JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2000, 51, 2, 359—368

T. KAMIŃSKI[1], B. GAWROŃSKA[2], K. DERECKA[2], S. OKRASA[1], J. PRZAŁA[1]

GENE EXPRESSION AND PEPTIDE LOCALIZATION FOR LH/hCG RECEPTOR IN PORCINE SMALL AND LARGE LUTEAL CELLS: POSSIBLE REGULATION BY OPIOID PEPTIDES

[1]Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, [2]Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

The aim of the present studies was to investigate (1) the presence of LH receptor (LHR) in porcine separated small (SLCs) and large (LLCs) luteal cells taken from mid-luteal corpora lutea and (2) the influence of opioid agonist, FK 33—824 (FK) on LHR gene expression in these cells. Immunocytochemistry revealed intense staining for LHR in both SLCs and LLCs. Reverse transcription-polymerase chain reaction (RT-PCR) and Southern hybridization were used to check the effect of FK and hCG on LHR gene expression. The LHR gene expression was observed in non-stimulated LLCs and in both types of cells after treatment with FK or hCG. FK stimulated LHR gene expression in SLCs and inhibited the gene expression in LLCs. Moreover, FK inhibited and potentiated stimulatory influence of hCG on the gene expression in LLCs and SLCs, respectively. These results suggest that LHR gene expression in porcine luteal cells can be regulated by opioid peptides.

K e y w o r d s: *LH receptor, opioid peptides, gene expression, RT-PCR, immunocytochemistry*

ABBREVIATIONS:

ABC complex	avidin-biotin-horseradish peroxide complex
BSA	bovine serum albumin
cDNA	complementary deoxyribonucleic acid
CL	corpus luteum
FK	FK 33–824
hCG	human chorionic gonadotrophin
LDL	low density lipoprotein
LH	luteinizing hormone
LHR	luteinizing hormone receptor
LLCs	large luteal cells
mRNA	messenger ribonucleic acid
PBS	phosphate buffered saline
RT–PCR	reverse transcription-polymerase chain reaction
SDS	sodium dodecyl sulphate
SLCs	small luteal cells
SSC	saline sodium citrate
TBS	Tris-hydroxylmethylaminomethane — buffered saline

INTRODUCTION

Corpora lutea (CL) are transient endocrine glands, which are composed of luteal and non-luteal cells. The steroidogenic luteal cells, which form the endocrine component of the gland, are comprised of two types of morphologically distinct cells: large luteal cells (LLCs) and small luteal cells (SLCs). Both types of luteal cells produce progesterone (P_4) and secretion of this steroid from mature porcine CL is stimulated by LH (1). It is also known that both SLCs and LLCs increase P_4 production under the influence of hCG in the pig (2). Specific receptors with high affinity for LH/hCG have been found in porcine luteal cells (3), granulosa cells (4) and theca interna cells (5).

In addition to LH, the main regulator of CL functions, many other factors can influence luteal functions, both directly and through modulating LH action. Earlier studies have indicated that endogenous opioid peptides are one group of these factors. β-Endorphin, one of the well-known representatives of opioids, has been found in the corpora lutea of sheep (6), pigs (7, 8) cows (9) and in human ovary (10). Met-enkephalin, another extensively examined opioid peptide, has been demonstrated in bovine, human and rabbit ovary (11—13). Peptides that belong to the dynorphin family have been identified in the CL of cows (9) and luteal cells of rats (14). Confirmation of local opioid precursor production in the ovaries has come by way of identification of mRNAs for proopiomelanocortin (15—17), proenkephalin (16) and prodynorphin (18). These findings and reports concerning the influence of opioids on steroidogenesis in human granulosa cells (19), in rat luteal cells (20) and in bovine luteal cells (21) suggest that opioid peptides exert autocrine and/or paracrine effects in the ovary. Moreover, results obtained in our laboratory (2) indicate that hCG-induced P_4 secretion by pig small luteal cells is reduced by an opioid agonist, FK 33—824. However, the mechanism of action of opioids on porcine luteal cells is only superficially understood.

The aim of this study was: (1) to check the localisation of LH receptor peptide and appropriate mRNA in isolated porcine SLCs and LLCs and (2) to elucidate the possible effect of the opioid agonist, FK 33—824 (FK) on the expression of LH receptors (LHR) in these cells.

MATERIALS AND METHODS

Animals

Ovaries were recovered from mature cross-bred gilts on Days 8—10 of the oestrous cycle in a local slaughterhouse and transported to the laboratory in cold PBS with penicillin (50 IU/ml), streptomycin (50 μg/ml) and gentamicin (50 μg/ml). The stage of the oestrous cycle was confirmed according to Akins and Morrissette (22).

Luteal cell dispersion and separation

Corpora lutea were dissected from ovaries, weighed, minced into small fragments (1—2 mm) and dispersed with 0.125% trypsin solution in F-12 medium (Sigma, St. Louis, MO, USA). The medium was supplemented with gentamicin (50 μg/ml) and nystatin (240 U/ml). Luteal cells were obtained by sequential dissociations of the luteal tissue (4 to 6 times, 10 min each) at 37°C and centrifugations (800 × g for 10 min). The cells were rinsed (3 times) with fresh medium, filtered through a nylon filter (75 μm mesh) to remove undigested fragments of the tissue and counted in a haemocytometer. Cell viability (100%) was determined by trypan blue dye exclusion.

To separate small and large luteal cell fractions unit gravity sedimentation system was used (CELSEP, Du Pont, USA). This system provides facilities for separation of cells differing by more than 0.5 microns in diameter with excellent resolution. Concentrated luteal cells (5×10^7) were resuspended in 50 ml of 1% Ficoll 400 (Pharmacia, Sweden) and then transferred to a previously prepared linear density gradient of Ficoll 400, using initial concentrations 2 and 4%. Distribution of the cells was carried out for two hours at room temperature. Fractions (20 ml) were collected and the cells washed (3 times) in M199 medium (Sigma, St. Louis, MO, USA) with the addition of 1g BSA/100 ml. Fractions 2—5 contained large cells (>30 μm in diameter) and fractions 17—28, small cells (10—20 μm). The viability of SLCs and LLCs after separation was 100% and 96.87 ± 2.23%, respectively. Contamination of large cells by small cells was 15.78 ± 4.58%, as determined by light microscopy on the basis of size and morphology. The fractions of small cells were completely free from contamination by large ones.

Luteal cell culture

Small and large luteal cells were incubated in M199 medium plus hydrocortisone (40 ng/ml; Sigma, St. Louis, MO, USA), transferrin (5 μg/ml; Sigma, St. Louis, MO, USA), insulin (2 μg/ml; Sigma, St. Louis, MO, USA), gentamicin (50 μg/ml), nystatin (240 U/ml), porcine LDL (100 μg/ml) containing 50 μg cholesterol, and 1% BSA. The concentrations of SLCs and LLCs used in the experiment were 10^6 cells/well and 10^5 cells/well, respectively. Small and large luteal cells were cultured in 1 ml of incubation medium in 24-well plastic plates or on glass coverslips (in experiments related to immunocytochemistry) for 12 h, at 37°C, and in a humidified atmosphere of 5% CO_2 : 95% air. Small and large luteal cells were treated with hCG (100 ng/ml; kindly provided by the National Hormone and Pituitary Agency, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, University of Maryland, USA) alone, FK (10^{-9}M; Sigma, St. Louis, MO, USA) alone or were co-treated with FK and hCG or naloxone (NAL, 10^{-5} M; Sigma, St. Louis, MO, USA). None of the treatments affected the viability of the cells.

Immunocytochemistry

Luteal cells were cultured on glass coverslips for 12 h, and the attached cells were fixed in 4% paraformaldehyd for 20 min. The coverslips were washed in 0.05 M Tris-hydroxylmethylaminomethane — buffered saline (TBS), then placed in ethanol in ascending concentration series. The cells were treated with 1% H_2O_2 in methanol for 30 min to block endogenous peroxidases, and then in 0.75% of glycine in TBS for 30 min to block free aldehyde groups. Following rinsing in TBS, the coverslips were incubated overnight with primary antibody (diluted 1:400; a rabbit polyclonal antiserum directed against the amino acids 15—38 sequence of the rat LH receptor; a courtesy of Dr Patrick C. Roche, Mayo Clinic, Rochester, MN, USA). Antibody binding was detected with the ABC complex (avidin-biotin-horseradish peroxide

complex; Vectastain ABC kit from Vector Laboratories Inc., Burlingame, CA, USA). Peroxidase activity was revealed using 3,3'-diaminobenzidine as a substrate. Two types of controls were performed: the primary antibody was (1) omitted or (2) substituted with non-specific immunoglobulin G (IgG) during the immunostainin procedure. Some coverslips were counterstained with hematoxylin for better visualisation of cell nuclei.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from SLCs, LLCs and control tissues (positive – ovary, negative – intestine) according to Chomczynski and Sacchi (23). The purity of RNA was determined by the A_{260}/A_{280} ratio in spectrophotometry. A pair of oligonucleotide primers (upper – located in exon 9, nucleotides from 738 to 761 and lower – located in exon 11, nucleotides from 1456 to 1475) was designed to generate fragments of 740 bp from variant A (full length receptor) and 470 bp from variant B (shorter, lacking transmembrane domain) of porcine LH receptor. RT-PCR was performed using Tth DNA polymerase (Promega, Madison, MI, USA) having both reverse transcriptase and DNA polymerase activities. Two micrograms of total RNA per 100 µl reaction mixture were reverse-transcribed at 42°C for one hour, denatured at 97°C for 3 min, and amplified for 30 cycles including denaturation at 96°C for 30 sec, primer annealing at 55°C for 30 sec and extension at 72°C for 1 min. The PCR products (20 µl) were electrophoresed on 1.5% (w/v) agarose gel and photographed under UV light.

Southern hybridization

The hybridization of the PCR products was performed according to Sambrook *et al.* (24). A fragment of pig LH receptor cDNA (nucleotides from 960 to 2089), labelled with [α-^{32}P]dCTP using the random prime method (Rediprime Kit, Amersham, UK), was used as a probe. The hybridization was performed at 65°C for 12 h. After hybridization, the blots were washed twice with 1 × SSC (150 mmol/l NaCl, 15 mmol/l sodium citrate, pH 7.0), containing 0.1% (w/v) sodium dodecyl sulphate (SDS), for 15 min at room temperature, twice with 0.2 × SSC/ 0.1% (w/v) SDS at 65°C for 15 min and twice in 0.1 × SSC/ 0.1% (w/v) SDS at 65°C for 15 min. The washed sheets were exposed to Kodak X-ray film for 1 or 24 h.

RESULTS

Localisation of LH receptors in isolated luteal cells

Immunostaining for LH receptors in isolated SLCs and LLCs is shown in Fig. 1a and in Fig. 1b, respectively. Positive immunostaining for LH receptor was detectable in both SLCs and LLCs. Cytoplasm and plasma membrane of SLCs exhibited strong immunostaining for LHR. Immunoexpression of LHR in LLCs was solely confined to plasma membranes. No LH receptor immunostaining was observed when the primary antibody was replaced with non-specific IgG (*Fig. 1c, Fig. 1 d*).

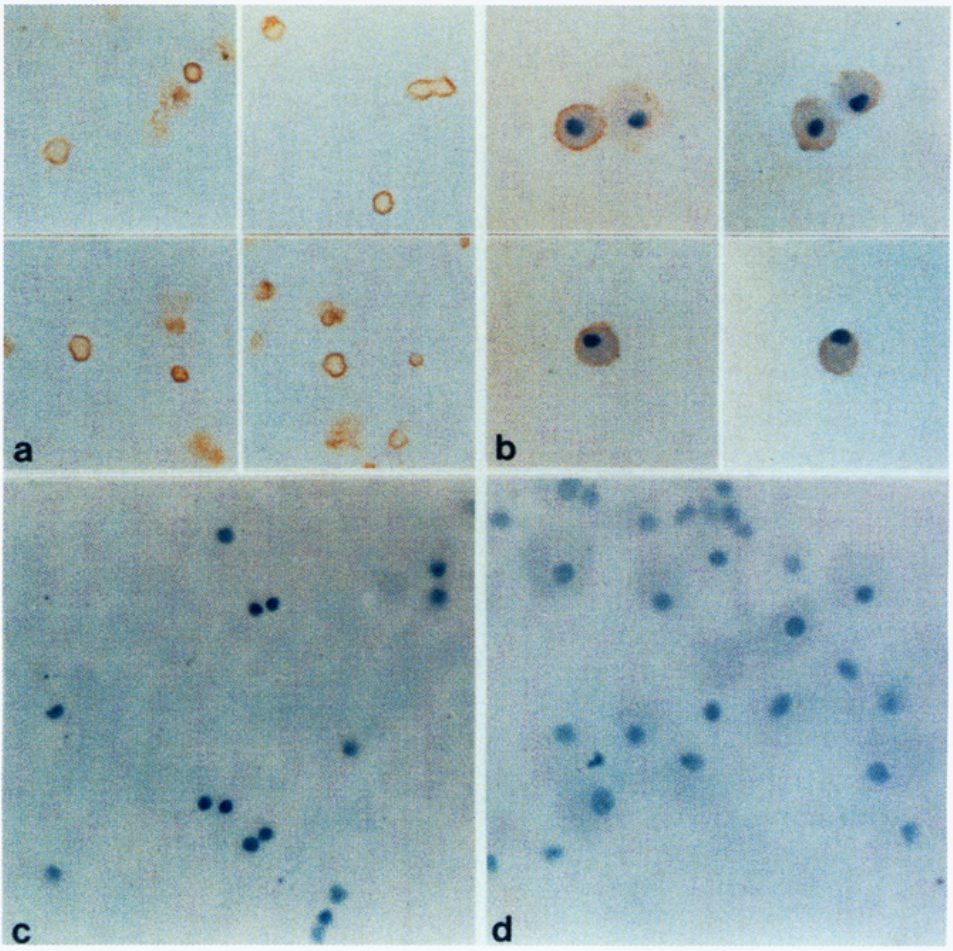

Fig. 1. Immunocytochemistry for LH receptors in porcine small (A) and large (B) luteal cells. C and D — control for small and large luteal cells, respectively, in which primary antibody was substituted with non-specific IgG.

Gene expression for LH receptor

Porcine LHR transcripts (variants A and B) were found in LLCs but not SLCs of the control groups. FK added alone to cultures stimulated LHR gene expression in SLCs but inhibited in LLCs. The influence of FK on the gene expression in SLCs was blocked by opioid antagonist, naloxone. Additionally, FK added with hCG potentiated stimulatory effect of hCG on SLCs and attenuated this effect in the case of LLCs. In studied groups RT-PCR products contained both variant A and variant B (*Fig. 2*).

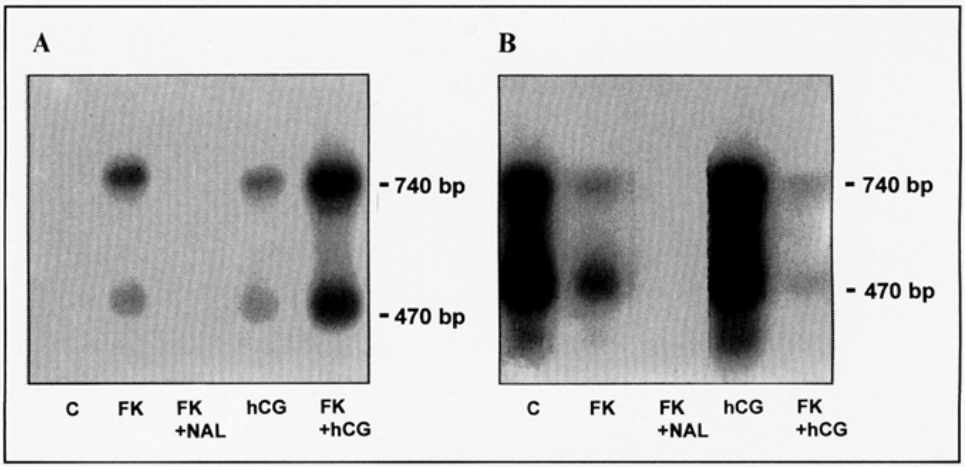

Fig. 2. Southern blot analysis of RT-PCR products of LH receptor transcripts in porcine small (A) and large (B) luteal cells cultured without treatment (control, C) or with FK 33—824 (FK, 10^{-9} M), hCG (100 ng/ml), naloxone (NAL, 10^{-5} M) added alone or in combinations. Labelled pig LH receptor cDNA was used as a probe.

There was no amplification of DNA after reverse transcription reaction in the mixture containing intestine total RNA (negative control). Ovary used as positive control demonstrated the presence of RT-PCR products corresponding to exons 9—11 (*Fig. 3*).

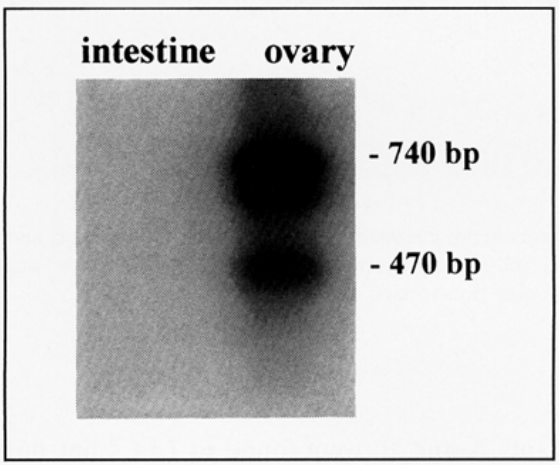

Fig. 3. Positive (ovary) and negative (intestine) control of RT-PCR. Labelled pig LH receptor cDNA was used as a probe.

DISCUSSION

In the present study immunocytochemistry revealed presence of receptor protein in both luteal cell types and Southern hybridization of RT-PCR products confirmed expression of LHR in isolated LLCs, non-stimulated and

stimulated by hCG and FK. In SLCs the gene for the LHR was expressed only after FK or hCG stimulation.

Papers concerning the changes in LHR in porcine luteal cells, depending on the type of cells and the phase of the oestrous cycle, have yielded inconsistent results. Meduri *et al.* (25) using immunocytochemical method found LHR mainly in the peripheral luteal cells derived from the theca. On the other hand, Gębarowska *et al.* (26) using autoradiography did not establish any differences in the ^{125}I-hCG binding between the peripheral and central region of mid-luteal CL of the pig. Moreover, Yuan and Lucy (27) detected by *in situ* hybridization mRNA for LHR in both SLCs and LLCs taken from CL on Day 10 of the cycle. Our data support the model, in which the receptors are present in both types of luteal cells. Similar occurrence of LHR (in SLCs and LLCs) is postulated for ovine (28) and human (29) CL but not for bovine CL (30), where only luteal theca cells contained LHR mRNA.

In spite of the presence of immunoreactive LHR in non-stimulated SLCs from Days 11—12 of the cycle, the lack of appropriate mRNA in these cells may indicate the absence of gene expression for LHR during this stage. It is well known that number of LH binding sites in porcine corpora lutea isolated between Days 10 and 14 of the cycle is declined (3). Collectively, the above data suggest that SLCs may lose the ability of LHR gene expression around Day 10 of the cycle.

Hybridization of RT-PCR products revealed the presence of two LHR gene products in luteal cells: longer (740 bp) and shorter (470 bp) ones. The shorter PCR product is probably a fragment of the alternative splicing transcript. Maturation of LHR mRNA in the alternative splicing process was earlier reported for pigs (31, 32) and rats (33, 34).

The LHR gene exhibited some changes in porcine luteal cells following treatments with FK and/or hCG suggesting that content of LHR transcript is hormonally regulated. In spite of the fact that RT-PCR and Southern blot used in our experiment are only semiquantitative methods, the obtained results indicate inhibitory effect of FK on the gene expression in LLCs and stimulatory in SLCs. Additionally, FK potentiated and inhibited the influence of hCG on the gene expression in SLCs and LLCs, respectively. The mechanism of opioid influence on porcine luteal cells is only superficially understood. Opioid peptides act through their own receptors, which occurrence in porcine luteal cells has been previously demonstrated (35). Moreover, the response of porcine SLCs and LLCs to opioids is mediated through cyclic nucleotides (2). Results of present experiment suggest that opioid action inside luteal cells may comprise in some way modulation of LHR. However, it seems that it is not the only mechanism of opioid action. Observed in presented experiment stimulatory effect of FK on hCG-induced LHR gene expression in SLCs is not in line with inhibitory influence of FK on hCG-stimulated P_4 secretion by pig SLCs reported in our earlier study (2). This effect of FK on P_4 output may take place rather at the level

of intracellular transducing systems than at the receptor level. Nevertheless, further studies using other methods, such a Northern blot, are necessary to completely elucidate the regulation of LHR in porcine luteal cells by opioid peptides.

In our experiment we observed stimulatory effect of hCG on LHR gene expression. It is to some degree surprising, especially in the light of many studies suggesting down-regulation of LHR after exposure to elevated concentrations of LH (33, 36, 37). However, it seems possible that LH/hCG added to the cell culture may at first transiently increase the content of LHR mRNA and then cause decline in the message level. Similar observations were also reported for MA-10 Leydig tumor cells (38). Moreover, up-regulation of LHR under influence of hCG was found in cultured granulosa cells isolated from oestrogen-primed rats (39) and in human granulosa cells (40). It can not be also ruled out that reduced number of LHR in luteal cell culture may be accompanied by increased LHR mRNA level. This kind of interdependence between the level of receptor protein and receptor mRNA was found for FSH receptors in porcine (41) and rat (42) granulosa cells exposed to FSH.

In summary, we have provided evidence that LHR is present in porcine SLCs and LLCs from mid-luteal CL by localisation of both its proteins and mRNAs. The LHR gene expression in luteal cells seems to be regulated by opioid peptides and hCG.

Acknowledgements: We thank Dr I Białłowicz and Mrs M Ledwożyw for their skilful technical assistance. We also acknowledge the gift of hCG from the National Hormone and Pituitary Agency, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, University of Maryland, School of Medicine, USA. This research was supported by the State Committee for Scientific Research (grant No 5P06D04209).

REFERENCES

1. Gregoraszczuk EL. Steroid hormone release in cultures of pig corpus luteum and granulosa cells: effect of LH, hCG, PRL and estradiol. *Endocrinol Exp* 1983; 17: 59—68.
2. Kamiński T, Siawrys G, Okrasa S, Przała J. Action of the opioid agonist FK 33—824 on porcine small and large luteal cells from the mid-luteal phase: effect on progesterone, cAMP, cGMP and inositol phosphate release. *Anim Reprod Sci* 1999; 56: 245—257.
3. Zięcik AJ, Shaw HJ, Flint APF. Luteal LH receptors during the oestrous cycle and early pregnancy in the pig. *J Reprod Fertil* 1980; 60: 129—137.
4. Daguet M Ch. Increase of follicle cell LH binding and changes in the LH level of follicular fluid during the preovulatory period in the sow. *Ann Biol Anim Biochem Biophys* 1979; 19: 165—167.
5. Channing CP, Kammerman S. Characteristic of gonadotropin receptors of porcine granulosa cells during follicle maturation. *Endocrinology* 1973; 92: 531—540.
6. Lim AT, Lolait S, Barlow JW, O WS, Zois I, Toh BH, Funder JW. Immunoreactive β-endorphin in sheep ovary. *Nature* 1983; 303: 709—711.
7. Przała J, Kamiński T, Siawrys G, Okrasa S. Large luteal cells are the source of immunoreactive β-endorphin in the pig: effects of hCG and TNFα on its secretion by luteal cells in vitro. *Endocrine Reg* 1999; 33: 117—123.

8. Przała J, Kamiński T, Okrasa S, Siawrys G, Bogacka I. The content of β-endorphin-like immunoreactivity in porcine corpus luteum (*in vivo*) and the potential roles of progesterone, oxytocin and prolactin in the regulation of β-endorphin release from luteal cells *in vitro*. *Reprod Dom Anim* 2000 (in press).
9. Ehrenreich H, Stock A, Schulz R. Opioids in bovine luteal cell cultures. *In: Materials of the 10th Symposium of Medicinal and Veterinary Sciences Society* 1985; Berlin (in German).
10. Aleem FA, Omar RA, El Tabbakh GH. Immunoreactive β-endorphin in human ovaries. *Fertil Steril* 1986; 45: 507—512.
11. Cupo A, Menezo Y, Bueno L. Enkephalin production by the corpus luteum. *Neuropeptides* 1987; 9: 237—245.
12. Li W-I, Wu H, Kumar AM. Synthesis and secretion of immunoreactive-methionine-enkephalin from rabbit reproductive tissues *in vivo* and *in vitro*. *Biol Reprod* 1991; 45: 691—697.
13. Petraglia F, Segre A, Facchinetti F, Campanini D, Ruspa M, Genazzani AR. β-Endorphin and met-enkephalin in peritoneal and ovarian follicular fluids of fertile and postmenopausal women. *Fertil Steril* 1985; 44: 615—621.
14. Lolait SJ, Autelitano DJ, Markwick AJ, Toh BH, Funder JW. Co-expression of vasopressin with β-endorphin and dynorphin in individual cells from the ovaries of Brattleboro and Long-Evans rats: immunocytochemical studies. *Peptides* 1986; 7: 267—276.
15. Melner MH, Young SL, Czerwiec FS, Lyn D, Puett D, Roberts JL, Koos RD. The regulation of granulosa cell proopiomelanocortin mRNA by androgens and gonadotropins. *Endocrinology* 1986; 119: 2082—2088.
16. Jin DF, Muffly KE, Okulicz WC, Kilpatrick DL. Estrous cycle- and pregnancy-related differences in expression of the proenkephalin and proopiomelanocortin genes in the ovary and uterus. *Endocrinology* 1988; 122: 1466—1471.
17. Sanders SL, Melner MH, Curry TE Jr. Cellular localization of ovarian proopiomelanocortin mRNA during follicular and luteal development in the rat. *Mol Endocrinol* 1990; 4: 1311—1319.
18. Douglass J, Cox B, Quinn, B, Civelli O, Herbert E. Expression of the prodynorphin gene in male and female mammalian reproductive tissues. *Endocrinology* 1987 120:707—713.
19. Facchinetti F, Ruspa M, Petraglia F, Segre A, Forabosco A, Genazzani AR. Met-enkephalin enhances FSH-dependent progesterone production from cultured granulosa cells. *J Clin Endocrinol Metab* 1986; 63: 1222—1224.
20. Kato T, Kumai A, Okamoto R. Effect of β-endorphin on cAMP and progesterone accumulation in rat luteal cells. *Endocrine J* 1993; 40: 323—328.
21. Varsano JS, Izhar M, Perk K, Shemesh M. Effect of β-endorphin on steroidogenesis by bovine luteal cells. *Reprod Fertil Dev* 1990; 2: 337—343.
22. Akins EL, Morrissette MC. Gross ovarian changes during estrous cycle of swine. *Am J Vet Res* 1968; 29: 1953—1957.
23. Chomczynski P, Sacchi N. Single — step method RNA isolation by acid guanidinum thiocinate-phenol-chloroform extraction. *Anal Bioch* 1987; 162: 156—159.
24. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual: Cold Spring Harbor Laboratory Press, 1989.
25. Meduri G, Vu Hai MT, Jolivet A, Takemori S, Kominami S, Driancourt MA, Milgrom E. Comparison of cellular distribution of LH receptors and steroidogenic enzymes in the porcine ovary. *J Endocrinol* 1996; 148: 435—446.
26. Gębarowska D, Zięcik AJ, Gregoraszczuk EL. Luteinizing hormone receptors on granulosa cells from preovulatory follicles and luteal cells throughout the oestrous cycle of pigs. *Anim Reprod Sci* 1997; 49: 191—205.
27. Yuan W, Lucy MC. Messenger ribonucleic acid expression for growth hormone receptor, luteinizing hormone receptor, and steroidogenic enzymes during the estrous cycle and pregnancy in porcine and bovine corpora lutea. *Dom Anim Endocrinol* 1996; 13: 431—444.
28. Smith GW, Gentry PC, Roberts RM, Smith MF. Ontogeny and regulation of luteinizing hormone receptor messenger ribonucleic acid within the ovine corpus luteum. *Biol Reprod* 1996; 54: 76—83.

29. Nishimori K, Dunkel L, Hsueh AJ, Yamoto M, Nakano R. Expression of luteinizing hormone and chorionic gonadotropin receptor messenger ribonucleic acid in human corpora lutea during menstrual cycle and pregnancy. *J Clin Endocrinol Metab* 1995; 80(4): 1444—1448.
30. Mamluk R, Wolfenson D, Meidan R. LH receptor mRNA and cytochrome P450 side-chain cleavage expression in bovine theca and granulosa cells luteinized by LH or forskolin. *Dom Anim Endocrinol* 1998; 15(2): 103—114.
31. Loosfelt H, Misrachi M, Atger M, Salesse R, Vuttai-Luu Thi MT, Jolivet A, Guiochon-Mantel A, Sar S, Jallal B, Garnier J, Milgrom E. Cloning and sequencing of porcine LH/hCG receptor cDNA: variant lacking transmembrane domain. *Science* 1989; 245: 525—528.
32. Derecka K, Pietila EM, Rajaniemi HJ, Zięcik AJ. Cycle dependent LH/hCG receptor gene expression in porcine nongonadal reproductive tissues. *J Physiol Pharmacol* 1995; 46: 77—85.
33. Lakkakorpi JT, Pietila EM, Aatsinki JT, Rajaniemi HJ. Human chorionic gonadotropin (CG) — induced down-regulation of the rat luteal LH/CG receptor results in part from the down regulation of its synthesis, involving increased alternative processing of the primary transcript. *Mol Endocrinol* 1993; 10: 153—162.
34. Koo YB, Ji I, Ji TH. Characterization of different size of rat luteinizing hormone/human chorionic gonadotropin receptor messenger ribonucleic acids. *Endocrinology* 1994; 134: 16—19.
35. Hamada H, Kishioka S, Yamoto M, Nakano R. (^{3}H) Naloxone binding sites in porcine ovarian follicles and corpora lutea during the ovarian cycle. *Europ J Endocrinol* 1995; 132: 622—626.
36. Conti M, Harwood JP, Hsueh AJW, Dufau ML, Catt KJ. Gonadotropin induced loss of hormone receptors and desensitization of adenylate cyclase in the ovary. *J Biol Chem* 1976; 251: 7729—7731.
37. Hoffman YM, Peegel H, Sprock MJE, Zhang Q, Menon KMJ. Evidence that human chorionic gonadotropin/luteinizing hormone receptor down-regulation involves decreased levels of receptor messenger ribonucleic acid. *Endocrinology* 1991; 128: 338—343.
38. Wang H, Segaloff DL, Ascoli M. Two distinct pathways for the lutropin/choriogonadotropin (LH/CG) — induced down-regulation of the LH/CG receptor: receptor-mediated endocytosis and a cAMP — dependent reduction in receptor mRNA. *J Biol Chem* 1991; 266: 780—785.
39. Piquette GN, Lapolt PS, Okikawa M, Hsueh AJW. Regulation of luteinizing hormone receptor messenger ribonucleic acid levels by gonadotropins, growth factors, and gonadotropin-releasing hormone in cultured rat granulosa cells. *Endocrinology* 1991; 128: 2449—2456.
40. Takao Y, Honda T, Ueda M, Hattori N, Yamada S, Maeda M, Fujiwara H, Mori T, Wimalasena J. Immunohistochemical localization of the LH/HCG receptor in human ovary: HCG enhances cell surface expression of LH/HCG receptor on luteinizing granulosa cells in vitro. *Mol Human Reprod* 1997; 3(7): 569—578.
41. Sites CK, Patterson K, Jamison CS, Friezner Degen SJ, LaBarbera AR. Follicle-stimulating hormone (FSH) increases FSH receptor messenger ribonucleic acid while decreasing FSH binding in cultured porcine granulosa cells. *Endocrinology* 1994; 134: 411—417.
42. Tilly JL, Lapolt PS, Hsuech AJW. Hormonal regulation of follicle-stimulating hormone receptor messenger ribonucleic acid levels in cultured rat granulosa cells. *Endocrinology* 1992; 130: 1296—1302.

R e c e i v e d: December 6, 1999
A c c e p t e d: March 27, 2000

Author's address: Tadeusz Kamiński, University of Warmia and Mazury in Olsztyn, Oczapowski Str 5, 10-718 Olsztyn, Poland