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The effects of *mu*-, *delta*- and *kappa*-opioid receptor activation on *in vitro* prolactin secretion by anterior pituitary cells of cyclic gilts

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ABSTRACT. The aim of the study was to determine an *in vitro* effect of specific agonists of opioid receptors on basal prolactin secretion and in the presence of dopamine or thyreoliberin (TRH) by porcine anterior pituitary cells. The cells were isolated from anterior pituitaries of gilts on days 8–10, 15–17 and 19–21 of the oestrous cycle and submitted to *in vitro* culture with *mu*-, *delta*- and *kappa*-opioid receptor agonists – FK 33-824, DPLPE and U 50,488, respectively. Differentiated effects of the opioid agonists on prolactin secretion by isolated pituitary cells of gilts in chosen days of the oestrous cycle were shown. In the midluteal phase (days 8–10), a reduced prolactin secretion was demonstrated after activation of *mu*-, *delta*- and *kappa*-opioid receptors under all tested conditions. In the early follicular phase (days 15–17), the activation of *mu*-, *delta*- and *kappa*-opioid receptors increased prolactin secretion under basal conditions, as well as *mu*- and *delta*-opioid receptors – in the presence of TRH, but the stimulation of *mu*- and *kappa*-opioid receptors reduced the hormone secretion in the presence of dopamine. In the late follicular phase (days 19–21), *kappa*-opioid receptor agonist stimulated prolactin secretion under all tested conditions. The activation of *mu*- and *delta*-opioid receptors increased prolactin secretion under basal conditions and in the presence of dopamine, but decreased – in the presence of TRH. The results suggest a possibility of diverse participation of endogenous opioids, depending on stage of the oestrous cycle, in the modulation of prolactin secretion at the pituitary level in gilts during the oestrous cycle.

Introduction

Prolactin is a pituitary hormone with a wide range of activities, including its involvement in the control of reproductive processes, related to the oestrous cycle, pregnancy and lactation (Freeman et al., 2000; Dusza and Ciereszko, 2007; Ignacak et al., 2012). In pigs, prolactin secretion during the oestrous cycle is altered. During the luteal phase, the plasma prolactin concentration is low, in the late luteal/early follicular phases characteristic and relatively short pulses correlated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) surges are observed, and during the preovulatory period substantial increase in its secretion occurs (Dusza and Krzymowska, 1979; Brinkley, 1981; Van de Wiel et al., 1981; Dusza et al., 1988). A key role in the regulation of prolactin secretion is played by dopamine and thyreoliberin (TRH)

(Freeman et al., 2000; Dusza and Ciereszko, 2007; Ignacak et al., 2012). Endogenous opioid peptides (EOPs) are also involved in the regulation of prolactin secretion (Tavakoli-Nezhad and Arbogast, 2010; Vuong et al., 2010). They are products of proteolytic cleavage of protein precursors, i.e. proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN) (Akil et al., 1984). POMC is a precursor for *β*-endorphin, demonstrating the greatest affinity to *mu*-opioid receptor, PENK is a precursor of Met- and Leu-enkephalin, which are preferentially bound by *delta*-opioid receptor, and the peptides derived from PDYN – dynorphin A, dynorphin B and *α*-neoendorphins act through *kappa*-opioid receptor (Satoh and Minami, 1995; Akil et al., 1998). The expression of genes encoding opioid precursors and receptors has been confirmed in the pituitary as well as ovary and uterus of the pig (Staszkiewicz et al., 2007; Wylot et al., 2008; Dziekoński et al., 2015a, b).

In the literature, there are only few studies trying to explain the role of EOPs in the prolactin secretion in pig during the oestrous cycle. In studies performed by Barb et al. (1986), an administration of non-specific opioid receptor antagonist, naloxone, stimulated prolactin secretion in gilts during the luteal phase (days 10–11 of the cycle), in contrast to gilts in the early and late follicular phases (days 15–17 and 18–19 of the cycle, respectively). In other study (Okrasa et al., 1990), naloxone did not affect plasma prolactin concentration in gilts during the early follicular phase, but caused its delayed decrease on day 20 of the oestrous cycle. In turn, bolus administration of *mu*-opioid receptor agonist, FK 33-824, did not influence prolactin secretion in gilts during the early follicular phase, whereas during the late follicular phase, both bolus and continuous treatments with this agonist stimulated prolactin secretion (Okrasa et al., 1990).

The central effect of EOPs on prolactin secretion by modulating the activity of dopaminergic hypothalamic systems is well documented (Tavakoli-Nezhad and Arbogast, 2010; Vuong et al., 2010), but knowledge about their action on its secretion at the pituitary level is very scarce. Several early studies concerning the latter problem rather excluded a possibility of EOP action at the pituitary level on prolactin secretion (Login and Macleod, 1979; Giudici et al., 1984; Buydens et al., 1986), while some others have proved an attenuation of inhibitory influence of dopamine on lactotrophs by opioids (Enjalbert et al., 1979; Voigt et al., 1983; Bentley and Wallis, 1986). Naloxone-sensitive cells were found in the porcine anterior pituitary gland (Szafranska and Tilton, 2000). The expression of genes encoding opioid precursors and receptors in the pituitary of cycling pigs has been demonstrated (Wylot et al., 2008). Modulation of luteinizing hormone (LH) secretion by the pig pituitary cells *in vitro* was described by Barb et al. (1990). In addition, it has been documented that opioid agonists (acting through *kappa*- and *delta*-opioid receptors) may reduce gonadotropin (LH and FSH) secretion at the pituitary level in cyclic gilts (Wylot et al., 2013). Taking into account the above observations, it might be hypothesized that opioids also modulate prolactin secretion at the pituitary level in cyclic gilts. To verify this assumption, the studies testing the effect of activation of three major opioid receptor types (*mu*, *delta* and *kappa*) on basal prolactin secretion as well as in the presence of dopamine or TRH by dispersed pituitary cells isolated from gilts during chosen periods of the oestrous cycle were undertaken. On the basis of secretory prolactin pattern in cyclic pigs, the midluteal, early and late follicular phases of the oestrous cycle were selected for the study.

Material and methods

Pituitary collection and isolation of anterior pituitary cells

Pituitaries were collected in the local slaughterhouse from mature cross-bred gilts (Large White × Polish Landrace) on days 8–10 (the midluteal phase), 15–17 (the early follicular phase) and 19–21 (the late follicular phase) of the oestrous cycle. The stage of the cycle was established basing on the ovarian morphology (Akins and Morrissette, 1968). Immediately after collection of each pituitary, anterior lobe was isolated and cut into smaller portions in a chilled Petri dish, then transferred to cooled Dulbecco medium containing 0.1% BSA (Sigma-Aldrich, St. Louis, MO, USA), nystatin (240 IU · ml^{-1}, Sigma-Aldrich, St. Louis, MO, USA) and gentamicin (100 µg · ml^{-1}; KRKA, Novomesto, Slovenia) and transported to the laboratory. All *in vitro* experiments were carried out according to the principles and procedures approved by the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn (Poland).

Isolation of pituitary cells was carried out under sterile conditions in accordance with the procedure described by Bogacka et al. (2002). Briefly, the cell isolation included: washing of the pituitary fragments in Dulbecco medium with the above mentioned antibiotics, cutting them into smaller pieces

and suspending in 3 ml of 0.3% trypsin solution (Biomed, Lublin, Poland), and then repeated digestion at 37 °C for 10 min with constant stirring on a magnetic stirrer. After each digestion, the supernatants containing the released cells were transferred into a sterile tube and centrifuged at 800 *g* for 10 min at 4 °C. The obtained cell pellets were washed three times in the preparation medium, then pooled and filtered through a sterile nylon filter in order to separate single cells from undigested tissue fragments. The filtrates were again centrifuged and the cell fraction – suspended in 1 ml of preparation medium. Subsequently, the cells were counted in the Bürker's chamber, their viability assessed with 0.4% trypan blue solution. For each isolation about 8–10 pituitaries were used in order to obtain an adequate number of cells to carry out full experimental design. Seven series (n = 7) of the cell cultures were performed within each stage of the oestrous cycle.

In vitro culture of anterior pituitary cells

Isolated cells were preincubated in McCoy-5A medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 2.5% bovine calf serum (Life Technologies, Carlsbad, CA, USA), 10% horse serum (Biomed, Lublin, Poland), Vitamins Solution (Sigma-Aldrich, St. Louis, MO, USA), Non-essential Amino Acid Solution (Sigma-Aldrich, St. Louis, MO, USA), nystatin (240 IU · ml^{-1}) and gentamycin (100 µg · ml^{-1}) on 24-well culture plates (Corning Inc., Corning, NY, USA) at a density of 3×10^5 cells per well for 48 h at 37 °C under controlled atmosphere (5% CO_2 and 95% air). Then, incubation of the cells in 1 ml of fresh McCoy-5A medium without serum was continued for additional 24 h. After the 72-hour preincubation, the cells reached high confluence (above 90%). Subsequently, the cells were rinsed with fresh medium and exposed to opioid agonists for 4 h to test the effects of the treatment on prolactin secretion. The effects of opioid receptor agonists (*mu* – FK 33-824, *delta* – DPLPE and *kappa* – U 50,488; all from Sigma-Aldrich, St. Louis, MO, USA) at concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1} on prolactin secretion under basal conditions as well as in the presence of dopamine (10^{-7} mol · l^{-1}) or TRH (10^{-7} mol · l^{-1}) were tested. The doses of opioid agonists were chosen based on previous studies (Kaminski et al., 2004; Wylot et al., 2013). All experiments were performed in duplicates. After incubation, the culture media were collected and stored at −72 °C for further analysis.

Radioimmunoassay of prolactin

Prolactin concentration in culture media was determined by radioimmunoassay (RIA) procedure, previously described by Dusza and Krzymowska (1979), using a rabbit primary antibody against porcine prolactin (Harbor UCLA Medical Center, Torrance, CA, USA) and the secondary precipitating antibody (Department of Animal Physiology, University of Warmia and Mazury, Olsztyn, Poland) (Szafranska et al., 2002). Porcine prolactin for standards and iodination was provided by A.F. Parlow (Harbor UCLA Medical Centre, Torrance, CA, USA). The hormone was labelled using ^{125}I (Hartmann Analytic, Braunschweig, Germany). Sensitivity of the assay was 0.047 ng per sample. The intra- and inter-assay coefficients of variation were below 10%.

Statistical analysis

The statistical analysis of data was performed using Statistica 10.0 program (StatSoft Inc., Tulsa, OK, USA). Concentrations of prolactin were shown as the means ± standard error of means (SEM). The influence of opioid agonists on prolactin secretion was analysed with the one-way analysis of variance (ANOVA) for repeated measurements and followed by Least Significant Difference post-hoc test. Values with $P < 0.05$ were considered to be statistically significant (*) and with $P < 0.01$ – highly significant (**).

Results

Differentiated influences of opioid receptor agonists on *in vitro* prolactin secretion by porcine anterior pituitary cells under basal conditions and in the presence of dopamine or TRH were observed depending on the stage of the oestrous cycle (Figures 1–3).

Luteal phase of the oestrous cycle

The activation of *mu*-, *delta*- and *kappa*-opioid receptors with specific agonists at all tested doses significantly reduced basal prolactin secretion as well as its secretion in the presence of dopamine (10^{-7} mol · l^{-1}) and TRH (10^{-7} mol · l^{-1}) by pituitary cells of gilts in the luteal phase (Figure 1). Under the basal conditions, the addition of *mu*-opioid receptor agonist decreased ($P < 0.01$) prolactin secretion from control value (17.53 ± 3.13 ng · ml^{-1}) to: 9.37 ± 2.86, 7.24 ± 1.71 and 7.76 ± 1.37 ng · ml^{-1}, *delta*- opioid receptor agonist to: 7.36 ± 1.37 ($P < 0.01$), 7.13 ± 1.02 ($P < 0.01$) and 10.85 ± 2.14 ng · ml^{-1} ($P < 0.05$), whereas *kappa*-opioid receptor agonist ($P < 0.01$) to:

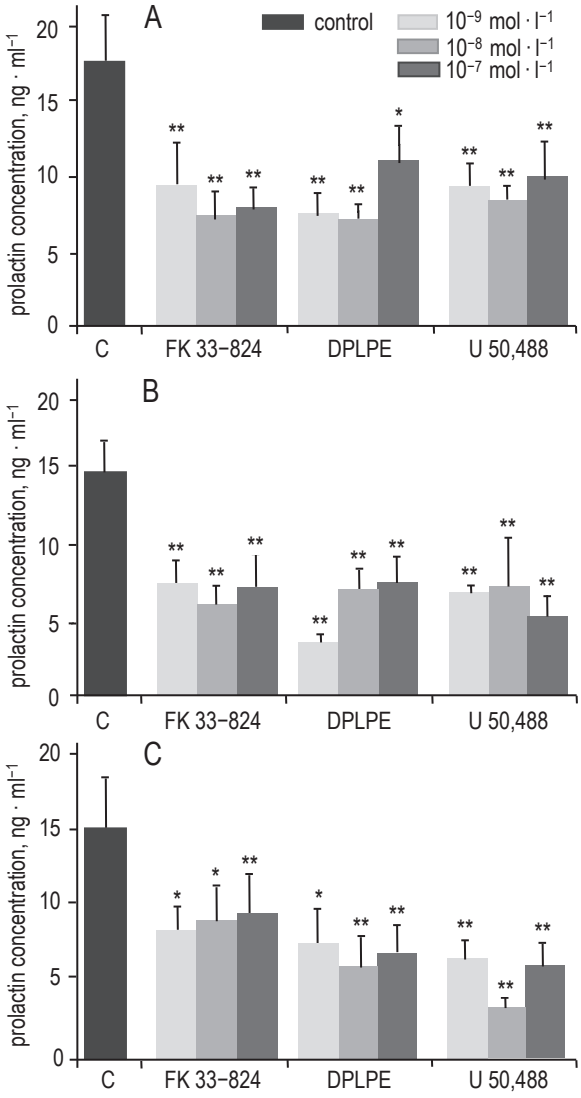

Figure 1. The effect of opioid receptor agonists (*mu* – FK 33-824, *delta* – DPLPE and *kappa* – U 50,488) at concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1} on prolactin secretion *in vitro*: A. under basal condition and in the presence of B. dopamine (10^{-7} mol · l^{-1}) or C. thyreoliberin (TRH) (10^{-7} mol · l^{-1}) by pituitary cells of gilts in midluteal phase (days 8–10) of the oestrous cycle. The concentration of prolactin was expressed per 10^5 cells as mean ± SEM (n = 7). Significant differences between treatments and respective control (C) are indicated with asterisks: * – $P < 0.05$, ** – $P < 0.01$

9.25 ± 1.47, 8.32 ± 0.88 and 9.87 ± 2.36 ng · ml^{-1} at concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively (Figure 1A).

In the presence of dopamine (10^{-7} mol · l^{-1}), the activation of *mu*-opioid receptor reduced prolactin secretion from 14.82 ± 2.01 ng · ml^{-1} to: 7.36 ± 1.30, 6.09 ± 1.30 and 7.24 ± 1.13 ng · ml^{-1}, *delta*-opioid receptor – to: 3.34 ± 0.38, 7.17 ± 1.34 and 7.24 ± 1.50 ng · ml^{-1}, whereas *kappa*-opioid receptor – to: 5.50 ± 1.71, 7.31 ± 3.08 and 5.15 ± 1.41 ng · ml^{-1} for the tested concentrations of agonists – 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1} ($P < 0.01$ for all differences), respectively (Figure 1B).

In the presence of TRH (10^{-7} mol · l^{-1}), the activation of opioid receptors by *mu*-opioid receptor agonist decreased prolactin secretion from 15.39 ± 2.81 ng · ml^{-1} to: 8.64 ± 1.09 ($P < 0.05$), 9.25 ± 2.38 ($P < 0.05$) and 7.39 ± 1.76 ng · ml^{-1} ($P < 0.01$), by *delta*-opioid receptor agonist ($P < 0.01$) to: 7.37 ± 2.12, 6.16 ± 2.05 and 7.21 ± 1.72 ng · ml^{-1}, while by *kappa*-opioid receptor agonist ($P < 0.01$) to: 6.77 ± 1.31, 3.46 ± 0.66 and 6.18 ± 1.59 ng · ml^{-1} for concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively (Figure 1C).

Early follicular phase of the oestrous cycle

The basal prolactin *in vitro* secretion by porcine anterior pituitary cells representing the early follicular phase was elevated ($P < 0.01$) by agonists of opioid receptors (Figure 2A); FK 33-824 (from 6.87 ± 2.29 to 14.97 ± 2.41 ng · ml^{-1} at concentration 10^{-7} mol · l^{-1}), DPLPE (to 15.69 ± 3.69 ng · ml^{-1} at concentration 10^{-7} mol · l^{-1}) and U 50,488 (to 14.60 ± 1.16 and 14.84 ± 1.55 ng · ml^{-1} for concentrations 10^{-8} and 10^{-7} mol · l^{-1}, respectively).

In the presence of dopamine, however prolactin secretion was significantly ($P < 0.05$) decreased in response to *mu*- and *kappa*-opioid receptor agonists: FK 33-824 (from 9.30 ± 0.92 ng · ml^{-1} to: 4.21 ± 0.77 and 4.76 ± 0.96 ng · ml^{-1} for concentrations 10^{-8} and 10^{-7} mol · l^{-1}, respectively) and U 50,488 (to: 4.90 ± 1.45, 4.82 ± 2.11 and 4.94 ± 1.24 ng · ml^{-1} for concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively). The effects of *delta*-opioid receptor agonist on prolactin secretion by the cells were negligible (Figure 2B).

In the presence of TRH, prolactin secretion was significantly elevated by treatment of the cells with *mu*-opioid receptor agonist from 9.73 ± 1.78 ng · ml^{-1} to: 19.22 ± 3.50 ($P < 0.05$), 18.23 ± 2.93 ($P < 0.05$) and 22.42 ± 3.44 ng · ml^{-1} ($P < 0.01$) for concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively, as well as with *delta*-opioid receptor agonist to 19.03 ± 3.72 ng · ml^{-1} ($P < 0.05$) at concentration 10^{-7} mol · l^{-1}. The activation of *kappa*-opioid receptor did not significantly influence prolactin secretion in the presence of TRH by the pituitary cells of gilts during this period (Figure 2C).

Late follicular phase of the oestrous cycle

The activation of opioid receptors elevated the basal secretion of prolactin by pituitary cells representing the late follicular phase of the cycle (Figure 3A). In response to *mu*-opioid receptor agonist (FK 33-824), prolactin concentration in culture medium was increased ($P < 0.01$) from 13.52 ± 2.36 ng · ml^{-1} to: 28.62 ± 2.60, 26.19 ± 2.97 and 24.96 ± 1.24 ng · ml^{-1} for concentrations

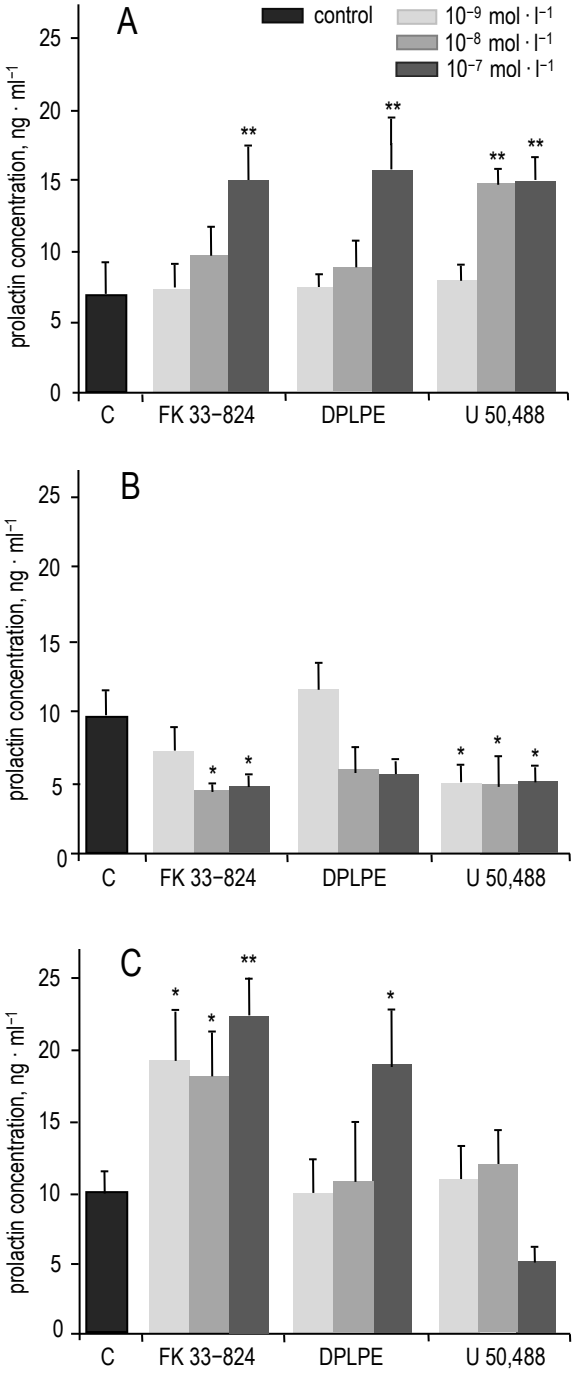

Figure 2. The effect of opioid receptor agonists (*mu* – FK 33-824, *delta* – DPLPE and *kappa* – U 50,488) at concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1} on prolactin secretion *in vitro*: A. under basal condition and in the presence of B. dopamine (10^{-7} mol · l^{-1}) or C. thyreoliberin (TRH) (10^{-7} mol · l^{-1}) by pituitary cells of gilts in early follicular phase (days 15–17) of the oestrous cycle. The concentration of prolactin was expressed per 10^5 cells as mean ± SEM (n = 7). Significant differences between treatments and respective control (C) are indicated with asterisks: * – $P < 0.05$, ** – $P < 0.01$

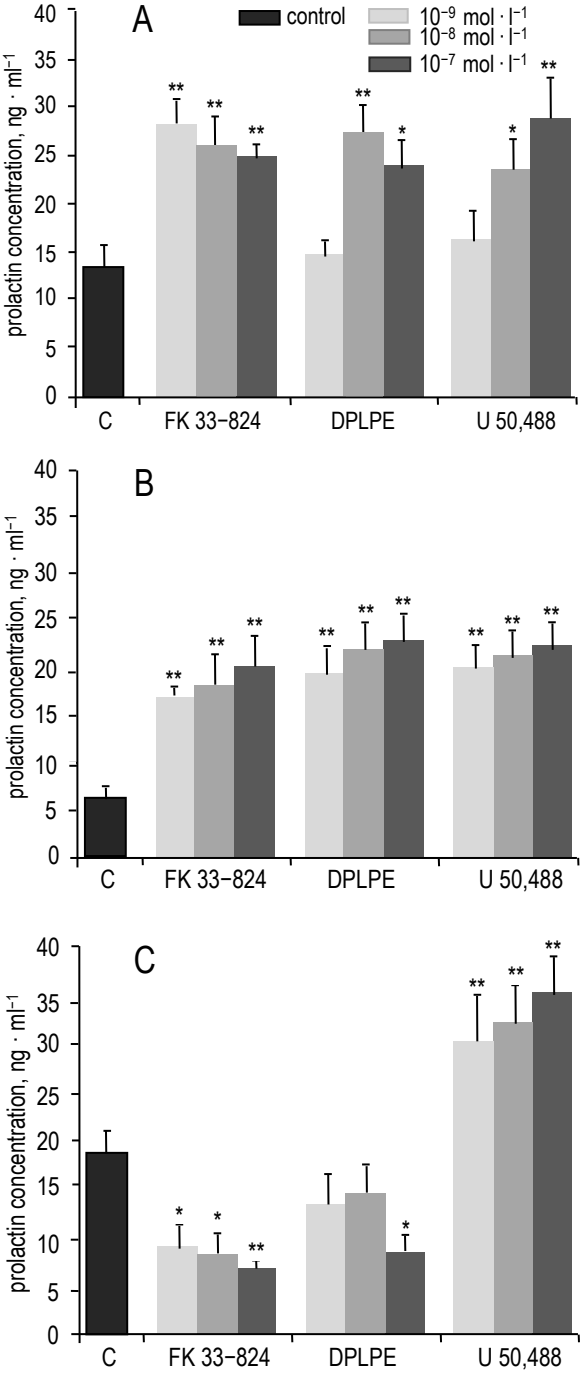

Figure 3. The effect of opioid receptor agonists (*mu* – FK 33-824, *delta* – DPLPE and *kappa* – U 50,488) at concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1} on prolactin secretion *in vitro*: A. under basal condition and in the presence of B. dopamine (10^{-7} mol · l^{-1}) or C. thyreoliberin (TRH) (10^{-7} mol · l^{-1}) by pituitary cells of gilts in late follicular phase (days 19–21) of the oestrous cycle. The concentration of prolactin was expressed per 10^5 cells as mean ± SEM (n = 7). Significant differences between treatments and respective control (C) are indicated with asterisks: * – $P < 0.05$, ** – $P < 0.01$

10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively. In turn, *delta*- and *kappa*-opioid receptor agonists at concentrations 10^{-8} and 10^{-7} mol · l^{-1} increased it to: 27.54 ± 2.96 ($P < 0.01$) and 23.90 ± 2.56 ng · ml^{-1} ($P < 0.05$), and to: 23.70 ± 2.75 ($P < 0.05$) and 28.81 ± 4.28 ng · ml^{-1} ($P < 0.01$), respectively.

In the presence of dopamine, treatment of the cells with *mu*-, *delta*- and *kappa*-opioid receptor agonists at all tested doses significantly ($P < 0.01$) stimulated prolactin secretion (Figure 3B). The *mu*-opioid receptor agonist raised it from 6.51 ± 1.15 ng · ml^{-1} to: 16.66 ± 0.72, 17.92 ± 3.32

and 19.81 ± 3.30 ng · ml^{-1}, *delta*-opioid receptor agonist – to: 19.58 ± 2.45, 22.12 ± 3.02 and 22.58 ± 2.65 ng · ml^{-1}, and *kappa*-opioid receptor agonist – to: 20.11 ± 2.05; 21.02 ± 3.23 and 21.97 ± 2.97 ng · ml^{-1} for concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively.

In the presence of TRH (Figure 3C), the pituitary cells from the late follicular phase reduced secretion of prolactin in response to *mu*-receptor agonist from 18.66 ± 2.42 ng · ml^{-1} to: 9.24 ± 2.22 ($P < 0.05$), 8.55 ± 2.11 ($P < 0.05$) and 6.77 ± 0.90 ng · ml^{-1} ($P < 0.01$) at concentrations 10^{-9}, 10^{-8}, 10^{-7} mol · l^{-1}, respectively, as well as to *delta*-opioid receptor agonist at concentration 10^{-7} mol · l^{-1} (to 8.56 ± 1.91 ng · ml^{-1}; $P < 0.05$). In turn, prolactin secretion was significantly increased ($P < 0.01$) by *kappa*-opioid receptor agonist (from 18.66 ± 2.42 ng · ml^{-1} to: 30.77 ± 4.60, 32.45 ± 3.96 and 35.47 ± 4.00 ng · ml^{-1} for concentrations 10^{-9}, 10^{-8} and 10^{-7} mol · l^{-1}, respectively).

Discussion

This study was undertaken to investigate the potential action of EOPs at the pituitary level on prolactin secretion in pigs during three chosen periods of the oestrous cycle with characteristic secretory pattern of the hormone, i.e. the midluteal, early and late follicular phases. It has been shown that opioid systems differently affect prolactin secretion during these stages of the oestrous cycle.

In the midluteal phase of the oestrous cycle, the inhibitory effect of *mu*-, *delta*- and *kappa*-opioid receptor agonists on *in vitro* prolactin secretion by porcine pituitary cells under basal conditions as well as in the presence of tested secretagogues (dopamine and TRH) was demonstrated. In the previous *in vivo* studies, it has been shown that blockage of opioid receptors through the administration of naloxone (a non-specific antagonist of these receptors) elevates prolactin secretion in the luteal phase gilts (Barb et al., 1986). This suggested an inhibitory influence of EOPs on prolactin secretion in pigs during this phase of the oestrous cycle, which is in accordance with our present data. Similar effect of naloxone on plasma prolactin concentrations was also observed in sows during late pregnancy (Willis et al., 1996). Conversely, naloxone administration during lactation decreased plasma prolactin concentrations in sows (Barb et al., 1986; Mattioli et al., 1986; Armstrong et al., 1988), excluding the first 78-hour period after farrowing, when the inhibitory effect of naloxone on prolactin secretion did not appear (De Rensis et al., 1993). Therefore, it was suggested that there are two routes of opioid action on prolactin secretion in pigs: one luteal-related, which inhibits prolactin secretion and the other, suckling-dependent, stimulating release of the hormone (Barb et al., 1991). The *in vivo* studies on gilts during luteal phase demonstrated that increased prolactin secretion in response to naloxone is synchronized with a release of LH, indicating an involvement of progesterone-dependent, opioidergic inhibitory pathway in the control of both pituitary hormones secretion (Barb et al., 1986). Moreover, in the cited paper, it has been considered that the naloxone-induced increase in prolactin secretion in the luteal phase gilts, in addition to hypothalamic effects, might be also mediated by a paracrine effect directed from gonadotrophs to lactotrophs. The present study confirms a potential participation of opioid systems at the pituitary level in inhibition of prolactin secretion in pigs during luteal phase.

In the early follicular phase, the effects of opioid receptors activation on prolactin secretion *in vitro* were differentiated; from stimulation to inhibition or lack of significant influence. Under basal conditions, agonists at higher concentrations increased prolactin secretion. In the presence of TRH, particularly activation of *mu*-opioid receptor as well as the highest dose of *delta*-opioid receptor agonist lead to a raise in prolactin secretion. On the other hand, in the presence of dopamine, agonists of *mu*- and *kappa*-opioid receptors significantly reduced prolactin secretion. In *in vivo* studies on gilts in the early follicular phase (days 15–17), some decrease in prolactin secretion was noted following treatment with naloxone, but authors expressed doubts that this change might be caused by the opioid antagonist since prolactin concentrations were descending during the pretreatment period (Barb et al., 1986). In other studies, neither naloxone nor *mu*-opioid receptor agonist, FK 33-824, affected plasma prolactin concentration in gilts at similar stage of the oestrous cycle (Okrasa et al., 1990). In turn, intracerebroventricular injections of *β*-endorphin significantly increased plasma prolactin concentrations in ewes during the early follicular phase (Curlewis et al., 1991). In the present *in vitro* study, during this stage of the cycle, opposing actions of opioid agonists on prolactin secretion by porcine pituitary cells, depending on the presence of TRH or dopamine, were observed. The most striking differences pertain to the action of *mu*- and *kappa*-opioid receptor agonists, i.e. FK 33-824 and U 50,488, respectively. The stimulatory effect of FK 33-824 on prolactin secretion noted under basal conditions became more evident in the presence of TRH, but in the presence of dopamine it changed to an inhibitory one. In turn, stimulation

of prolactin secretion by U 50,488 found under basal conditions did not appear in the presence of TRH, but in the presence of dopamine this agonist manifested an inhibitory action. This observation suggests an implication of pituitary opioid systems (acting mainly through *mu*- and *kappa*-opioid receptors) in the action of dopamine and TRH on prolactin secretion, which might be connected with the modelling of the hormone secretory pattern specific for the early follicular phase of the oestrous cycle. Prolactin secretion during this stage of the cycle is characterized by the presence of several pulses (Dusza et al., 1988), therefore it requires both stimulation (during initiation of the pulse) and inhibition (connected with the pulse termination and duration of intervals between pulses) of the hormone secretion. In contrast to previous *in vivo* studies on gilts, our *in vitro* experiment indicates that participation of EOPs in control of pulsatile prolactin secretion during this period of the cycle through auto- and/or paracrine interactions at the level of the pituitary is possible. However, these interactions need to be better delineated in further experiments.

In the late follicular phase, the stimulation of prolactin secretion by the pituitary cells dominated, and it occurred in response to all tested agonists under basal conditions as well as in the presence of dopamine. In the presence of TRH, only *kappa*-opioid receptor agonist increased prolactin secretion, whereas *mu*- and *delta*-opioid receptor agonists reduced it. In gilts during the late follicular phase of the oestrous cycle (days 18–19), decreased plasma prolactin concentrations were observed after *in vivo* treatment with naloxone, but these changes were not fully convincing due to large fluctuations in the hormone concentrations (Barb et al., 1986). In later study, a significant but delayed decrease in prolactin secretion was observed following naloxone administration to gilts on day 20 of the oestrous cycle (Okrasa et al., 1990). This is in line with the study, in which FK 33-824 (*mu*-opioid receptor agonist), administered either as a bolus injection or continuous infusion, increased plasma prolactin concentration in gilts during the late follicular phase (Okrasa and Tilton, 1992). Predominantly a stimulatory effect of opioid agonists on *in vitro* prolactin secretion by porcine pituitary cells representing the late follicular phase of the cycle, revealed in the present study, might be associated with increasing secretory potential of lactotrophs during preovulatory period.

The present study indicates that co-treatment of pituitary cells derived from cyclic gilts with agonists of opioid receptors markedly affects their response to major prolactin secretagogues, dopamine or TRH. In the midluteal phase, the activation of these receptors unanimously reduced prolactin secretion in response to both dopamine and TRH. In the early follicular phase, *mu*- and *kappa*-opioid receptor agonists inhibited prolactin secretion in response to dopamine, whereas *mu*- and *delta*-opioid receptor agonists stimulated it in the presence of TRH. In turn, during the late follicular phase, all tested opioid receptor agonists stimulated prolactin secretion in response to dopamine, whereas in the presence of TRH, *kappa*-opioid receptor agonist stimulated prolactin secretion, but agonists of *mu*- and *delta*-opioid receptors reduced it. Therefore, it seems that EOPs have a potential to influence the responsiveness of lactotrophs to dopamine and TRH. Furthermore, the responsiveness observed in this study under *in vitro* conditions seems to be determined by the *in vivo* status of gilts used in the experiment, i.e. the stage of the oestrous cycle characterized by differentiated concentrations of steroid hormones in blood plasma. Studies performed on rats have yielded data suggesting the effect of steroids on the responsiveness of pituitary cells to hypothalamic major prolactin secretagogues. The upregulation of mRNA for TRH receptor in rat pituitary cells by estradiol has been demonstrated (Kimura et al., 1994). The modulatory effect of estradiol on prolactin secretion induced by TRH is achieved through membrane estrogen receptors and PI3K/Akt signalling pathway (Sosa et al., 2012). It was also documented that steroid hormones may affect the expression of dopamine receptor (D2) on pituitary cells of female rats (Joubert-Bression et al., 1990). Namely, estradiol reduced the number of binding sites for dopamine but progesterone increased it. Moreover, estradiol was able to affect the D2 receptor splicing in a lactotroph cell line (MMQ), increasing the ratio of long isoform (D2L) to short isoform (D2S) of dopamine receptor by stimulation of the D2L receptor expression. This effect of estradiol was abolished by progesterone (Guivarc'h et al., 1998). However, a greater stimulatory effect of estradiol on prolactin production and cell proliferation was observed in lactotrophs-derived cell line expressing D2S receptor than that with D2L receptor (Sengupta and Sarkar, 2012). Collectively, our results and cited data allow to suppose that EOPs – affecting prolactin secretion at the pituitary level – may interact with major its secretagogues (dopamine and TRH) in a steroid-dependent manner.

It has to be mentioned that prolactin secretion remains under control of multiple factors acting at hypothalamic and/or pituitary level (Freeman et al., 2000; Dusza and Ciereszko, 2007; Ignacak et al., 2012). The influence of several factors on *in vitro* prolactin

secretion by porcine anterior pituitary cells has been confirmed. Oxytocin stimulated prolactin secretion by the cells derived from the luteal phase gilts (Kotwica et al., 2006). In addition, gonadotropin-releasing hormone (GnRH), oxytocin and vasoactive intestinal peptide (VIP) stimulated prolactin secretion by anterior pituitary cells isolated from ovariectomized (OVX) gilts mainly 60–66 hours after administration of estradiol benzoate (i.e. during the estrogen-induced LH surge) or following treatment with progesterone, but did not influence the hormone secretion by the cells derived from untreated OVX gilts (Bogacka et al., 2002). Adrenergic agents (acting through α- and β-receptors) also appeared to affect prolactin secretion *in vitro* by pituitary cells of gilts, treated and untreated with steroids (Siawrys et al., 2003). In addition, the differentiated modulation of prolactin secretion by pituitary cells *in vitro* following treatment with agonists of opioid receptors was described herein for pigs at various stages of the oestrous cycle. The present study has brought data which apparently confirm a view pertaining to the participation of EOPs in the mechanism modulating prolactin secretion at the pituitary level in the pig during the oestrous cycle.

Conclusions

Activation of opioid receptors (*mu*, *delta* and *kappa*) may exert a modulatory effect on prolactin secretion at the pituitary level in cyclic gilts. The effects of specific agonists of opioid receptors appeared to be differentiated dependently on the stage of the oestrous cycle and the presence of tested prolactin secretagogues; dopamine or thyreoliberin (TRH). Therefore, opioid peptides seem to be a part of modulatory system functioning at the pituitary level, which supports physiological pattern of prolactin secretion in pigs during the oestrous cycle. Nevertheless, the mechanism of their differentiated action on prolactin secretion, revealed in this study, requires deeper elucidation in future research.

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References

Akil H., Owens C., Gutstein H., Taylor L., Curran E., Watson S., 1998. Endogenous opioids: overview and current issues. Drug Alcohol Depend. 51, 127–140, https://doi.org/10.1016/S0376-8716(98)00071-4

Akil H., Watson S.J., Young E., Lewis M.E., Khachaturian H., Walker J.M., 1984. Endogenous opioids: biology and function. Ann. Rev. Neurosci. 7, 223–255, https://doi.org/10.1146/annurev.ne.07.030184.001255

Akins E.L., Morrissette M.C., 1968. Gross ovarian changes during estrous cycle of swine. Am. J. Vet. Res. 29, 1953–1957

Armstrong J.D., Kraeling R.R., Britt J.H., 1988. Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J. Reprod. Fertil. 83, 301–308, https://doi.org/10.1530/jrf.0.0830301

Barb C.R., Barrett J.B., Wright J.T., Kraeling R.R., Rampacek G.B., 1990. Opioid modulation of LH secretion by pig pituitary cells *in vitro*. J. Reprod. Fertil. 90, 213–219, https://doi.org/10.1530/jrf.0.0900213

Barb C.R., Kraeling R.R., Rampacek G.B., 1991. Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Domest. Anim. Endocrinol. 8, 15–27, https://doi.org/10.1016/0739-7240(91)90036-J

Barb C.R., Kraeling R.R., Rampacek G.B., Whisnant C.S., 1986. Influence of stage of the estrous cycle on endogenous opioid modulation of luteinizing hormone, prolactin, and cortisol secretion in the gilt. Biol. Reprod. 35, 1162–1167, https://doi.org/10.1095/biolreprod35.5.1162

Bentley A.M., Wallis M., 1986. Effects of two enkephalin analogues, morphine sulphate, dopamine and naloxone on prolactin secretion from rat anterior pituitary glands *in vitro*. J. Endocrinol. 109, 313–320, https://doi.org/10.1677/joe.0.1090313

Bogacka I., Siawrys G., Okrasa S., Kaminski T., Przala J., 2002. The influences of GnRH, oxytocin and vasoactive intestinal peptide on LH and PRL secretion by porcine pituitary cells. J. Physiol. Pharmacol. 53, 439–451

Brinkley H.J., 1981. Endocrine signaling and female reproduction. Biol. Reprod. 24, 22–43, https://doi.org/10.1095/biolreprod24.1.22

Buydens P., Velkeniers B., Golstein J., Vanhaelst L., 1986. Dissociation of the central and peripheral effects of naloxone on PRL secretion. Horm. Metab. Res. 18, 575–576, https://doi.org/10.1055/s-2007-1012377

Curlewis J.D., Naylor A.M., Rhind S.M., McNeilly A.S., 1991. Effects of beta-endorphin on pulsatile luteinizing hormone and prolactin secretion during the follicular phase in the ewe. J. Neuroendocrinol. 3, 123–126, https://doi.org/10.1111/j.1365-2826.1991.tb00252.x

De Rensis F., Cosgrove J.R., Foxcroft G.R., 1993. Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol. Reprod. 48, 970–976, https://doi.org/10.1095/biolreprod48.5.970

Dusza L., Ciereszko R., 2007. Gonadotropins and prolactin: regulation of secretion and action on target cells. In: T. Krzymowski (Editor). Biology of animal reproduction. Physiological Regulation of Reproductive Processes in Females (in Polish). University of Warmia and Mazury, Olsztyn (Poland), pp. 95–138

Dusza L., Okrasa S., Ciereszko R., Kotwica G., Tilton J.E., 1988. Endocrine changes associated with spontaneous luteolysis in sows. I. Temporal relationships among prolactin, prostaglandin $F_{2\alpha}$, progesterone and LH. Anim. Reprod. Sci. 17, 115–122, https://doi.org/10.1016/0378-4320(88)90051-6

Dusza L., Krzymowska H., 1979. Plasma prolactin concentrations during the oestrous cycle of sows. J. Reprod. Fertil. 57, 511–514, https://doi.org/10.1530/jrf.0.0570511

Dziekoński M., Żmijewska A., Franczak A., Kotwica G., Czelejewska W., Okrasa S., 2015a. The expression of mRNAs for opioid precursors in endometrium of cyclic and early pregnant pigs; effects of IL-1β, IL-6 and TNFα. J. Anim. Feed Sci. 24, 118–126, https://doi.org/10.22358/jafs/65637/2015

Dziekoński M., Żmijewska A., Franczak A., Kotwica G., Kościukiewicz K., Czelejewska W., Okrasa S., 2015b. The expression of mRNAs for opioid receptors in endometrium of cyclic and early pregnant pigs; effects of IL-1β, IL-6 and TNFα. J. Anim. Feed Sci. 24, 332–340, https://doi.org/10.22358/jafs/65616/2015

Enjalbert A., Ruberg M., Arancibia S., Priam M., Kordon C., 1979. Endogenous opiates block dopamine inhibition of prolactin secretion *in vitro*. Nature 280, 595–597, https://doi.org/10.1038/280595a0

Freeman M.E., Kanyicska B., Lerant A., Nagy G., 2000. Prolactin: structure, function, and regulation of secretion. Physiol. Rev. 80, 1523–1631, https://doi.org/10.1152/physrev.2000.80.4.1523

Giudici D., D'Urso R., Falaschi P., Negri L., Melchiorri P., Motta M., 1984. Dermorphin stimulates prolactin secretion in the rat. Neuroendocrinology 39, 236–244, https://doi.org/10.1159/000123985

Guivarc'h D., Vincent J.-D., Vernier P., 1998. Alternative splicing of the D2 dopamine receptor messenger ribonucleic acid is modulated by activated sex steroid receptors in the MMQ prolactin cell line. Endocrinology 139, 4213–4221, https://doi.org/10.1210/endo.139.10.6246

Ignacak A., Kasztelnik M., Sliwa T., Korbut R.A., Rajda K., Guzik T.J., 2012. Prolactin – not only lactotrophin. a "new" view of the "old" hormone. J. Physiol. Pharmacol. 63, 435–443

Joubert-Bression D., Brandi A.M., Birman P., Peillon F., 1990. Effect of oestradiol on dopamine receptors and protein kinase C activity in the rat pituitary: binding of oestradiol to pituitary membranes. Ciba Found Symp. 153, 156–168

Kaminski T., Siawrys G., Bogacka I., Okrasa S., Przala J., 2004. The influence of opioid peptides on steroidogenesis in porcine granulosa cells. Reprod. Domest. Anim. 39, 25–32, https://doi.org/10.1046/j.1439-0531.2003.00471.x

Kimura N., Arai K., Sahara Y., Suzuki H., Kimura N., 1994. Estradiol transcriptionally and posttranscriptionally up-regulates thyrotropin-releasing hormone receptor messenger ribonucleic acid in rat pituitary cells. Endocrinology 134, 432–440, https://doi.org/10.1210/endo.134.1.8275956

Kotwica G., Staszkiewicz J., Skowroński M.T., Siawrys G., Bogacka I., Franczak A., Kurowicka B., Kraziński B., Okrasa S., 2006. Effects of oxytocin alone and in combination with selected hypothalamic hormones on ACTH, *β*-endorphin, LH and PRL secretion by anterior pituitary cells of cyclic pigs. Reprod. Biol. 6, 115–131

Login I.S., Macleod R.M., 1979. Failure of opiates to reverse dopamine inhibition of prolactin secretion in vitro. Eur. J. Pharmacol. 60, 253–255, https://doi.org/10.1016/0014-2999(79)90227-9

Mattioli M., Conte F., Galeati G., Seren E., 1986. Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J. Reprod. Fertil. 76, 167–173, https://doi.org/10.1530/jrf.0.0760167

Okrasa S., Weigl R., Jeng M., Tilton J., 1990. Concentrations of prolactin, LH and FSH after naloxone administration in follicular-phase gilts. Anim. Reprod. Sci. 22, 39–46, https://doi.org/10.1016/0378-4320(90)90036-F

Okrasa S.C., Tilton J.E., 1992. Concentrations of prolactin and LH after administration of Met-enkephalin analogue (FK 33-824) to gilts during the follicular phase. Anim. Reprod. Sci. 27, 195–207, https://doi.org/10.1016/0378-4320(92)90058-L

Satoh M., Minami M., 1995. Molecular pharmacology of the opioid receptors. Pharmacol. Ther. 68, 343–364, https://doi.org/10.1016/0163-7258(95)02011-X

Sengupta A., Sarkar D.K., 2012. Estrogen inhibits D2S receptor-regulated Gi3 and Gs protein interactions to stimulate prolactin production and cell proliferation in lactotropic cells. J. Endocrinol. 214, 67–78, https://doi.org/10.1530/JOE-12-0125

Siawrys G., Bogacka I., Okrasa S., Kamiński T., Przała J., 2003. The effects of GnRH and adrenergic agents on PRL and *β*-endorphin secretion by porcine pituitary cells *in vitro*. Acta Vet. Hung. 51, 353–369, https://doi.org/10.1556/AVet.51.2003.3.10

Sosa L.d.V., Gutiérrez S., Petiti J.P., Palmeri C.M., Mascanfroni I.D., Soaje M., De Paul A.L., Torres A.I., 2012. 17β-Estradiol modulates the prolactin secretion induced by TRH through membrane estrogen receptors via PI3K/Akt in female rat anterior pituitary cell culture. Am. J. Physiol. Endocrinol. Metab. 302, 1189–1197, https://doi.org/10.1152/ajpendo.00408.2011

Staszkiewicz J., Skowronski M.T., Siawrys G., Kaminski T., Krazinski B.E., Plonka K., Wylot B., Przala J., Okrasa S., 2007. Expression of proopiomelanocortin, proenkephalin and prodynorphin genes in porcine luteal cells. Acta Vet. Hung. 55, 435–449, https://doi.org/10.1556/AVet.55.2007.4.3

Szafranska B., Tilton J.E., 2000. Free intracellular calcium ($[Ca2^{+}]_i$) in opioid sensitive cells of the porcine anterior pituitary. J. Physiol. Pharmacol. 51, 541–554

Szafranska B., Ziecik A., Okrasa S., 2002. Primary antisera against selected steroids or proteins and secondary antisera against *γ*-globulins – an available tool for studies of reproductive processes. Reprod. Biol. 2, 187–204

Tavakoli-Nezhad M., Arbogast L.A., 2010. Mu and kappa opioid receptor expression in the mediobasal hypothalamus and effectiveness of selective antagonists on prolactin release during lactation. Neuroscience 166, 359–367, https://doi.org/10.1016/j.neuroscience.2009.12.066

Van de Wiel D.F.M., Erkens J., Koops W., Vos E., Van Landeghem A.A., 1981. Periestrous and midluteal time courses of circulating LH, FSH, prolactin, estradiol-17β and progesterone in the domestic pig. Biol. Reprod. 24, 223–233, https://doi.org/10.1095/biolreprod24.2.223

Voigt K.H., Frank D., Düker E., Martin R., Wuttke W., 1983. Dopamine-inhibited release of prolactin and intermediate lobe-POMC-peptides: different modulation by opioids. Life Sci. 33, Suppl. 1, 507–510, https://doi.org/10.1016/0024-3205(83)90552-0

Vuong C., Van Uum S.H.M., O'Dell L.E., Lutfy K., Friedman T.C., 2010. The effects of opioids and opioid analogs on animal and human endocrine systems. Endocr. Rev. 31, 98–132, https://doi.org/10.1210/er.2009-0009

Willis H.J., Cosgrove J.R., Foxcroft G.R., 1996. Opioidergic control of luteinizing hormone and prolactin secretion in late gestation in the sow. Biol. Reprod. 55, 318–324, https://doi.org/10.1095/biolreprod55.2.318

Wylot B., Staszkiewicz J., Okrasa S., 2008. The expression of genes coding for opioid precursors, opioid receptors, β-LH subunit and GnRH receptor in the anterior pituitary of cyclic gilts. J. Physiol. Pharmacol. 59, 745–758

Wylot B., Tworus K., Okrasa S., 2013. The effects of *mu*-, *delta*- and *kappa*-opioid receptor activation on luteinizing and follicle-stimulating hormone secretion from porcine pituitary cells. J. Physiol. Pharmacol. 64, 505–511