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Effect of short-term and prolonged stress on the biosynthesis of gonadotropin-releasing hormone (GnRH) and GnRH receptor (GnRHR) in the hypothalamus and GnRHR in the pituitary of ewes during various physiological states

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ABSTRACT

Using an ELISA assay, the levels of GnRH and GnRHR were analysed in the preoptic area (POA), anterior (AH) and ventromedial hypothalamus (VM), stalk/median eminence (SME); and GnRHR in the anterior pituitary gland (AP) of non-breeding and breeding sheep subjected to short-term or prolonged stress. The ELISA study was supplemented with an analysis of plasma LH concentration. Short-term footshock stimulation significantly increased GnRH levels in hypothalamus in both seasons. Prolonged stress elevated or decreased GnRH concentrations in the POA and the VM, respectively during *anoestrus*, and lowered GnRH amount in the POA-hypothalamus of follicular-phase sheep. An up-regulation of GnRHR levels was noted in both, anoestrous and follicular-phase animals. In the non-breeding period, a prolonged stress procedure increased GnRHR biosynthesis in the VM and decreased it in the SME and AP, while in the breeding time the quantities of GnRHR were significantly lower in the whole hypothalamus. In follicular-phase ewes the fluctuations of GnRH and GnRHR levels under short-term and prolonged stress were reflected in the changes of LH secretion, suggesting the existence of a direct relationship between GnRH and GnRH-R biosynthesis and GnRH/LH release in this period.

The study showed that stress was capable of modulating the biosynthesis of GnRH and GnRHR; the pattern of changes was dependent upon the animal's physiological state and on the time course of stressor application. The obtained results indicate that the disturbances of gonadotropin secretion under stress conditions in sheep may be due to a dysfunction of GnRH and GnRHR biosynthetic pathways.

1. Introduction

It is generally accepted that prolonged or chronic stress has a profound inhibitory effect on reproductive function through the suppression of GnRH/LH secretion

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(Karsch and Battaglia, 2002; Breen and Karsch, 2006; Dobson et al., 2012). However, the immediate response of GnRH neurons to acute stress depends primarily on the central neural mechanism(s) (Rivier and Rivest, 1991), and at least in some circumstances may be stimulatory (Tomaszewska and Przekop, 1999; Tanebe et al., 2000; Łapot et al., 2007). Prolonged stress additionally modulates GnRH secretion with the participation of hormones, neurotransmitters and neuropeptides activated during the time-course of stressful stimuli. All of these compounds released from the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes act mainly within the hypothalamus and the anterior pituitary gland to mediate the generally suppressive influence of stress on gonadotropin secretion (Tanebe et al., 2000; Dobson et al., 2003; Maeda and Tsukamura, 2006).

In contrast to the fairly well-known neuroendocrine mechanisms of GnRH release under stress conditions, the molecular processes governing the secretion of GnRH/gonadotropins still remain poorly understood. Currently available data on rats and ewes have shown that the expression of GnRH mRNA and GnRHR mRNA under stress conditions, similarly as gonadotropin secretion, is highly dependent on the kind of stress, its duration, the physiological state of the animal and its sex (Tanebe et al., 2000; Ciechanowska et al., 2007; Herman et al., 2012). It is likely that various stressors affect the transcriptional activity of both genes by modulating the steroid-receptive hypothalamic inputs onto GnRH cells in different physiological states. Indeed, it was earlier suggested that in rats (Kang et al., 1995; Leonhardt et al., 1995; Roth et al., 1997; Seong et al., 1998) and sheep (Ciechanowska et al., 2010) some hypothalamic neurotransmitters which participate in the oestrogen-mediated control of GnRH release may also be involved in the regulation of GnRH and GnRHR transcriptional activities. However in ewes, as a seasonally breeding species, the pattern of these actions differs between the anoestrous and oestrous periods (Pompolo et al., 2003; Sergeeva and Jansen, 2009; Ciechanowska et al., 2010; Clarke and Smith, 2010).

Despite a number of studies on GnRH/LH release in animals under the application of various types of stressors, there is no coherent evidence as to how stress affects the molecular mechanisms related to the biosynthesis of GnRH and GnRHR, especially when considering the relationships between the transcription and translation processes. To clarify the mRNA–protein correlation, in the present study we analysed the influence of short-term and prolonged stress on the levels of post-translational products of genes encoding the GnRH ligand and GnRH receptor. It seemed that a more precise definition of a complex interplay between the molecular and cellular processes governing GnRH/LH secretion under stress conditions may contribute significantly to a better understanding of the mechanisms underlying the stress-induced reproductive dysfunction. Thus, these studies aimed at expanding an important medical issue, in both veterinary and human medicine, especially in cognitive appraisals of causes of female infertility and gonadotropin secretion disorders.

2. Material and methods

2.1. Animals

The studies were performed on eighteen 3–4-year-old Polish Merino ewes during the middle of the non-breeding season (April–May) or during the middle of the breeding time (October–November). The sheep were kept indoors in individual pens and exposed to natural light. Food and water were available *ad libitum*. The sheep were well adapted to the experimental conditions; they always had visual contact with their neighbours, even during the process of blood collection, in order to prevent stress associated with social isolation. The oestrous cycle in the ewes was tested by running them with a vasectomised ram twice daily; only ewes that showed two consecutive normal oestrous cycles were chosen for the subsequent experiments. The day of onset of *oestrus* is referred to as day 0.

2.2. Stress procedure

For anoestrous and for follicular-phase ewes there were three groups (control, short-term and prolonged stress) with 6 animals per group. A state of stress was induced by applying the repetitive bursts of a 3 mA alternating current of 0.5 s on and 1 s off, arranged in a series of ten, during a 20-min period in every hour. These pulses were delivered to the electrodes on the feet of the animals at the level of the metacarpus in the programmed schedule: 3 h (from 08.00 to 11.00) during one day (the sixteenth day in the case of the oestrous cycle) for the ewes subjected to short-term stress and 5 h daily (from 08.00 to 13.00) over four days (from the 13th to 16th day of the oestrous cycle) in case of the animals subjected to prolonged stress. This procedure was described in detail by Przekop et al. (1985).

To determine the LH concentration and its secretion profile, a series of blood samples were collected *via* indwelling jugular catheters at 10-min intervals for five hours. In the anoestrous animals, blood collection was performed: for the first time on day prior to the stress procedure and for the second time, on the day of the last stimulation. In the follicular-phase ewes, the blood was collected: first on the 16th day of the oestrous cycle prior to the stress procedure and the second time on the 16th day of the next oestrous cycle during the last day of stimulation. Thus, each ewe served as its own control for LH analysis. Plasma samples were stored at −20 °C until assayed.

Immediately after the last blood collection the ewes were euthanised and tissues were obtained from the POA-hypothalamus for the analysis of GnRH and GnRHR biosynthesis. Non-stressed (intact) ewes served as controls for brain structure assay. After each ewe had been euthanised its brain was rapidly removed from the skull and the stalk/median eminence (SME) was isolated. Hypothalamic blocks were sectioned sagittally and dissected from both sides into three parts (*i.e.* preoptic area – POA, anterior hypothalamus – AH, ventromedial hypothalamus – VM) according to the stereotaxic atlas of the ovine