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Novel biological and possible applicable roles of LH/hCG receptor

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Abstract

Luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptors are widely expressed in gonadal cells, however, the presence of these receptors has also been demonstrated in several other non-gonadal female and male tissues. The expression level of non-gonadal LH/hCG receptors is much lower than in gonads, although their expression is regulated by similar mechanisms and they also exert biological effects using similar signaling pathways. Hormonally regulated LH/hCG receptor expression in the oviduct suggests that LH could be involved in the regulation of its contraction, gametes/embryos transport and synchronization of the fertilization. One of the major roles of the myometrial LH/hCG receptors may also be the stimulation of growth and maintenance of the uterine relaxation during pregnancy. In pigs, LH seems to be one of the pleiotropic factors which influence the endometrial prostaglandin $F_{2\alpha}$ synthesis and initiation of the luteolysis. The LH/hCG receptor expression in several cancer cells provides new possibilities for developing new strategies for targeted cancer therapy based on lytic LH/hCG conjugates.

Key words: LH/hCG receptor; uterus; oviduct; cancer

Introduction

Approximately 30 years ago the luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor was demonstrated to be an integral component of the granulosa, and luteal cells in the ovary and the Leydig cells of testes. In the available literature, the first report on non-gonadal LH/hCG binding sites was described in the porcine uterus (Ziecik et al., 1986). However, the first unintentional discovery of LH/hCG receptors out of gonads was made by Hannu Rajaniemi in his doctoral dissertation¹. In this one autoradiogram clearly shows binding of ¹²⁵I-labeled LH not only in the ovaries but also in the uterus (unpublished data/PhD thesis/University of Turku, Turku Finland/personal communication). The presence of LH/hCG receptors in the uterus was next demonstrated in rabbit (Sawitzke and Odell, 1991; Jensen and Odell, 1988), rat (Sawitzke and Odell, 1991; Bonnamy et al., 1990), mouse (Ziecik et al., 1992a; Mukherjee et al., 1994) and human (Reshef et al., 1990). Although non-gonadal LH/hCG receptors have been described in many species, the most numerous investigations were performed in humans and pigs. To date it is clear, that LH/hCG receptors are widely distributed in non-gonadal tissues including the female and male reproductive tract (oviduct, uterus and male accessory sex organs), placenta, mammary gland, brain, adrenal cortex, T lymphocytes and urinary bladder (Table 1; for a review see Rao, 1996). Recently, the association of neuronal LH/hCG receptor expression with sensory, memory, reproductive behavior and autonomic structures has also been identified (Apaja et al., 2004).

The pig is a species distant in phylogeny from humans, but unexpectedly, the transgenic pig became the most convenient animal donor for xenotransplantation of many organs for humans (Polejaeva et al., 2000). In this article we describe the distribution of LH/hCG receptors expression in the female reproductive tract and their involvement in the porcine oviduct and uterine function and compare these functions to human and other

¹Rajaniemi, H. 1971. Distribution of pituitary gonadotrophic hormones in experimental animal and binding to target tissues. University of Turku, Turku, Finland.

mammals. Moreover, we discuss new approaches to the development of novel therapies of LH/CG receptor expressing cancers.

Structure and regulation of expression

The LH/hCG receptor gene structure has been defined in the pig, human, rat and mouse (Loosfelt et al., 1989; Tsai-Morris et al., 1991; Huhtaniemi et al., 1992; Atger et al., 1995). In various species, this gene contains 11 exons, separated by 10 introns. Exons 1-10 encode the 5'-untranslated region and most of the extracellular domain. The last exon encodes seven characteristic membrane segments and the intracellular domain containing the C-terminal tail. The predicted mass of the porcine LH/hCG receptor polypeptide core is 75 kDa. For example, the glycosylated mature receptor expressed in the testes is 85 kDa, but less abundant smaller isoforms are also observed (Loosfelt et al., 1989). Transfection of mammalian cells with the cDNA for the rat or the human LH/hCG receptor bring about three distinct receptor forms with molecular masses of 75-85 kDa, 85-95 kDa, and 165-200 kDa. The 85-95 kDa band corresponds to the mature LH/hCG receptor present at the cell surface. In the pig LH/hCG receptor, two minimal critical sequences in the extracellular domain, fragments 21-24 and 102-107, are involved in hormone binding, and the receptor activation centre was localised within the sequence 111-121 (Davis et al., 1995). Two segments of the receptor that participate in hormone-receptor interaction are located in the inner surface of the horseshoe and vicinal loops but not on the α -helical stretches forming the outer circumference.

The primary transcriptional start sites of the LH/hCG receptor gene both in humans and rats lie within 180 bp 5' to the translational ATG codon, and deletion analysis has localized the promoter within this region (Tsai-Morris et al., 1993, 1995; Geng et al., 1999). In rat cell lines, expression of the LH/hCG receptor gene is constitutively inhibited by

upstream sequences -180 to -2678 bp (Tsai-Morris et al., 1993, 1995), whereas in human cell lines, only small inhibitory effects were observed (Geng et al., 1999). This region contains seven activator protein-1 (AP1) binding sites as well as a hemipalindromic cyclic adenosine monophosphate (cAMP) response element (hCRE) domain, which have the potential to bind the Jun/Fos and cAMP response element-binding protein (CREB)/modulator protein (CREM)/activating transcription factor (ATF) bZIP transcription factor families, respectively. The human and rat LH/hCG receptor gene is TATA-less and contains EREs direct repeats and two specificity proteins (Sp) 1-4 CG binding domains (I and II) within the proximal promoter (Phillips et al., 2005).

The distal -180 to -2678 bp region of the promoter, although capable of binding members of the Jun family via the multiple AP1 binding domains within this region, has no major role in regulating expression of the LH/hCG receptor gene in myometrial cells (Phillips et al., 2005). In contrast, the two Sp1-4 CG boxes within the +1 to -180 bp proximal promoter are central to expression of the gene in the myometrium (Geng et al., 1999), as reported in other human cell lines (Zhang and Dufau, 2003). However, not only Sp1/Sp3 proteins are involved in this process in the myometrium, but Sp4 and a novel Sp-like factor(s) also take part in transcriptional regulation of this gene. It would appear that Sp1/Sp3/Sp4 and Sp-like proteins are all involved in recruiting histone deacetylase (HDAC) complexes to the promoter, preventing chromatin remodeling and transcriptional activation (Phillips et al., 2005).

Fallopian tube

LH/hCG receptor mRNA expression has been shown in human (Lei et al., 1993c) and porcine (Derecka et al., 1995) fallopian tubes. Further immunohistochemical experiments revealed that LH/hCG receptor protein expression locate in the epithelium of tubal mucosa, smooth muscle cells, and the blood vessel endothelium in pigs (Gawronska et al., 1999). However, in

the mouse, LH/hCG receptors were detected in the serosa and subepithelial cells, but not in the epithelium of the oviduct (Zheng et al., 2001). This discrepancy may reflect the species differences or results from different experimental techniques.

The hormonal status of the pig may also influence the receptor expression levels in the porcine oviduct (Gawronska et al., 1999). Injection of estradiol benzoate was able to upregulate the expression of LH/hCG binding sites in the oviduct (Gawronska et al., 1999). The direct action of estradiol on the LH/hCG receptor status was also shown in humans, where estradiol caused up-regulation of these receptors in cultured oviductal epithelial cells (Han et al., 1996). An abundant concentration of LH/hCG receptor protein was observed in oviducts obtained from estradiol- and progesterone-treated gilts. Additionally, western blotting analysis revealed the presence of 75, 48 and 45 kDa immunoreactive receptor proteins in the porcine oviduct, analogously to the porcine corpus luteum (CL; Gawronska et al., 1999, 2000).

A hormonal profile dependent differential distribution of LH/hCG receptors in various oviductal cells, may imply the possibility of a direct involvement of LH in the regulation of the function of fallopian tube. It has been shown that spontaneous contractions of the porcine oviduct could be modulated by LH during various stages of the estrous cycle (Table 2; Gawronska et al., 1999). *In vitro* experiments showed that LH can modulate the oviduct contractility in gilts primed with both estradiol and progesterone but not in gilts primed only with progesterone, estradiol, or corn oil (Gawronska et al., 1999, 2000). The mechanism underlying the effects of LH on the oviduct is not clear yet, however, it is possible that its action is based on direct stimulation of local factors, such as prostaglandins (PGs) or nitric oxide (NO). Results of Han et al. (1996) support this concept, since hCG treatment increased cyclooxygenase-2 (COX-2; the key-enzyme in PGs synthesis) mRNA and protein levels and PGE₂ production in human oviductal mucosa cells *in vitro*. It seems that the preovulatory

surge of LH, beside its major action on the follicle rupture and luteinization of granulosa and theca cells, plays also an important role in the control of oviduct contraction. LH might also be a major factor responsible for opening the ampulla-isthmic junction for spermatozoa and synchronization of fertilization in the ampulla. Moreover, the known relaxing action of LH may facilitate the transport of embryos through the isthmus toward the uterus. Further studies are required to substantiate the hypotheses of LH roles in the fallopian tube.

Myometrium

LH/hCG receptor in myometrium was first identified in pigs (Ziecik et al., 1986), later its expression was confirmed in rabbit (Jensen and Odell, 1988) and in human (Reshef et al., 1990). The expression of LH/hCG receptors in the myometrium is higher in the progesterone-dominated phase of the estrous cycle than in the estrogen-dominated phase. The receptors are also expressed in the myometrium during pregnancy in humans and pigs and in the postpartum and postmenopausal periods in women (Ziecik et al., 1986; Reshef et al., 1990; Zuo et al., 1994). Cyclic expression patterns of LH/hCG receptors in the human menstrual and porcine estrous cycles, along with the known effects of estradiol and progesterone on myometrial LH/hCG receptor expression in ovariectomized pigs (Ziecik et al., 1992b) may suggest that estradiol directly upregulates and progesterone acts through estradiol-primed tissue to increase LH/hCG receptor levels in the myometrium as well as in the endometrium (Stepien et al., 1999) and fallopian tubes (Gawronska et al., 2000). The presence of LH/hCG receptors in the myometrium raises an intriguing possibility that LH/hCG may also have a direct effect on uterine function in addition to its indirect action via ovarian steroid hormones. There are data suggesting that LH/hCG receptors may play a role in the hyperplasia and hypotrophy of the uterus (Korneyi et al., 1993) and in the uterine motility having an inhibitory

effect on the frequency and amplitude of spontaneous myometrial contractions in the porcine uterus (Fig. 1; Flowers et al., 1991).

Myometrial LH/hCG receptors in women are down-regulated following the onset of labor in both term and preterm deliveries (Zuo et al., 1994) suggesting that hCG may have a role in maintaining uterine quiescence during pregnancy. Slattery et al. (2001) clearly demonstrated that hCG exerts a significant concentration-dependent relaxant effect on human myometrial tissue obtained in late pregnancy. This inhibitory effect was not estrogen-dependent as occurred in other tissue types. These findings clearly demonstrate that hCG has a relaxing effect on the myometrium and thus its clinical utility in the treatment of preterm labor should be estimated. Preliminary treatments, which involved administration of LH/hCG or placebo to women in preterm labor, showed significant improvement in terms of pregnancy prolongation and smaller numbers of infants with birth weights less than 2500 g in the treatment group².

In the porcine myometrial cells, hCG activates two different signaling systems, adenylate cyclase and phospholipase C (Kisieleska et al., 1996). The exact mechanism of the inhibitory effect of hCG is unknown but direct reduction of intracellular calcium availability (Eta et al., 1994), down regulation of myometrial gap junction (Ambrus and Rao, 1994) and changes in the synthesis of eicosanoids (Zuo et al., 1994) are very likely to be involved in this mechanism. The action of hCG on myometrium is primarily mediated via coupling of LH/hCG receptors to the adenyly stimulatory G protein, G_{α_s} , and the formation of cAMP. Increased accumulation of cAMP in the myometrium during pregnancy as a result of stimulation of the adenyly cyclase pathway by hCG may also be potentiated by the increase in levels of G_{α_s} - and G_{α_s} -coupled adenyly cyclase activities (Europe-Finner et al., 1993). The molecular processes by which expression of the G_{α_s} gene may be regulated in human

² Ali, F.M., Fateen, B., Ezzet, A., Badawy, H., Ramadan, A., El-tobge A., 2000. Treatment of preterm labor with human chorionic gonadotropin: a new modality. *Obstet. Gynecol.* 95, 104.

myometrium during pregnancy appears to involve binding of Sp-like transcription factors to GC boxes within the promoter region of the gene that stimulates expression of the $G\alpha_s$ gene via a protein kinase A (PKA)-dependent mechanism (Phillips et al., 2002). Recently, Phillips et al. (2005) showed that Sp1/Sp3/Sp4 and Sp-like proteins are involved in recruiting HDAC complexes to the proximal promoter in the human myometrium, preventing chromatin remodeling and resulting in transcriptional regression of the gene. These data suggest that administration of the HDAC inhibitor, trichostatin A, to the human myometrial cells *in vitro* and *in vivo* substantially removes this silencing effect on expression of the gene. Therefore, the use of this and similar agents could be considered to upregulate myometrial LH/hCG receptor levels and subsequent maintenance of the uterine relaxation during fetal maturation.

Endometrium

First observations of endometrial LH/hCG binding sites were made in the pig (Ziecik et al., 1986). The LH/hCG receptors were also characterized in the bovine endometrium (Freidman et al., 1995). The highest concentration of these receptors in the bovine endometrial tissue was observed on days 15 to 17 of the estrous cycle and decline afterwards to a nadir. In human endometrium, expression of gonadotropin receptors was also demonstrated in the proliferative and secretory phases of the menstrual cycle, the LH/hCG receptors immunoreactivity in cells from the secretory phase being higher than in corresponding cells from the proliferative phase of the cycle (Reshef et al., 1990). In pigs, endometrial LH/hCG receptor expression was investigated at the protein and mRNA levels. Western blot analysis revealed the presence of the predominant 75 kDa immunoreactive species in crude membrane preparations of the porcine endometrium, analogously to the CL (Stepien et al., 1999). A higher concentration of this protein was observed on days 10 and 15 of the estrous cycle than on days 5 and 19. Expression of LH/hCG receptors in the

endometrium and endometrial blood vessels of the pig was also demonstrated using autoradiography with ^{125}I -labeled hCG (Wasowicz and Ziecik, 2000).

A higher level of endometrial LH/hCG receptors before and at the time of luteolysis in pigs and cows suggested the possible role of LH in PGs synthesis (Fig. 2). Indeed, LH induces COX protein expression and increases $\text{PGF}_{2\alpha}$ release from bovine endometrium (Shemesh et al., 1994). In both species, pulsatile release of $\text{PGF}_{2\alpha}$ from the endometrium is responsible for initiation of luteolysis, but the stimulus for its secretion has not been well elucidated (Ziecik et al., 2001). We showed that in pigs LH is able to stimulate $\text{PGF}_{2\alpha}$ secretion from endometrial tissue (Stepien et al., 1999) as well as separately cultured stromal and luminal epithelial cells (Fig. 3; Blitek and Ziecik, 2005). Incubation of endometrial explants with LH resulted in an increase of $\text{PGF}_{2\alpha}$ output in a dose-dependent manner on days 5-16 of the estrous cycle. However, the strongest effect was found during luteolysis (days 14-16) of the estrous cycle. Moreover, a LH-stimulated upregulation of COX protein has also been shown (Stepien et al., 1999).

LH is released in a pulsatile manner during the luteal phase of the estrous cycle (Parvizi et al., 1976; Van de Wiel et al., 1981). Since both systemic infusion (Ziecik et al., 2001) and intramuscular injection of hCG (Guthrie and Bolt, 1983) were reported to be able to induce $\text{PGF}_{2\alpha}$ release *in vivo*, we thus hypothesized that the endogenous LH pulses may provoke PGs secretion from the porcine endometrium. This hypothesis has been supported by the observations that much higher correlation exists between LH and 13,14-dihydro-15-keto $\text{PGF}_{2\alpha}$ (PGFM) peaks (79.2%, Ziecik et al., 2001) than between oxytocin and PGFM (30%, Kotwica et al., 1999). At the time of luteolysis, the number of endometrial LH/hCG receptors reaches the maximum in pigs. However, after initiation of luteolytic secretion of $\text{PGF}_{2\alpha}$ on days 14-16 of the estrus cycle, concentration of endometrial LH/hCG receptors starts to decline (Stepien et al., 1999). Interestingly, during the same period of the estrous cycle, the

number of LH/hCG receptors in the porcine CL also declines (Fig. 2; Ziecik et al., 1980). Other results showed that injection of hCG during the late luteal phase of the estrous cycle increased concentrations of peripheral PGFM in cows (Fields and Shemesh, 2004).

The “luteolytic” action of LH is restricted only to the late luteal phase of the porcine estrous cycle. After fertilization, the developing blastocyst is able to overcome this effect and restores the classical luteotropic action of LH. Estrogen produced by blastocyst seems to act as an anti-luteolysin and causes a redirection of endometrial $\text{PGF}_{2\alpha}$ secretion from the uterine vasculature (endocrine) into the uterine lumen (exocrine) (Bazer and Thatcher, 1977; Krzymowski and Stefanczyk-Krzymowska, 2002). Additionally, the presence of blastocysts in the uterine lumen probably alters the release of the final product of PG synthesis in the pig. It appears to happen by induction of PGE_2 secretion instead of $\text{PGF}_{2\alpha}$ (Moeliono et al., 1977; Akinlosotu et al., 1988) or at least by changing the $\text{PGE}_2:\text{PGF}_{2\alpha}$ ratio to favor luteotropic PGE_2 effects (Davis and Blair, 1993; Ziecik et al., 2001). Our recent unpublished data suggest that up-regulation of PGE synthase in conceptus on days 10-12 of pregnancy may be important in modulating ratio for the maternal recognition and establishment of pregnancy in pigs³. Moreover, the luteolytic effect of $\text{PGF}_{2\alpha}$ on luteal cells collected from mature and regressing porcine CL can be abolished by the increase of the $\text{PGE}_2:\text{PGF}_{2\alpha}$ ratio *in vitro* (Gregoraszczuk and Michas, 1999). Recently, we have also found that exogenous estrogen administration between days 11 and 15 after the onset of estrus results in elevation of LH-induced PGE_2 release from cultured porcine endometrium (G. Bodek and A.J. Ziecik – unpublished data). This data is in agreement with earlier published data of Christenson et al. (1994) on PGs output in non-mated and mated gilts. In contrast to non-mated gilts, PG secretion in mated gilts reached a peak earlier with a domination of PGE_2 synthesis. Garverick et al. (1982) suggested that estrogen of conceptus origin is an additional stimulus for the

³Waclawik, A., Blitek, A., Kaczmarek, M., Ziecik, A.J., 2005. Expression patterns of mPGES-1 and PGFS in pig trophoblast. The 4th Joint Meeting of the UK Fertility Societies, Warwick, UK, 3-6 April.

maintenance of LH/hCG receptors in the CL by a mechanism that is independent of the porcine uterus.

It is very likely that LH mediates its actions upon the porcine endometrial cells via two intracellular second messenger systems (Stepien and Ziecik, 2002). The first one involves the activation of adenylate cyclase and production of cAMP and the second one the stimulation of phosphoinositide-specific phospholipase C and the activation of protein kinase C. The same signaling pathways mediate LH action in the ovary (Marsh, 1975; Davis et al., 1987) and myometrium of pigs (Kisieleska et al., 1996) as well as in the bovine cervical tissues (Mizrachi and Shemesh, 1999). Taken together, it appears that LH/hCG action on porcine endometrium induces $\text{PGF}_{2\alpha}$ production which contributes to the regression of the CL (Fig 2). LH binding to the endometrial receptors in pigs and cows and LH induced secretion of PGE_2 in the placentoma of the ewe suggest that LH/hCG together with other embryonic factors could be applied for development of new strategies for improvement of reproductive efficiency in domestic animals (Weems et al., 2003).

Uterine blood flow

LH/hCG receptors are expressed in the vascular smooth muscle layer of arteries and veins of the porcine broad ligament (Ziecik et al., 1995). Small vessels supplying and draining blood from the uterus, except the uterine artery, show higher concentrations of LH/hCG receptors during the luteal than the follicular phase of the estrous cycle. Uterine veins, but not arteries, of proestrus/estrus cows express LH/hCG receptors (Shemesh et al., 1997). Presence of LH/hCG receptors in the uterine vascular system may be necessary for LH-induced COX-2 expression and increased PGs synthesis in the uterine veins (Shemesh et al., 1997). Therefore, the maximal stimulation of the LH/hCG receptor during proestrus/estrus stage may serve to increase the amounts of prostanoids reaching the regressing CL. It happens either directly by

increasing prostanoid production in the vascular bed or indirectly by increasing the blood flow to the ovary.

Administration of hCG to gilts in the luteal phase and also to estrogen and progesterone primed ovariectomized gilts shows that LH/hCG may directly influence the blood flow (Ziecik et al., 1996). The uterine blood flow response was biphasic after hCG treatment. The first early phase occurred about 1 hour after hCG injection and the second later phase about 1 hour after the onset of the peak of estradiol release. It has been known for some time that both single injection and infusion of hCG into gilts in the luteal phase of the estrous cycle can significantly elevate estradiol-17 β concentrations in systemic blood 3 hours later (Ziecik et al., 1996). Therefore, the first phase of the uterine blood flow response in gilts could directly be induced by hCG, but the second is more likely an estradiol effect. It has been shown that estrogen induces uterine vasodilatation in the pig (Ford, 1985), sheep (Van Buren et al., 1992) and women (Kurjak et al., 1991). Toth et al. (1994) suggested that hCG could regulate formation of vasoactive eicosanoids via the COX pathway of arachidonic acid metabolism in uterine arteries. According the above authors the vasodilatory effect could be due to the hCG-induced increase of PGI₂ and consequent decrease of thromboxane A₂ synthesis.

The precise mechanism involved in vasoactive action of LH/hCG is not yet known. Recently, it has been demonstrated that LH caused a significant attenuation of vascular response to norepinephrine (NE) in the third order branches of porcine uterine artery⁴ whereas NE induced a dose-dependent contraction of all experimental and control arteries, addition of LH caused a sideways shift of the dose-response curve to NE. The corresponding EC₅₀ values (μ mol/l) were 2.2 $\pm$ 0.4 in phosphate buffered saline (PBS) and 3.3 $\pm$ 0.4 (p <0.05) in LH pre-treated vessels. Also intraperitoneal administration of hCG caused attenuation of the

⁴Skipor, J., Kowalik, A., 2005. Luteinizing hormone attenuates the vascular response to norepinephrine. The 4th Symposium of the Society for Biology of reproduction, Cracow, Poland, 22-24 September.

contractile responses of isolated rat aortic rings to phenylephrine, angiotensin II, CaCl_2 and KCl (Ezimokhai et al., 2000). This effect of hCG could be contributed to the attenuated vascular responses in normal pregnancy.

Targeted treatment of the gonadal and non-gonadal tumors through LH/hCG receptors

Presently available cancer treatments are mostly based on chemotherapeutic drugs cytotoxic not only to rapidly dividing tumor cells but also to normal cells. The major problem of cancer chemotherapy is that it has severe side effects (Boman, 1995) and the search for an effective, non-toxic to normal cells dose is still difficult. Therefore, one focus of the ongoing research in this field is to evaluate more selective ways to delivery chemotherapeutic agents to the primary tumor and their metastases, which allow the use of higher doses without side effects to normal cells (Magrath, 1994). Since specific binding sites for LH/hCG receptor are present in breast (Lojun et al., 1997; Meduri et al., 1997), uterine (Lei et al., 1993b; Lin et al., 1994; Singh et al., 1995), ovarian and prostate cancers (Emons et al., 1992; Tao et al., 1997; Dirnhofer et al., 1998), targeted treatment of these tumors based on cytotoxic or lytic LH/hCG conjugates could provide a more specific and efficacious tool for ablation of these malignancies than conventional chemotherapeutics.

Recently, a new approach based on the selectivity of certain carrier molecules for specific binding sites in tumor tissues has been proposed (Leuschner et al., 2001; Hansel et al., 2001). Several synthetic, alpha-helical, membrane disrupting peptides have been linked with a 15-amino acid fragment (81-95) of β chain of hCG (Leuschner and Hansel, 2005). The 81- to 95-amino acid region of hCG β was selected as ligand, since it is the most potent region of the full size hCG involved in the interaction between hormone and receptor (Morbeck, 1993). The ability of selective destruction of Hecate-CG β , Phor14-CG β and Phor21-CG β conjugates has been tested in numerous *in vitro* and *in vivo* experiments (Hansel et al. 2001;

Leuschner et al. 2001, 2003; Gawronska et al., 2002; Bodek et al., 2003, 2005a, b; Zaleska et al., 2003, 2004).

We have investigated *in vitro* the susceptibility of various gonadal and non-gonadal cancer cell types, expressing and non-expressing LH/hCG receptors, to Hecate-CG β conjugate treatment (Zaleska et al., 2003; Bodek et al, 2003, 2005a). In a dose- and time-dependent manner, the conjugate selectively destroyed cells possessing LH/hCG in lower concentrations than Hecate alone and the efficacy strongly correlate with the number of LH/hCG receptors. In LH/hCG receptor non-expressing cells, Hecate-CG β conjugate was effective only in high concentrations, similar to Hecate alone (Bodek et al, 2005a).

Administration of Hecate-CG β conjugate has efficiently inhibited the growth of diethylstilbestrol/dimethylbenz[a]anthracene (DES/DMBA)-induced rat mammary tumors *in vivo*, although we were able to document the expression of LH/hCG in tumors in only 17% of the treated rats (Zaleska et al., 2004). Interestingly, the treatment did not affect estradiol, LH, follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) blood plasma concentrations. However, our recent results have revealed a significant decrease of prolactin (PRL) and growth hormone (GH) levels in the blood plasma of Hecate-CG β conjugate treated animals (our unpublished results), which may indicate an unknown systemic effect of Hecate-CG β conjugate treatment. To date it is not clear how Hecate-CG β conjugate effectively reduces the volume of DES/DMBA-induced rat mammary tumors, further studies are required to elucidate the *in vivo* mechanisms of action of the conjugate in mammary gland tumors in rats.

Increasing concentrations of Hecate-CG β conjugate have been shown to inhibit binding of hCG to the receptors. Thus, it seems that binding abilities of used fragment of hCG β were not influenced by the conjugation process (Bodek et al., 2003). Interestingly, the lytic activity of Hecate-CG β conjugate can be limited by the hCG. Short-term *in vitro*

preincubation of human prostate cancer cells (PC-3) with 100 ng/ml of gonadotropin led to saturation of LH/hCG receptors and protected these cells against the toxicity of Hecate-CG β conjugate (Bodek et al., 2005a).

Taken together, several *in vitro* and *in vivo* experiments proved the concept that Hecate-CG β conjugate can be effective in targeted destruction of cells expressing LH/hCG receptors. This unique combination of toxic moiety (Hecate) and fragment of β chain of hCG may be used in a development of potential targeted therapy of gonadal and non-gonadal tumors expressing LH/hCG receptors without causing side effects. However, further studies are needed in order to determine the detailed mechanism of Hecate-CG β conjugate action and regulation of conjugate cell/receptor specific destruction, as well as the minimal effective dose and the best method of administration.

Table 1. Evidence of LH/hCG receptor in non-gonadal tissues of human and pig

Tissue	Human	Pig
Myometrium	(+) Reshef et al., 1990	(+) Ziecik et al., 1986
Endometrium	(+) Reshef et al., 1990	(+) Ziecik et al., 1986
Oviduct	(+) Lei et al., 1993c	(+) Gawronska et al., 1999
Broad ligamnet	(?)	(+) Ziecik et al. 1995
Uterine vessels	(+) Toth et al., 1994	(+) Wasowicz and Ziecik, 2000
Cervix	(+) Lin et al., 2003	(+) Stepien et al., 2000
Mammary gland	(+) Lojun et al., 1997	(+) Meduri et al, 1997
Placenta	(+) Reshef et al., 1990	(?)
Umbilical cord	(+) Rao et al., 1993	(+) Wasowicz et al., 1999
Brain	(+) Lei et al., 1993a	(?)
Adrenals	(+) Pabon et al., 1996b	(?)
Skin	(+) Pabon et al., 1996a	(?)
Lymphocytes	(+) Lin et al., 1995	(?)
Epididymis	(+) Rao, 2001	(+) A.J. Ziecik, unpublished data
Prostate	(+) Dirnhofer et al., 1998	(?)

(+) - evidence of LH/hCG receptor presence; (?) - lack of data

Table 2. Mean ($\pm$ SEM) frequency and amplitude of spontaneous contraction of the pig isthmus collected during different stages of the estrous cycle and incubated with LH (100 ng/ml) (modified from Gawronska et al., 1999).

Stage of the estrous cycle	Frequency		Amplitude	
	II	III	II	III
Days 18-2 (peri-ovulatory)	101.1 $\pm$ 9.3	77.4 $\pm$ 7.8	83.4 $\pm$ 8.6	68.4 $\pm$ 7.3*
Days 5-8 (early luteal)	94.5 $\pm$ 5.9	98.7 $\pm$ 0.7	105.8 $\pm$ 4.0	79.2 $\pm$ 3.8*
Days 13-15 (late luteal)	104.3 $\pm$ 6.9	82.3 $\pm$ 11.8	93.1 $\pm$ 6.9	59.4 $\pm$ 15.1*
Days 16-17 (early follicular)	94.9 $\pm$ 3.1	99.5 $\pm$ 4.21	112.7 $\pm$ 11.9	112.8 $\pm$ 15.7

The responses are expressed as percentage of initial values (period I: from 10 to 0 min before hormone treatment; period II: from 0 to 10 min; and period III: from 20 to 30 min after LH addition). * $P < 0.05$ versus period I in rows.

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Legends for figures

Fig. 1. Human chorionic gonadotropin (hCG) inhibits amplitude of myometrial contractions in vitro (b) comparing to control (a) and prostaglandin $F_{2\alpha}$ stimulation (c) (Flowers et al., 1991).

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Fig. 2. Luteolysis in pigs. (A) Porcine CL is insensitive to exogenous $PGF_{2\alpha}$ until day 12 of the estrous cycle (insensitive period). Number of $PGF_{2\alpha}$ receptors increases dramatically on days 13 and remains high on days 14-17 (sensitive period; according to Gadsby et al., 1990).

(B) Release of $PGF_{2\alpha}$ from endometrium is initiated and maintained by pleiotropic action of many factors including LH, oxytocin and tumor necrosis factor (TNF) α . LHR – LH/hCG receptor; OTR – oxytocin receptor; TNFR – TNF α receptor; PR – progesterone receptor. LHR in corpus luteum and PR in the endometrium are down regulated after day 13 of the estrous cycle.

Fig. 3. The effect of LH on $PGF_{2\alpha}$ secretion by cultured porcine stromal (A) and luminal epithelial (B) cells obtained at days 10-12 of the estrous cycle. LH (0.1 – 100 ng/ml) was added for 6 h in stromal and 12 h in luminal epithelial cells culture. Data are presented as mean $\pm$ SEM. **, $P < 0.01$; ***, $P < 0.001$ when compared to control value (Blitek and Ziecik, 2005). Reproduced with permission.