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; Ignacik 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 F₂α (PGF₂α) surges are observed, and during the pre-ovulatory 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) (Akiel et al., 1984). POMC is a precursor for β-endorphin, demonstrating the greatest affinity to μ-opioid receptor, PENK is a precursor of Met- and Leu-enkephalin, which are preferentially bound by δ-opioid receptor, and the peptides derived from PDYN – dynorphin A, dynorphin B and α-neoendorphins act through κ-opioid receptor (Satoh and Minami, 1995; Akiel et al., 1998). The expression of genes encoding opioid receptors and precursors 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 μ-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 κ- and δ-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 (μ, δ and κ) 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 × 10⁶ cells per well for 48 h at 37 °C under controlled atmosphere (5% CO₂ 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 ¹²⁵I (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:
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^-8, 10^-7, 10^-6 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 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 10^8 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).
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) (10⁻⁸ 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 10⁶ cells as mean ± SEM (n = 7). Significant differences between treatments and respective control (C) are indicated with asterisks: * – P < 0.05, ** – P < 0.01

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 10⁻⁸, 10⁻⁷, 10⁻⁵ 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 vivo 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 modulation 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 vitro 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 autocrine 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-Bresillon 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 ovario-ectomized (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


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Wybory 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