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THE EFFECTS OF GnRH AND ADRENERGIC AGENTS ON PRL AND β-ENDORPHIN SECRETION BY PORCINE PITUITARY CELLS *IN VITRO*

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The direct effects of α- and β-adrenergic agents on PRL and β-endorphin (β-END) secretion *in vitro* by porcine pituitary cells have been investigated. Pituitary glands were obtained from mature gilts, which were ovariectomised (OVX) one month before slaughter. Ovariectomised gilts, assigned to four groups, were primed with: (1) vehicle (OVX); (2) and (3) oestradiol benzoate (EB; 2.5 mg/100 kg b.w.) at 30–36 h (OVX+EB I) and 60–66 h (OVX+EB II) before slaughter, respectively; and (4) progesterone (P_4; 120 mg/100 kg b.w.) for 5 consecutive days before slaughter (OVX+P_4). Isolated anterior pituitary cells were submitted to 3.5 h incubation in the presence of GnRH, α- and β-adrenergic agonists [phenylephrine (PHEN) and isoproterenol (ISOP), respectively], or α- and β-adrenergic blockers [phentolamine (PHENT) and propranolol (PROP), respectively]. The culture media were assayed for PRL (exp. I) and β-endorphin-like immunoreactivity (β-END-LI) (experiment II). In experiment I, GnRH did not influence PRL release by pituitary cells in all experimental groups. Some of tested doses of adrenergic agonists, PHEN and ISOP, increased PRL release from pituitary cells of OVX gilts, but not from those of OVX+EB I animals. In the OVX+EB II group, PHEN alone, but ISOP with PROP, potentiated PRL secretion by the cells. In OVX+P_4 animals, PHEN alone or in combination with PHENT and also ISOP alone or with PROP enhanced PRL output from the cells. In experiment II, addition of GnRH increased β-END-LI release from pituitary cells only in the OVX+EB II group. PHEN and PHENT potentiated β-END-LI secretion by pituitary cells in OVX+EB II and OVX+P_4 groups, while ISOP and PROP increased β-END-LI secretion by the cells of OVX and OVX+EB II animals. In turn, in the OVX+EB I group, effect of PHENT and PROP on PRL secretion by pituitary cells was inhibitory. In conclusion, our results suggest that adrenergic agents can modulate PRL and β-END secretion by porcine pituitary cells in a manner dependent on the hormonal status of gilts.

Key words: Pig-endocrinology, adrenergic agents, PRL, β-endorphin, GnRH, pituitary

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The pituitary is a site of synthesis and secretion of several hormones, including prolactin (PRL) and β-endorphin (β-END). PRL is synthesised and stored within lactotrophs of anterior lobe, while β-END within corticotrophs of the anterior lobe and melanotrophs of intermediate lobe of the pituitary gland (Bloom et al., 1977; Eipper and Mains, 1978; Lamberts and MacLeod, 1990). Secretion of these substances from anterior pituitary (AP) is controlled by stimulatory and inhibitory influences of hypothalamic origin. Many neuropeptides, such as thyrotropin-releasing hormone (TRH), vasopressin, neurotensin, galanin and the recently discovered PRL-releasing peptide (PrRP) could promote PRL release from pituitary (Lamberts and MacLeod, 1990; Ben-Jonathan and Liu, 1992; Hammond et al., 1996; Hinuma et al., 1998). In turn, release of β-END – proopiomelanocortin (POMC) processing product, co-released with ACTH – is mainly enhanced by corticotropin-releasing hormone (CRH), as well as by substance P, cholecystokinine (CCK), vasopressin, oxytocin (OT) and vasoactive intestinal peptide (VIP) (Matsumura et al., 1982, 1983; Sweep and Wiegant, 1989; Bogacka et al., 2002*a*). Catecholamines and gonadotrophin-releasing hormone (GnRH) are other candidates, which may influence pituitary PRL and β-END secretion (Denef and Baes, 1982; Gambacciani et al., 1988; Kerdelhue et al., 1988; Shin and Barton, 1993; Denef, 1994; Colthorpe et al., 2000). Studies primarily performed on laboratory rodents have established that catecholamines are involved in the regulation of prolactin secretion (Vijayan and McCann, 1978; Weiner and Ganong, 1978; Deaver and Dailey, 1982) and β-END secretion (Vermes et al., 1980; Berkenbosch et al., 1981; Pettibone and Mueller, 1982). Besides acting at the level of hypothalamus, catecholamines can also directly influence the pituitary gland functions. β-Adrenergic receptors have been detected in the anterior pituitary of rats (Denef and Baes, 1982; Perkins et al., 1985) and pigs (Perkins et al., 1985). It was also shown that stimulation of α- and β-adrenergic receptors by selective agonists induces release of pituitary β-END (Vermes et al., 1980; Berkenbosch et al., 1981; Pettibone and Mueller, 1982). Moreover, GnRH was found to stimulate differentiation of lactotrophs and to increase their number in the rat pituitary during postnatal period (Van Bael et al., 1994). GnRH may also affect β-END release from anterior pituitary (Gambacciani et al., 1988; Kerdelhue et al., 1988).

It is known that endocrine functions of the anterior pituitary gland are additionally modulated by ovarian steroids. Oestradiol was found to stimulate PRL gene transcription, PRL synthesis and secretion (Shull and Gorski, 1986; Song et al., 1989). Moreover, oestradiol treatment alone and in combination with progesterone has been shown to increase the percentage of immunoreactive lactotrophs among anterior pituitary cells in ovariectomised (OVX) rats (Livingstone et al., 1998). Oestrogens are also capable of affecting the anterior pituitary β-END content and secretion (Petraglia et al., 1982; Kerdelhue et al., 1988).

Generally, data on the action of catecholamines on PRL and β-END secretion by pituitary cells under different physiological or experimental conditions are limited and such studies have not been conducted on pigs. Moreover, it seems that under certain physiological conditions (e.g. proper steroid milieu) GnRH, a major stimulator of LH secretion, may induce additional effects in this gland. The studies were thus designed to examine the direct effect of GnRH, α- and β-adrenergic stimulators and blockers on secretion of PRL and β-endorphin-like immunoreactivity (β-END-LI) from dispersed pituitary cells obtained from OVX gilts primed, or not, with either oestradiol benzoate (EB) or progesterone (P_4).

Materials and methods

Chemicals

Dulbecco's modified Eagle's medium, McCoy's 5a medium, GnRH, phenylephrine (PHEN), phentolamine (PHENT), isoproterenol (ISOP), MEM-non-essential amino acids, gentamicin, nystatin, Hepes, bacitracin, glucose, IBMX and steroid standards were purchased from Sigma (St. Louis, MO, USA). Porcine LH was kindly supplied by the National Hormone and Pituitary Agency, University of Maryland, USA. Labelled hormones: (1,2,6,7-^{3}H) progesterone and (2,4,6,7-^{3}H) oestradiol were from Amersham, UK. Oestradiol benzoate, progesterone, propranolol (PROP) and antibiotics (penicillin, streptomycin) were obtained from Polfa (Poland). Porcine β-endorphin and antisera against β-endorphin were obtained from Peninsula Laboratories Inc. (Belmont, CA, USA). Prolactin was isolated from porcine pituitaries and kindly provided by Prof. Kazimierz Kochman (The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna near Warsaw, Poland). Trypsin was product of the Laboratory of Sera and Vaccines (Lublin, Poland) and trypan blue of Chemapol (Czech Republic). Dowex anion-exchange resin (AG 1-X8; formate form, 100–200 mesh) was purchased from Bio-Rad Laboratories (Austria). Other reagents not mentioned in the text were from Sigma.

Experimental animals

The studies were carried out in accordance with the principles and procedures of the Animal Ethics Committee at the University of Warmia and Mazury. Mature gilts, at 7–8 months of age, were ovariectomised under general anaesthesia and one month later randomly assigned to one of the following treatment groups: (1) OVX – i.m. injection of corn oil (placebo), 1 ml/100 kg b.w.; (2) OVX+EB I – injection of 2.5 mg EB/100 kg b.w. at 30–36 h before slaughter; (3) OVX+EB II – injection of 2.5 mg EB/100 kg b.w. at 60–66 h before slaughter;

(4) OVX+P_4 – injection of P_4 (120 mg/100 kg b.w.) for 5 consecutive days before slaughter. Gilts within these four experimental groups represented different hormonal status: (1) OVX – the regulation of hypothalamo-pituitary pathway independent of ovarian steroids; (2) OVX+EB I and (3) OVX+EB II – negative and positive feedback between oestradiol and LH (a preovulatory-like surge of LH), respectively; (4) OVX+P_4 – luteal phase of the oestrous cycle. Numbers of animals within groups used in particular experiments are detailed in the figures. For confirmation of the hormonal status of gilts the peripheral blood samples were collected during slaughtering to establish the plasma LH, 17β-oestradiol (E_2), and P_4 concentrations.

Anterior pituitary cell culture

Tissues from each animal were processed and cultured separately. The cells were prepared for culturing as described previously (Siawrys et al., 2002). Briefly, the anterior lobe pieces were prepared in Dulbecco's medium containing 0.1% bovine serum albumin (BSA), antibiotics, nystatin and then dispersed with 0.3% trypsin (15 ml/g tissue). After centrifugation the supernatant was discarded, and the cell pellet was washed three times and resuspended in McCoy's 5a medium containing horse serum (10%) and fetal calf serum (2.5%). The cells were diluted with culture medium to 10^6 cells/ml. The cell suspension was then placed in 24-well plates and cultured under the atmosphere of 95% air and 5% CO_2 for 3 days at 37 °C. Subsequently, culture media were changed, plates were rinsed and pituitary cells were cultured (3.5 h) in McCoy's 5a medium (without sera) containing ascorbic acid (0.57 nM) and bacitracin (20 μM) in the absence (control) or presence of tested factors, in two independent experiments.

Experiment I: Effects of GnRH and α- and β-adrenergic agents on PRL secretion. In this experiment, the cultured cells within each group of gilts were divided into eleven subgroups treated with: GnRH – 100 ng/ml; PHEN (α-adrenergic agonist) – 10 nM, 100 nM, 1 μM; PHENT (α-adrenergic blocker) – 1 μM, alone and in combination with PHEN (100 nM); ISOP (β-adrenergic agonist) – 10 nM, 100 nM, 1 μM; PROP (β-adrenergic blocker) – 1 μM, alone and in combination with ISOP (100 nM). The media of the control cultures did not contain the aforementioned factors.

Experiment II: Effects of GnRH and α- and β-adrenergic agents on β-END-LI secretion. In this experiment, adrenergic agonists (100 nM) and antagonists (1 μM) were separately used (without combinations) at only one chosen concentration.

The doses of studied factors were chosen on the basis of data from the literature (Denef and Baes, 1982; Pettibone and Mueller, 1982; Perkins et al., 1985) and our preliminary experiments (Siawrys et al., 2002). Following 3.5 h

incubation the media from experiments I and II were collected and stored at –20 °C until assayed for PRL (experiment I) and β-END-LI (experiment II).

Hormone assays

Steroid hormones. Plasma concentration of E_2 was analysed according to the method described by Hotchkiss et al. (1971). Cross-reactivity of the antiserum against E_2 has been published previously by Szafrańska and Tilton (1993). The sensitivity of the assay was 10.33 pg/ml. The intra-assay coefficient of variation was 6.25%.

Plasma P_4 level was analysed according to the method described by Ottobre et al. (1989). The specificity of the antibodies for P_4 has been reported by Dziadkowiec et al. (1982). The sensitivity of the assay was 22 pg/ml. The intra-assay coefficient of variation was 5.65%.

Luteinising hormone. Plasma concentration of LH was determined by RIA procedure, previously described by Ziecik et al. (1978). Cross-reactivity of the antiserum against LH has been published previously by Szafrańska and Tilton (1993). The sensitivity of the assay was 0.08 ng/ml. The intra-assay coefficient of variation was 8.54%.

PRL. PRL concentration in the culture media was determined by RIA procedure, previously described by Dusza and Krzymowska (1979). Porcine PRL was used for iodination and standards. The goat antiserum against porcine PRL did not exhibit cross-reactivity with porcine LH and FSH. The sensitivity of the assay was 0.09 ng/ml. The intra- and inter-assay coefficients of variation were 4.64% and 6.62%, respectively.

β-END-LI. β-END-LI in media was determined by the double-antibody RIA procedure described by Okrasa et al. (1995) and Przała et al. (2001). The rabbit antiserum against β-endorphin exhibited equimolar cross-reactivity (100%) with β-endorphin and β-lipotropin, but did not exhibit any cross-reactivity with α-endorphin, γ-endorphin, met-enkephalin, ACTH and α-MSH. The antiserum against rabbit γ-globulin was produced in our Department (Szafranska et al., 2002). Porcine β-endorphin was used for iodination and standards. The sensitivity of the assay was 25 pg/ml. The intra- and inter-assay coefficients of variation were 5.93% and 6.02%, respectively.

Statistical analysis

Concentrations of hormones (LH, E_2 and P_4) in plasma of experimental gilts are expressed as means ± SEM. All data from *in vitro* studies for PRL and β-END-LI are presented as the percentage (means ± SEM) of basic secretion (= 100%), established in control pituitary cell incubation for each experimental animal. Data were subjected to a one-way analysis of variance (ANOVA) and

significant differences between control and treatment mean values within each experimental group were determined by Student's *t*-test. The differences between means were assumed as significant for $p < 0.05$ and highly significant for $p < 0.01$.

Results

Plasma LH, E_2 and P_4 concentrations in experimental gilts

Mean plasma E_2 concentrations in gilts of the OVX, OVX+EB I, and OVX+EB II groups were 15.95 ± 0.78 pg/ml, 29.05 ± 1.64 pg/ml and 23.07 ± 1.64 pg/ml, respectively. In gilts of the same groups, plasma LH concentrations were 1.56 ± 0.01, 0.45 ± 0.06 and 2.88 ± 0.43 ng/ml, respectively. The changes ($p < 0.01$) in LH levels in the OVX+EB I and OVX+EB II groups in comparison to OVX gilts have thus confirmed the negative and positive feedback, respectively, between oestradiol and LH. Plasma P_4 concentrations in the OVX and OVX+P_4 groups were 0.70 ± 0.03 ng/ml and 9.42 ± 0.25 ng/ml, respectively.

Experiment I: Effects of GnRH and α- and β-adrenergic agents on PRL secretion in vitro

Basal PRL secretion *in vitro* by porcine pituitary cells was not significantly ($p > 0.05$) changed following *in vivo* pretreatment of OVX gilts with EB at 30–36 h (562.67 ± 27.41 ng/ml) and 60–66 h before slaughter (541.51 ± 43.34 ng/ml) in comparison with OVX animals (627.24 ± 31.67 ng/ml). Progesterone priming of OVX gilts *in vivo* was also without effect on subsequent PRL release *in vitro* by pituitary cells (643.71 ± 30.74 ng/ml).

In all groups of animals (OVX, OVX+EB I, OVX+EB II and OVX+P_4) the addition of GnRH to culture media was not followed by significant ($p > 0.05$) changes in PRL release from pituitary cells (Figs 1, 2a, 2b and 3).

In the present study, the α- and β-adrenergic agents have caused increases in PRL secretion by the cells depending on the hormonal status of gilts. Treatment with α-adrenergic agonist, PHEN (10 nM and 1 μM; $p < 0.05$ and $p < 0.01$, respectively), or with β-adrenergic agonist, ISOP (10 nM and 1 μM; $p < 0.05$ and $p < 0.01$, respectively), increased PRL release by pituitary cells from OVX gilts (Fig. 1). In contrast, neither α-adrenergic nor β-adrenergic agents affected PRL secretion by pituitary cells derived from OVX+EB I animals (Fig. 2a). PRL secretion by pituitary cells from OVX+EB II gilts was potentiated ($p < 0.05$) by α-adrenergic agonist, PHEN (100 nM) (Fig. 2b). The stimulatory effect of PHEN (100 nM) was blocked by α-adrenergic antagonist, PHENT. However, simultaneous treatment of the pituitary cells with β-adrenergic agonist (ISOP; 100 nM) and antagonist (PROP; 1 μM) slightly increased ($p < 0.05$) PRL secretion in spite

of the lack of significant responses to these agents separately used. Pituitary cells derived from OVX+P_4 gilts (Fig. 3) increased PRL secretion in response to α-adrenergic agonist, PHEN (100 nM, $p < 0.05$), and blocker, PHENT (1 μM, $p < 0.01$), applied alone or together ($p < 0.05$). Moreover, β-adrenergic agonist, ISOP (100 nM, 1 μM; $p < 0.01$), and blocker, PROP (1 μM; $p < 0.01$), alone, as well as ISOP (100 nM) with PROP (1 μM; $p < 0.05$), significantly potentiated PRL release from these cells.

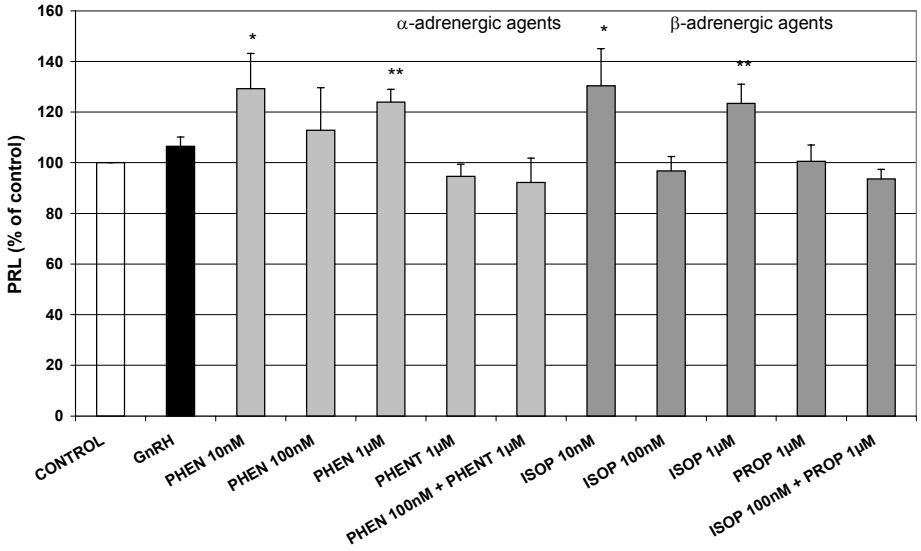

Fig. 1. Effects of GnRH and adrenergic agents on PRL release by cultured pituitary cells derived from OVX gilts (n = 7). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of PRL secretion by control cells (= 100%). Basal (control) secretion of PRL was 627.24 ± 31.67 ng/ml. Significant differences *vs*. control: $^{*}p < 0.05$; $^{**}p < 0.01$

Experiment II: Effects of GnRH and α- and β-adrenergic agents on β-END-LI secretion in vitro

Basal β-END-LI secretion by pituitary cells of OVX gilts was 11.90 ± 1.08 ng/ml. Pretreatment of OVX gilts *in vivo* with EB (30–36 h and 60–66 h before slaughter) was without significant effect on β-END-LI release from their pituitary cells *in vitro* (10.34 ± 2.22 ng/ml and 14.61 ± 2.03 ng/ml, respectively), while with P_4 increased ($p < 0.05$) β-END-LI secretion by these cells (15.71 ± 1.08 ng/ml).

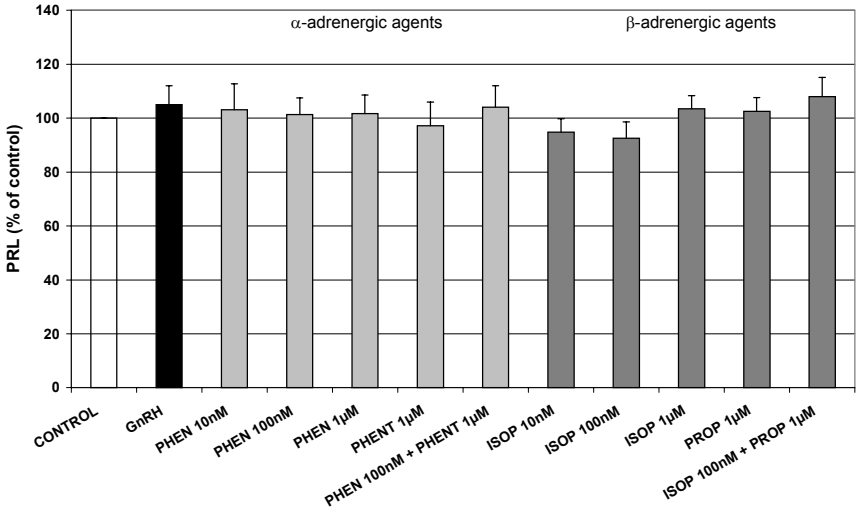

Fig. 2a. Effects of GnRH and adrenergic agents on PRL release by cultured pituitary cells derived from OVX+EB I gilts (n = 9). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of PRL secretion by control cells (= 100%). Basal (control) secretion of PRL was 562.67 ± 27.41 ng/ml

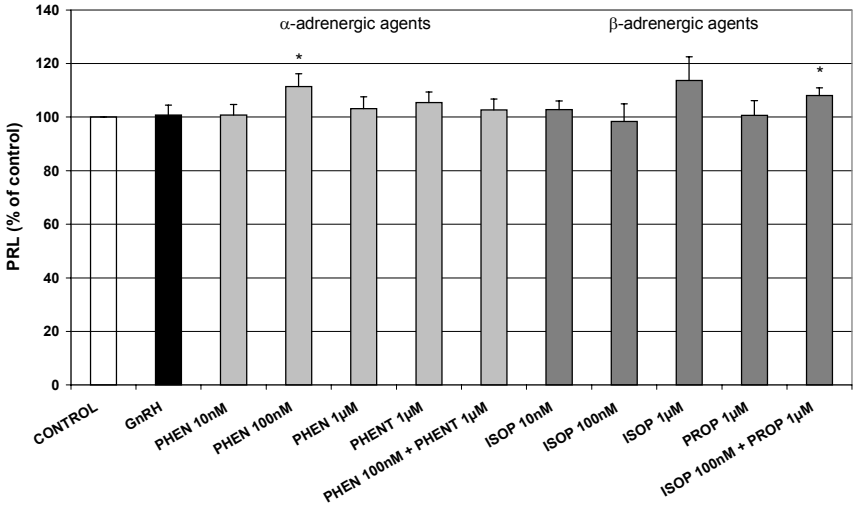

Fig. 2b. Effects of GnRH and adrenergic agents on PRL release by cultured pituitary cells derived from OVX+EB II gilts (n = 10). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of PRL secretion by control cells (= 100%). Basal (control) secretion of PRL was 541.51 ± 43.34 ng/ml. Significant differences *vs*. control: $^*p < 0.05$

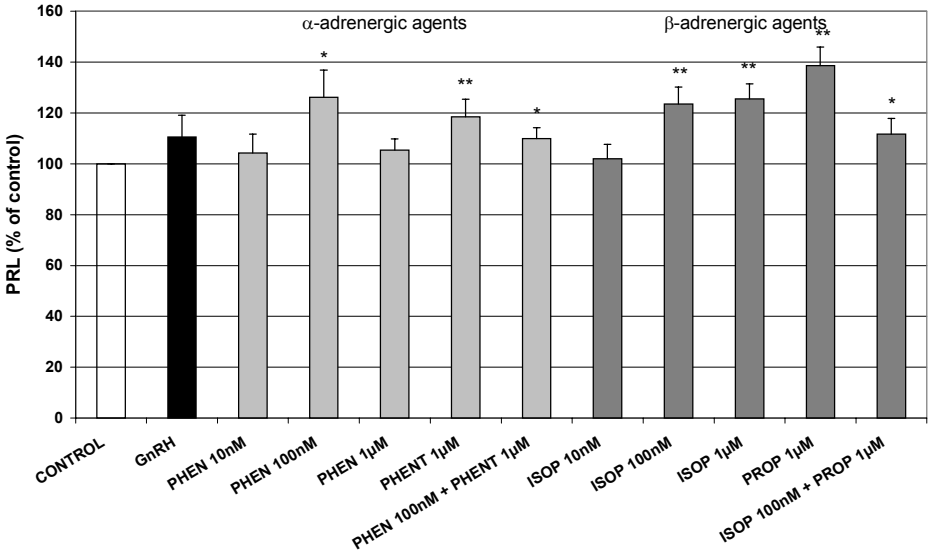

Fig. 3. Effects of GnRH and adrenergic agents on PRL release by cultured pituitary cells derived from OVX+P_4 gilts (n = 8). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of PRL secretion by control cells (= 100%). Basal (control) secretion of PRL was 643.71 ± 30.74 ng/ml. Significant differences *vs*. control: $^{*}p < 0.05$; $^{**}p < 0.01$

GnRH enhanced ($p < 0.05$) β-END-LI secretion by pituitary cells of OVX gilts treated with EB 60–66 h before slaughter (Fig. 5b). However, its effects on pituitary cells derived from gilts of other groups were negligible (Figs 4, 5a and 6).

The stimulation of α- and β-adrenergic receptors caused some changes in β-END-LI secretion by the cells depending on the hormonal status of gilts. β-adrenergic agonist, ISOP (100 nM), and antagonist, PROP (1 μM), significantly ($p < 0.05$ and $p < 0.01$, respectively) stimulated β-END-LI release from pituitary cells of OVX gilts (Fig. 4). In contrast, α-adrenergic agents, PHEN (100 nM) and PHENT (1 μM), failed to alter β-END-LI secretion by pituitary cells taken from these animals (Fig. 4). Treatments with adrenergic blockers, PHENT (1 μM) and PROP (1 μM), decreased ($p < 0.01$ and $p < 0.05$, respectively) β-END-LI release by pituitary cells from the OVX+EB I gilts, while the effects of adrenergic agonists, PHEN (100 nM) and ISOP (1 μM), were negligible (Fig. 5a). In turn, in the presence of both α- and β-adrenergic agents, β-END-LI secretion by pituitary cells of OVX+EB II animals (PHEN; $p < 0.05$; PHENT, ISOP and PROP; $p < 0.01$) was potentiated (Fig. 5b). In the OVX+P_4 group, only α-adrenergic agents, agonist (PHEN) and blocker (PHENT), were able to stimulate ($p < 0.05$) β-END-LI output from these cells (Fig. 6).

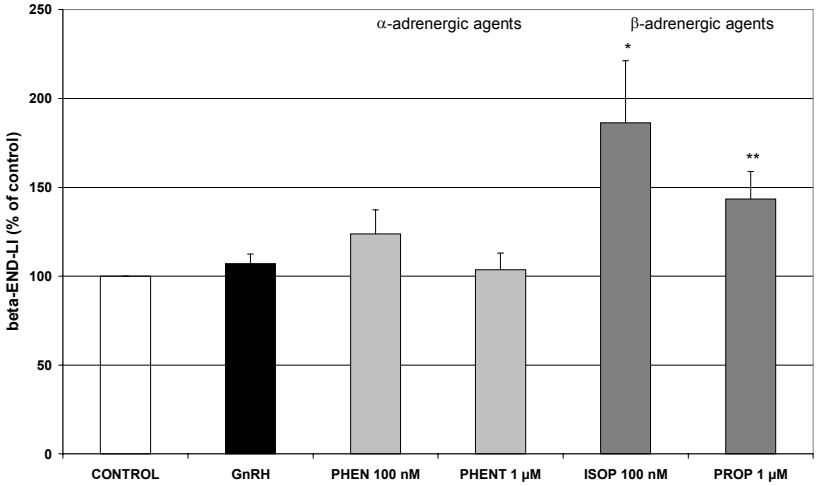

Fig. 4. Effects of GnRH and adrenergic agents on β-END-LI release by cultured pituitary cells derived from OVX gilts (n = 6). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of β-END-LI secretion by control cells (= 100%). Basal (control) secretion of β-END-LI was 11.90 ± 1.08 ng/ml. Significant differences vs. control: $^{*}p < 0.05$; $^{**}p < 0.01$

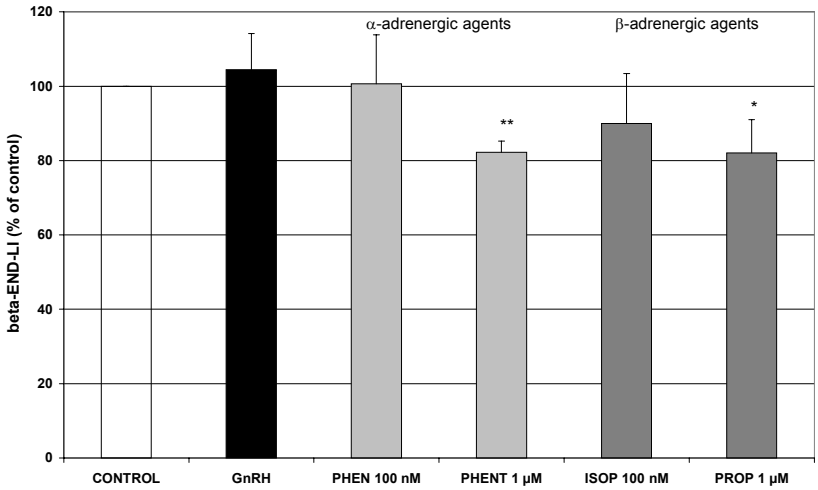

Fig. 5a. Effects of GnRH and adrenergic agents on β-END-LI release by cultured pituitary cells derived from OVX+EB I gilts (n = 4). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of β-END-LI secretion by control cells (= 100%). Basal (control) secretion of β-END-LI was 10.34 ± 2.22 ng/ml. Significant differences vs. control: $^{*}p < 0.05$; $^{**}p < 0.01$

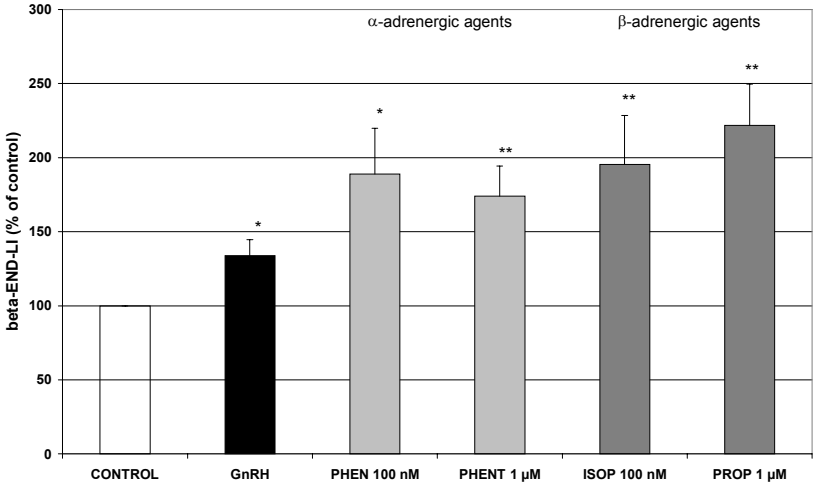

Fig. 5b. Effects of GnRH and adrenergic agents on β-END-LI release by cultured pituitary cells derived from OVX+EB II gilts (n = 4). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of β-END-LI secretion by control cells (= 100%). Basal (control) secretion of β-END-LI was 14.61 ± 2.03 ng/ml. Significant differences *vs*. control: $^{*}p < 0.05$; $^{**}p < 0.01$

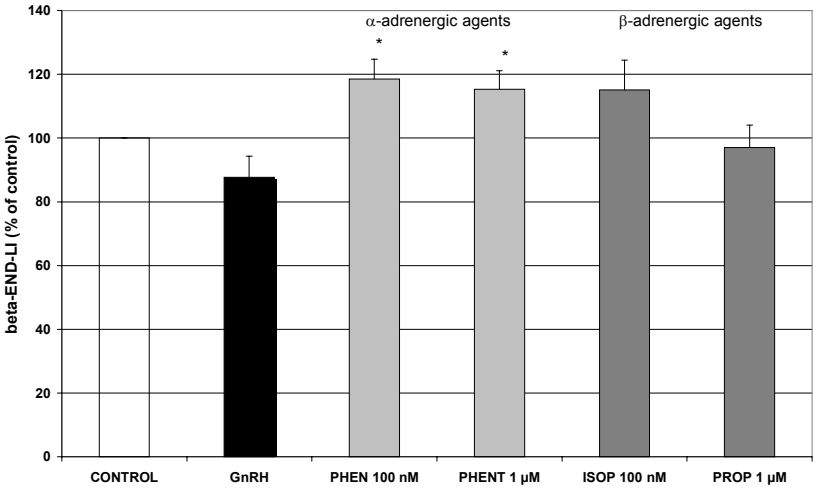

Fig. 6. Effects of GnRH and adrenergic agents on β-END-LI release by cultured pituitary cells derived from OVX+P_4 gilts (n = 7). The cells (10^6/well) were treated for 3.5 h with medium alone (control), GnRH (100 ng/ml), phenylephrine (PHEN; α-adrenergic agonist), phentolamine (PHENT; α-adrenergic blocker), isoproterenol (ISOP; β-adrenergic agonist) or propranolol (PROP; β-adrenergic blocker). Data are presented as the percentage (mean ± SEM) of β-END-LI secretion by control cells (= 100%). Basal (control) secretion of β-END-LI was 15.71 ± 1.08 ng/ml. Significant differences *vs*. control: $^{*}p < 0.05$

Discussion

The effect of GnRH, α- and β-adrenergic agents on PRL secretion by pituitary cells

In the present study, GnRH did not influence the secretion of PRL by pituitary cells taken from gilts of all experimental groups. In earlier *in vivo* studies performed on cyclic gilts, a bolus injection of GnRH during an opioid agonist (FK 33-842) infusion did not evoke remarkable changes in PRL secretion comparing to this hormone response to FK 33-824 alone administration (Okrasa and Tilton, 1992). Lafuente et al. (1995) also failed to observe GnRH effect on plasma PRL concentration in adult female rats. Andries and Denef (1995) revealed a stimulatory effect of GnRH on PRL secretion by pituitary cells derived from infant female rats during a restricted period of postnatal life (from day 3 to about day 9 after birth), but not from adult rats (Denef, 1994). These authors suggested that GnRH induces lactotroph development, and its action is mediated by an intracellular signal from gonadotrophs. Our data suggest that the effect of GnRH on PRL secretion does not occur in mature gilts, like in adult female rats.

There are studies indicating that catecholamines (e.g. dopamine, epinephrine, norepinephrine) or adrenergic agents directly alter anterior pituitary functions (Denef and Baes, 1982; Swartz and Moberg, 1986; Li, 1989; Shin and Barton, 1993; Colthorpe et al., 2000). In this research, we have observed small (up to approx. 40%), but significant, stimulatory effects of α- and/or β-adrenergic agents on basal PRL secretion *in vitro* by porcine pituitary cells, dependently on hormonal status of gilts. Pituitary cells derived from OVX gilts either primed, or not, with P_4 appeared to be the most susceptible to the action of both α- and β-adrenergic agents. In contrast, priming of experimental gilts with EB evidently reduced responsiveness of the cells to adrenergic agents in *in vitro* experiments performed 30–36 or 60–66 h after the treatment. Cultured pituitary cells of adult ewes diminished PRL release in the presence of α-adrenergic agonist (PHEN) and biphasically responded to β-adrenergic agonist (ISOP), with increased and decreased secretion of this hormone (Colthorpe et al., 2000). However, the anterior pituitary tissue from castrated ram lambs, studied in an *in vitro* perfusion system, did not change PRL secretion in response to catecholamines (norepinephrine, epinephrine) and β-adrenergic agent (ISOP) (Swartz and Moberg, 1986). Likewise, in female rats, there was no effect of α-adrenergic agonist (PHEN) (Caron et al., 1978; Swennen and Denef, 1982) and β-adrenergic agonist (ISOP) (Caron et al., 1978) on PRL secretion by pituitary cells. Studies performed with pituitary cells derived from male rats have yielded discrepant results. Perkins et al. (1985) did not observe any influence of β-adrenergic agonist (ISOP) on PRL release from pituitary cells, whereas Denef and Baes (1982) and Shin and Barton (1993) have demonstrated a stimulatory effect of the agonist on PRL secretion. Moreover, on the basis of pharmacological studies, Shin and Bar-

ton (1993) concluded that β_2-adrenergic receptors are implicated in the stimulation of PRL secretion and then suggested their presence within lactotrophs. Collectively, it seems that an appearance of adrenergic agent influence on PRL secretion by pituitary cells in mammals depends on species, sex and physiological status of animals, as well as on the experimental model applied. Our results indicate that moderate stimulatory influence of α- and β-adrenergic compounds on PRL secretion may occur in sexually matured gilts deprived of ovarian steroids or substituted with P_4, but the effect markedly diminishes in oestrogenic milieu.

In addition, a short commentary pertaining to stimulatory action of adrenergic antagonists, used alone or in combination with the agonist, on PRL secretion by pituitary cells of OVX gilts primed with P_4 is required. The paradoxical responses observed in our study might arise, among others, from quantitative and/or qualitative changes in adrenergic receptor population on pituitary cells in response to treatment with P_4. Alternatively, the *in vivo* pretreatment of gilts with P_4 could, in consequence, modify paracrine interactions in further pituitary cell culture. A comparable phenomenon concerning α-adrenergic receptors and *in vivo* secretion of prolactin in adult male rats has been noted by Jurcovicova et al. (1989*a, b*), as well as in the present study in relation to both types of adrenergic receptors and β-END secretion. However, further studies are needed to completely elucidate this problem.

The effect of GnRH, α- and β-adrenergic agents on β-END secretion by pituitary cells

In the present study, GnRH significantly increased β-END-LI secretion by pituitary cells obtained only from gilts being in the phase of positive feedback between oestradiol and LH (OVX+EB II). The research of Gambacciani et al. (1988) has shown increased β-END release from hemipituitaries of OVX female rat perfused with medium containing GnRH (80 nM) for 45 min, while this decapeptide was ineffective during a shorter perfusion period (15 min). The study of Kerdelhue et al. (1988) also demonstrated a stimulatory effect of GnRH on β-END release from perfused pituitary tissue of pro-oestrous rats. In our study, we noted stimulatory effect of GnRH on pituitary β-END secretion exclusively in gilts representing the positive feedback between oestradiol and LH, i.e. 60–66 h after the treatment with EB (the stage of maximum LH secretion). The observation possibly reflected a relationship, which under physiological conditions may be a part of mechanism responsible for 'extinction' of the preovulatory LH surge. This hypothesis was discussed in our previous report (Bogacka et al., 2002*b*).

A stimulatory effect of β-adrenergic agents on β-END secretion by pituitary cells of OVX gilts was observed in the present study. Priming with P_4 has abolished responsiveness of the cells to β-adrenergic compounds, but increased their susceptibility to α-adrenergic agents. The most evident influences of adrenergic compounds (both α and β) on β-END secretion by pituitary cells appeared

in gilts during the positive feedback between oestradiol and LH (OVX+EB II) in contrast to the negative feedback-phase gilts (OVX+EB I). The potentiated stimulatory action of adrenergic system on the anterior pituitary β-END secretion during advanced stages of the LH surge, like that of GnRH, may play an important role in the process of its termination, since this opioid peptide is a potential, local inhibitory factor affecting LH secretion. The study of Barb et al. (1990) has shown that β-END is capable of decreasing LH release from cultured porcine pituitary cells. On the basis of our data, endogenous catecholamines can be appended to a group of factors – besides GnRH (as considered above), OT, and VIP (Bogacka et al., 2002*b*) – which, transiently acting in concert (through pituitary β-END), may participate in the LH surge termination in sows.

Studies performed on laboratory animals investigated the role of adrenergic agents in the regulation of pituitary β-END secretion in other contexts than that considered in our research. The influences of adrenergic agents on β-END secretion in male rats were demonstrated in earlier *in vivo* and *in vitro* studies performed by Pettibone and Mueller (1982). They suggested a participation of α- and β-adrenergic receptors in stimulation of β-END release from anterior and neurointermediate pituitary lobes, respectively. However, in the study of Vermes et al. (1980) adrenergic factors did not affect β-END secretion by anterior pituitary cells of female rats. The action of adrenergic compounds on β-END secretion by neurointermediate lobe cells was confirmed in many studies (Vermes et al., 1980; Sweep and Wiegant, 1989; Némethy et al., 1998). Overall, our results and the cited data – although divergent in several aspects – imply that numerous factors, including hormonal status of studied animals or experimental protocol (e.g. duration of cell incubation), may condition the pituitary β-END response to treatments with various adrenergic agents.

Generally, this paper presents the first evidences for a participation of adrenergic agents in modulation of PRL and β-END secretion by anterior pituitary cells of the pig. The modulatory action of adrenergic compounds was found to be dependent on hormonal status of the studied animals. Moreover, in a similar manner GnRH appeared to affect β-END secretion by porcine anterior pituitary cells.

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