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Prolactin role in the bovine uterus during adenomyosis

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ABSTRACT

Adenomyosis is uterine dysfunction defined as the presence of endometrial glands within the myometrium. It is suggested that adenomyosis is estrogen-dependent pathology, and prolactin (PRL) also affects its development. In the uterus of ruminants, PRL stimulates gland proliferation and function. We hypothesized that in the bovine uterus, the expression of PRL and its receptors (PRLRs) during adenomyosis is disturbed and modulated by estradiol (E_2). Uterine tissues were collected postmortem from cows; epithelial, stromal, and myometrial cells were isolated; and cultured and treated with E_2. Material was divided into 2 groups: control (nonadenomyotic) and uteri with adenomyosis. In adenomyotic uterine tissue, PRL and its long-form receptor protein were increased, as determined by Western blotting. Immunohistostaining showed that during adenomyosis, PRL and its receptors are highly expressed in adenomyotic lesions. In cultured myometrial cells, protein expression of PRL and its receptors was increased during adenomyosis. Estradiol decreased PRLRs protein expression in nonadenomyotic stromal cells and in adenomyotic myometrial cells, and increased PRL secretion by adenomyotic myometrial cells. Moreover, PRL secretion was increased in untreated epithelial and stromal cells during adenomyosis. On the other hand, in stromal cells, PRLRs messenger RNA and protein expression was decreased, as determined by real-time PCR and Western blotting, respectively. Obtained results show that significant changes in PRL and PRLRs expression are observed in uterine tissue and cells during adenomyosis, which were also affected by E_2. These data suggest involvement of PRL in adenomyosis development and the link between PRL and E_2 actions during the dysfunction in cows.

1. Introduction

Adenomyosis is proliferative uterine dysfunction defined by the presence of endometrial glands deep within the myometrium [1]. It is mainly recognized in women; nevertheless, this condition occurs also in cows and may disturb reproductive performance of the animals [2–4]. Despite several hypotheses, the exact etiology of the dysfunction remains unknown. It is suggested that adenomyosis is estrogen-dependent dysfunction [3,5]. In cows, it was shown that estradiol receptor alpha protein expression is increased in uterine tissue during adenomyosis. Moreover, estradiol (E_2) concentrations in uterine tissue and blood are also higher in adenomyotic cows compared with healthy ones [3]. Another possible mechanism of adenomyosis development involves metaplasia of uterine stem cells in the myometrium under E_2 influence [6]. In our recent studies, we identified stem cell markers in the bovine uterus [7], and we showed that its expression is changed during adenomyosis [8]. Despite not knowing the precise mechanism of adenomyosis development, we do know that it involves the excessive proliferation of endometrial cells, which is possibly caused by abnormal hormonal stimuli, including a possible prolactin (PRL) role [2,3,9].

Prolactin is a pituitary hormone that acts multidirectionally on a wide range of tissues. It plays multiple

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systemic roles, including in growth and development, salt and water balance, reproductive and immune processes, and behavior and metabolism regulation. However, lactogenic activity of PRL is best characterized [10]. Biological action of PRL is mediated by its long-form receptor (lPRLR), which is distributed in almost all mammalian tissues [11]. On the other hand, the short form of PRLR (sPRLR) is suggested to be an inhibitor of PRL's biological activity, through limiting the ability of PRL to bind with lPRLR. Prolactin may also be produced locally in multiple tissues, including those in the reproductive organs, where it acts in para- and autocrine manner [10,12]. It has been shown that PRL is produced in particular in the human decidua and uterus [13,14], in myometrium [15,16] and in the ovary [17]. In cows, it has been demonstrated that PRL and its receptors are expressed in corpus luteum [11,18]. Prolactin receptors are also distributed in other reproductive tissues in many species [10], likewise in bovine uterus [19,20]. In the uterus of ruminants, PRL plays a role in pregnancy establishment and maintenance [21,22]. It stimulates the development of uterine glands and their secretory functions, therefore contributing to a proper environment for embryo implantation [23]. Moreover, PRL enhances E_2 actions in the uterus and stimulates estradiol receptor expression in the endometrium [10].

During adenomyosis in women, the blood level of PRL is increased [24]. Moreover, experimental adenomyosis is induced in rodents by pituitary grafting into the uterus, which also results in increased PRLRs expression in the tissue [9,25]. In dairy cows during lactation, blood levels of PRL are elevated, which may affect high-yielding dairy cows' reproductive performance [26,27]. These findings and the role PRL plays in the uterus suggest that this hormone could be involved in pathogenesis of adenomyosis. We hypothesized that in the bovine uterus, the expression of PRL and its receptors during adenomyosis is disturbed and modulated by E_2. Therefore, the aim of the present study was to evaluate messenger RNA (mRNA) and protein expression of PRL and its receptors, both long and short form, in bovine uterine tissue and cultured cells isolated from uteri with developed adenomyosis in comparison with control tissue and cells. In addition, to understand better the link between PRL and E_2 actions during adenomyosis, PRL, PRLRs expression, and PRL secretion were also determined in in vitro–cultured uterine cells after treatment with E_2.

2. Materials and methods

2.1. Material collection

A total of 20 uteri from Holstein/Polish Black and White cows (75%/25%, respectively) 5- to 7-years old, were collected postmortem (at day 8 to 10 of the estrous cycle). Material was acquired at the Warmia meat processing plant (Biskupiec, Poland) and transported on ice to the laboratory within 40 min. Day of the estrous cycle was evaluated by macroscopic observation of the ovaries and uterus [28] and confirmed by determination of progesterone (P_4) levels in peripheral blood plasma, using RIA. Peripheral blood samples were collected from the jugular vein, and information about the age of each cow was acquired. The reasons for culling animals from the herd were economic considerations and herd renewal. Bovine mammary gland tissue was also obtained and used as positive control for PRL and its receptors' protein expression.

Uterine tissue fragments (cross-sections, ie, endometrium and myometrium) were obtained from horn ipsilateral to corpus luteum and divided into 3 pieces: one fixed in 4% paraformaldehyde (PFA) in 0.1-M PBS (pH 7.4) for histo- and immunohistochemical staining, one frozen and stored at −86°C for further mRNA and protein expression determination in whole uterine tissue, and one used for immediate isolation and culture of uterine epithelial, stromal, and myometrial cells.

2.2. Histochemical staining and preliminary division of the material

Uterine tissue was fixed in 4% PFA and processed for a standard hematoxylin and eosin staining protocol. Stained cross-sections of the tissue were observed under a light microscope (Nikon FXA, Tokyo, Japan). Adenomyosis evaluation was performed as described previously [3,8]; 11 animals were classified as normal/control (without uterine glands within myometrium), and 9 cows were classified as adenomyotic (ADENO group).

2.3. Uterine cell isolation, in vitro culture, and treatment

Epithelial, stromal, and myometrial cells were isolated ($n = 7$ for control group and $n = 6$ for ADENO group) by enzymatic dissociation as previously described [7,29]. After isolation, cells were suspended in culture medium (Dulbecco's Modified Eagle medium, DMEM; Sigma, D5796, St. Louis, MO, USA) supplemented with 10% of fetal calf serum (FCS; Sigma, 12133C) and antibiotics (gentamicin/amphotericin B; Life Technologies, 1153727, Paisley, UK). The cells of each layer of the uterus were seeded separately at a density of 1×10^6 living cells/mL in 1 mL and 2 mL culture medium per well in collagen-coated 24-well and 6-well plates, respectively, (Biocoat; BD Bioscience, 4408, 4400, Bedford, MA, USA) and cultured at 37.5°C in a humidified atmosphere of 5% CO_2, 95% air. Media were changed every 2 d. Purity of the cell cultures was rated by 4 independent observations under the light microscope, based on the cells' morphology [30], and was evaluated for approximately 90% to 95% for each cell type. Epithelial cells grew in colonies and exhibited spherical shape, and stromal cells maintained fibroblast-like morphology, whereas myometrial cells exhibited fusiform appearance [30]. After 70% of the confluence was reached (at approximately the fourth day of culture), culture media were replaced with stimulation media (DMEM; Sigma D5796) supplemented with 0.1% of BSA (Sigma, A2058) and antibiotics (gentamicin/amphotericin B; Life Technologies, 1153727) containing E_2 (Sigma, E8875; 10^{-7} M) or no stimulant (untreated cells). Cells were incubated with stimulation media for 24 h. Concentration and incubation time for E_2 were chosen based on literature [31,32] and preliminary study (data not shown).

After stimulation, total mRNA, cell lysates, and culture media were collected. Cell culture homogeneity was also