficiencies secondary to acute stress and viral diseases (Esquifino et al., 2004). Melatonin was also identified as stimulator of the inflammation by activating monocytes and enhancing production of IL-1, IL-6 and TNFα (Haldar and Ahmad, 2010). It is generally accepted that immune function is enhanced in LN period (Haldar and Ahmad, 2010; Weil et al., 2014). However, the role of melatonin in the regulation of the course of the inflammatory response may be more ambiguous. On the one hand melatonin might promote early phases of inflammation, but on the other hand contribute to its attenuation in order to avoid

complications of chronic inflammation (Herman et al., 2015). It was also found that melatonin administration normalize *IL1*, *IL6* and *TNF* mRNA levels during cardiac inflammation which supports the thesis about dual role of melatonin in modulation of the inflammation (Mauriz et al., 2013).

Although, we did not state the influence of LPS on the expression of mRNA encoding *IL1R1* and *IL6R* in the *PT*, it does not preclude that these receptors may be involved in the immune-endocrine interaction. Probably relatively high basal expression of mRNA for these receptors in the *PT* caused that endotoxin did not affect these genes expression, but the expression of these receptor is enough to enable the transmission of inflammatory signals carried out by their ligands during an LPS-induced immune/inflammatory challenge. Our study not only suggests that proinflammatory cytokines such as IL-1β, IL-6 and TNFα may act on the *PT* cells due to the existence of their corresponding receptors but also that these molecules are locally synthesized in this pituitary structure because significant gene expression of *IL1B*, *IL6* and *TNF* was found in the *PT*. It is worth mentioning that the expression of mRNAs encoding these cytokines in the *PT* has not been described yet. Although obtained data concern only transcription of the genes encoding proinflammatory cytokines in the *PT*, taking into account that cytokines are not accumulated in the cell but they are continuously released during stimulation (Zagury et al., 2001), it is assumed that the changes in the cytokines expression are generally parallel to their gene transcription. The synthesis of proinflammatory cytokines mRNA have been previously found in other parts of the pituitary gland. In rat IL-1 mRNA was detected in pituicytes and thyrotrops (Koenig et al., 1990), while in the human expression of this mRNA was noted only in pituitary tumor cells (Tsagarakis et al., 1998). Additionaly studies carried out on rats and mice anterior pituitary showed that IL-1, IL-6 and TNFα are secreted by folliculo-stellate cells (Jovanović et al., 2014), and the presence of stellate cells is necessary for IL-6 secretion *in vitro* (Vankelecom et al., 1989). Expression of proinflammatory cytokines can be modulated by numerous factors including LPS or other cytokines (Gabay and Kushner, 1999). It is well established that IL-6 synthesis is induced by IL-1, as well as LPS, both *in vivo* and *in vitro* (Muramami et al., 1993). Moreover, it was proved that IL-6 secretion is inhibited by glucocorticoids (Tsagarakis et al., 1998). TNFα is a main stimulator of IL-1, which in turn can regulate its own receptor (Gabay and Kushner, 1999). Our study showed that endotoxin-induced expression of *IL6* and *TNF* gene in the ovine *PT* was higher during LN than SN period. This difference may also result from the stimulatory effect of melatonin on the proinflammatory cytokines synthesis.

The results of the present study suggest that the immune-endocrine interaction occurs at the level of the *PT*. The local synthesis of proinflammatory cytokines and expression of their corresponding receptors in the *PT* may have a profound effect on the pituitary hormones secretion because the changes occurring in the *PT* may influence the secretory activity of the *PD* cells (Lafarque et al., 2004). However, there is no data concerning the influence of proinflammatory mediators on the hormone secretion from the *PT*, these cytokines may affect the pituitary hormones release from the anterior pituitary cells (Arzt et al., 1998). It was also found that IL-1β increased the adrenocorticotropic hormone, LH, GH and TSH secretion in primary cultures of rat pituitary cells, while IL-6 stimulated prolactin, GH, FSH and LH release from rat pituicytes culture (Tsagarakis et al., 1998). On the other hand, the *ex vivo* study performed on the sheep pituitary explants showed that IL-1β suppressed LH secretion (Herman et al., 2013).

Conclusions

The study showed that mRNAs for proinflammatory cytokines and their receptors are expressed in the *pars tuberalis* (*PT*). Photoperiod has particularly influenced the response of proinflammatory cytokines on lipopolysaccharide treatment, however minor influence was observed on basal gene expression of proinflammatory cytokines in *PT*. Obtained results indicate that the *PT* may be one of the gateways for immune-endocrine interactions. The fact that the photoperiod influences to some extend the nocturnal synthesis of proinflammatory cytokines and their corresponding receptors in the *PT* during inflammatory condition suggests that these interactions could be influenced by the circulating melatonin.

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