INVITED REVIEW

Biology of the gonadotropin-releasing hormone system in gynecological cancers

Carsten Gründker, Andreas R Günthert, Silke Westphalen and Günter Emons

Department of Gynecology and Obstetrics, Georg-August-University, D-37070 Göttingen, Germany

(Correspondence should be addressed to G Emons, Department of Obstetrics and Gynecology, Georg-August-University of Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany; Email: emons@med.uni-goettingen.de)

Abstract

The expression of GnRH and its receptor as a part of an autocrine regulatory system of cell proliferation has been demonstrated in a number of human malignant tumors, including cancers of the breast, ovary and endometrium. Dose-dependent antiproliferative effects of GnRH agonists in cell lines derived from these cancers have been observed by various investigators. GnRH antagonists also have marked antiproliferative activity in most breast, ovarian and endometrial cancer cell lines tested, indicating that the dichotomy of GnRH agonists and antagonists might not apply to the GnRH system in cancer cells. The classical GnRH receptor signal-transduction mechanisms, known to operate in the pituitary, are not involved in the mediation of antiproliferative effects of GnRH analogs in cancer cells. Rather, the GnRH receptor interacts with the mitogenic signal transduction of growth factor receptors and related oncogene products associated with tyrosine kinase activity, via activation of a phosphotyrosine phosphatase, resulting in downregulation of cancer cell proliferation. In addition, GnRH activates nuclear factor κ B and protects the cancer cells from apoptosis. Furthermore, GnRH induces activation of the c-Jun N-terminal kinase/activator protein-1 (AP-1) pathway independent of the known AP-1 activators, protein kinase or mitogen activated protein kinase.

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Introduction

Hypothalamic gonadotropin-releasing hormone

The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH), also called luteinizing hormone releasing hormone (LHRH), has an important role in the control of mammalian reproduction (1–3). It is released from the hypothalamus in a pulsatile manner and stimulates the synthesis and release of luteinizing hormone and follicle-stimulating hormone. In addition to this well-documented classic hypophysiotropic action, GnRH might have a role as a modulator of the activity of diverse systems in the brain and many peripheral organs (1, 4–10). An autocrine/paracrine function of GnRH has been suggested to exist, for instance, in the placenta (11–14), granulosa cells (15–17), myometrium (18) and lymphoid cells (19–21). In addition, it is probable that such GnRH-based autocrine systems are present in a number of human malignant tumors, including cancers of the breast, ovary, endometrium and prostate.

In this article, our present knowledge on the common features and the mechanisms of action of GnRH in

human gynecological cancers will be reviewed. In addition, new knowledge on GnRH-II will be reported.

The GnRH receptor

GnRH binds to a specific GnRH receptor. Cloning of the GnRH receptor from several mammalian species (7, 22–28) has revealed that the receptor is a member of the large superfamily of seven transmembrane domain receptors that bind to G-proteins (7, 29, 30). Upon hormone binding, the GnRH receptor normally acts via pertussis toxin-insensitive G-proteins, most probably belonging to the G-protein α_q family (31). Through G-protein α_q , probably, phospholipases and calcium channels are activated as the next steps in signal transduction.

GnRH receptors in human reproductive tissues

There is growing evidence of auto- and paracrine GnRH systems in human reproductive tissues. In normal human extrapituitary tissues, including breast, placenta, endometrium, ovary and testis, controversial

data have been obtained relating to the presence of GnRH receptors (5–7). Northern blot analysis failed to detect GnRH receptor mRNAs in any of the non-pituitary tissues examined (24). However, using RT-PCR, these mRNAs were recently identified in granulosa luteal cells (16). The GnRH receptor transcript was identical to that found in pituitary gonadotrophs (32). During spontaneous luteinization, GnRH and GnRH receptor expression were increased, suggesting that GnRH has a role in controlling corpus luteum function (32). Kang *et al.* (33) demonstrated activation of mitogen-activated protein kinase (MAPK) by GnRH agonists in granulosa luteal cells. Nathwani *et al.* (32) were able to demonstrate a downregulation of GnRH mRNA by estradiol and a biphasic effect of estradiol on GnRH receptor mRNA expression, with an initial stimulation and a secondary downregulation. In addition, regulation of estrogen receptors α and β by GnRH agonists has been demonstrated, as the expression of both receptors is downregulated (34). The data published on the normal human ovary are controversial. Using RT-PCR, Ohno *et al.* (8) failed to detect GnRH receptor expression in the normal ovary. In contrast, Minaretzis *et al.* (17) detected GnRH receptor mRNA in 9 of 10 human ovaries, using RT-PCR. Srkalovic *et al.* (35) detected GnRH receptors in 54% of normal human ovaries, by radioligand binding experiments. To date, the only functional data in this field are the experiments of Kang *et al.* (36). In human ovarian surface epithelium (hOSE) cell cultures, they demonstrated the existence of GnRH receptors and a biphasic antiproliferative effect of the GnRH agonist, [D-Ala⁶]GnRH. This effect was blocked by the GnRH antagonist, antide (36). In the normal human endometrium and myometrium, Reshkin *et al.* (37) demonstrated expression of a high percentage of GnRH receptors, by radioligand binding experiments. Using RT-PCR and Southern blot, Ikeda *et al.* (38) were able to demonstrate GnRH, but not GnRH receptors, in the endometrium and the decidua. In contrast, Raga *et al.* (39) detected GnRH and GnRH receptors in the endometrium by RT-PCR and immunohistochemistry. As a dynamic pattern was found, with increasing concentrations in the luteal phase, the authors suggested that GnRH may have a paracrine/autocrine role in the early stages of

implantation by modulating trophoblastic secretion of human chorionic gonadotropin (39). Interestingly, similar findings were made in the Fallopian tube (40). GnRH receptors have even been detected in endometriosis: using RT-PCR and Southern blot, Borroni *et al.* (41) were able to demonstrate the presence of these receptors in 13 of 13 samples of ovarian endometriosis; however, in cell cultures of these lesions, the GnRH agonist, leuporelin, exerted antiproliferative effects in just 5 of 13 cases. Kottler *et al.* (42) demonstrated co-expression of GnRH and its receptor in malignant and non-malignant tissues of the human breast. As GnRH agonists inhibit breast epithelial cell growth, these data raise the possibility of an autocrine/paracrine role for GnRH in the human mammary gland (42).

To summarize: the expression of GnRH and of GnRH receptors, in addition to autocrine and paracrine effects of GnRH, have been shown in several benign reproductive tissues. In contrast to the situation with respect to the pituitary and the gynecologic tumors, the physiological role of these receptors is only marginally elucidated. Further understanding in this field might improve both reproductive medicine and the understanding of tumor biology.

GnRH in human gynecological cancers

Expression of GnRH and GnRH receptors

In earlier studies it was shown that breast, ovarian and endometrial cancers express receptors for GnRH (1, 2, 4, 43–45). Two types of GnRH binding sites exist, one of low affinity and high capacity, the other of high affinity and low capacity. The latter is comparable to the pituitary GnRH receptor (4, 46, 47). In 1992, cloning, sequencing and expression of the human pituitary GnRH receptor were reported (23, 24). These findings stimulated intensive research, leading to the demonstration of GnRH receptor gene transcripts in ovarian and endometrial cancer cell lines and in about 80% of the respective primary tumors (23, 48–50) (Table 1). In ovarian and endometrial cancer specimens and cell lines expressing mRNA for the pituitary GnRH receptor, high-affinity/low-capacity binding sites were found in

Table 1 Expression of GnRH and GnRH receptors (GnRH-R), and biological effects of GnRH analogs. Data were obtained in primary cancer, respective tumor cell lines, or both. Numbers in parentheses indicate the available data on percentages of primary cancers expressing GnRH, GnRH-R or the respective mRNAs. For details and references, see text. From Emons *et al.* (60), with permission. © 1997 Elsevier Science.

Tumor type	Expression of GnRH		Expression of GnRH-R		Inhibitory effects of GnRH analogs	
	Immuno/bioactivity	mRNA	Radioreceptor assay	mRNA	Proliferation	Mitogenic signal transduction
Breast cancer	+	+	+	+	+	+
Ovarian cancer	+ (>80%)	+ (>80%)	+ (>80%)	+ (>80%)	+	+
Endometrial cancer	+ (>80%)	+ (>80%)	+ (>80%)	+ (>80%)	+	+

close relation to the pituitary GnRH receptor (48–52). Kakar *et al.* (25) demonstrated that the nucleotide sequence of GnRH receptors in human breast and ovarian tumors is identical with that found in pituitary. Data available today suggest that about 50% of breast cancers (53) and approximately 80% of ovarian and endometrial cancers express high-affinity binding sites for GnRH. For prostate cancer, fewer findings have been published (46), but systematic investigations might lead to comparable results.

Harris *et al.* (54) reported the expression of the mRNA for GnRH in two human breast carcinoma cell lines. Recently, two groups independently demonstrated GnRH immunoreactivity and bioactivity, and the expression of mRNA for GnRH by cell lines and in the majority of biopsy samples of ovarian and endometrial cancers (50, 55, 56). As breast, ovarian and endometrial cancers express GnRH and its receptor, it seems reasonable to speculate that, in many of these tumors, there is a local regulatory system based on GnRH. The same situation was found in prostate cancer cells (45).

Direct antitumor effects of GnRH analogs

The *in vitro* proliferation of a variety of human cancer cell lines can be inhibited by agonistic or antagonistic analogs (or both) of GnRH in a dose- and time-dependent manner (4, 47, 51, 52, 57–59) (Fig. 1). In most cancer cells except for the ovarian cancer cell line EFO-27, GnRH antagonists act like agonists, indicating that the dichotomy of GnRH agonists and antagonists does not exist in tumor cells (51, 60). Using the human ovarian cancer cell line OV-1063 xenografted into nude mice, Yano *et al.* (61) demonstrated a signifi-

cant inhibition of tumor growth by chronic treatment with the GnRH antagonist, cetrorelix, but not with the GnRH agonist, triptorelin. As both GnRH analogs induced a comparable suppression of the pituitary–gonadal axis, the authors speculated that *in vivo* antitumor effects of cetrorelix were exerted directly on GnRH receptors in tumors (62). The findings on direct antitumor effects of GnRH analogs in ovarian and endometrial cancer reported by several other groups are completely or partly in agreement with those described earlier (47, 62–64). In contrast, other investigators failed to detect direct antitumor effects of GnRH analogs in human ovarian and endometrial cancer cell lines, or observed them only at extremely high concentrations of GnRH analog (65–67). These discrepancies might be explained by the likelihood that the majority of the cell lines used by these authors did not express high-affinity GnRH receptors (47, 68). Alternatively, differences in culture or experimental conditions and in the types of GnRH analogs used might be responsible for the observed variance. In the case of prostate cancer, several groups reported direct antiproliferative effects of GnRH analogs *in vitro* and in animal *in vivo* models, which could be mediated through specific GnRH-binding sites (1, 46, 69–73).

Proliferation of ovarian cancer cells was significantly increased after treatment with an antiserum to GnRH, suggesting that GnRH produced by human ovarian cancer cells acts as a negative autocrine regulator of proliferation (74) (Fig. 2). In contrast, Arencibia & Schally (75) have recently reported that, in ES-2 human ovarian cancer cells, the GnRH agonist triptorelin in a concentration of 10 ng/ml stimulated the proliferation *in vitro* after 48 h, but was inhibitory after 72 h and in concentrations of 1000 ng/ml. The GnRH

Figure 1 Effects of 6 days of treatment with increasing concentrations of GnRH agonist triptorelin (left panel) or antagonist cetrorelix (right panel) on the proliferation of the endometrial cancer cell line, Ishikawa. Cell number is expressed as percent of the control (C; vehicle only = 100%). Each column represents the mean $\pm$ S.E. of data obtained from three (triptorelin) or four (cetrorelix) independent experiments run in quadruplicate in three or four different passages of the cell line. Analysis of variance: $P < 0.001$. Significant differences ($P < 0.01$) compared with: ^aControl; ^b 10^{-11} mol/l; ^c 10^{-9} mol/l; ^d 10^{-7} mol/l (Newman–Keuls test). Experiments using endometrial cancer cell line Hec-1A and ovarian cancer cell line EFO-21 gave comparable results. From Emons *et al.* (52), with permission. © 1993 The Endocrine Society.

Figure 2 Time-course experiments of proliferation of the human ovarian cancer cell line EFO-21 in the presence of an antiserum against known GnRH. Cells were incubated with normal rabbit serum (left bars) or with anti-GnRH antiserum (middle bars) or without rabbit serum (right bars). Cell number is given as a percentage of the controls (100%). The bars represent the mean $\pm$ S.E.M. of three independent experiments, carried out using different passages of the cell line in quadruple determinations. ^a $P < 0.001$ compared with control and normal rabbit serum; ^b $P < 0.01$ compared with control; ^c $P < 0.05$ compared with normal rabbit serum. From Emons *et al.* (74), with permission. © 2000 Society of the European Journal of Endocrinology.

antagonist cetrorelix inhibited growth of the ES-2 cell line only in a concentration of 1000 ng/ml. The *in vitro* incubation of ES-2 ovarian cancer cells with an GnRH antibody inhibited cell proliferation in a time- and concentration-dependent manner. These results suggest that GnRH may function as an autocrine growth factor in this ovarian cancer cell line (75). Differences between tumor cell lines (e.g. variances in G-protein coupling and signaling) might explain these discrepancies.

Molecular mechanisms mediating the direct antitumor effects of GnRH

In view of the apparent similarity of GnRH receptors in peripheral cancers to those in the pituitary gonadotrophs, it seemed reasonable to speculate that GnRH signal transduction pathways in tumors might also be comparable to those operating in the pituitary, such as phospholipase C (PLC) and protein kinase C (PKC). Our group performed extensive studies in human ovarian and endometrial cancer cell lines. These cell lines express GnRH and GnRH receptors, and their proliferation is inhibited by GnRH analogs (50–52). Although we were able to demonstrate clearly the activation of PLC, PKC and adenylyl cyclase in these tumor cells by pharmacological stimuli, the GnRH agonist triptorelin, in concentrations that were inhibitory to proliferation, had no effects on the activity of these signaling systems (76). We found, however, that the mitogenic effect of growth factors in these cell lines could be counteracted

by triptorelin, indicating an interaction with the mitogenic signal transduction pathway (76). Comparable data were obtained by Moretti *et al.* (77) in the human prostatic cancer cell lines LNCaP and DU 145. These findings were in accord with reports that GnRH analogs reduced expression of growth factor receptors and their mRNA (61, 77, 78) or growth-factor-induced tyrosine kinase activity, or both (76, 77, 79–84). Growth-factor-induced tyrosine phosphorylation is probably counteracted by GnRH analogs through activation of a phosphotyrosine phosphatase (PTP) (76, 77, 80, 81, 83, 84), which is probably coupled to the GnRH receptor through a G-protein α_i in human reproductive tract tumors (85). Using an antibody against tyrosine phosphate, we were able to show that the GnRH agonist triptorelin and the antagonist cetrorelix reduce epidermal growth factor (EGF)-induced tyrosine auto-phosphorylation of EGF receptors to 60–80% of that in controls. This effect was completely inhibited using the phosphatase inhibitor, vanadate (86) (Fig. 3).

The concept of an inhibition of mitogenic signal transduction by GnRH analogs in human cancer cells was further corroborated by our group. We demonstrated that EGF-induced activation of MAPK, an enzyme further downstream in the growth factor signaling cascade (87), was virtually completely blocked in ovarian and endometrial cancer cells treated with the GnRH agonist triptorelin (76). By quantitative RT-PCR and western blotting, we showed that the EGF-induced expression of the immediate early gene *c-fos*, a mechanism still further downstream in mitogenic signaling, was completely abrogated in breast, ovarian and endometrial cancer cells by treatment with the GnRH agonist triptorelin or with the GnRH antagonist cetrorelix (88) (Fig. 4). The same effects were seen by Motta's group in the prostatic cancer cell line LNCaP by treatment with the GnRH agonist, goserelin (77, 89).

The reasons for the differences in GnRH signal transduction in the pituitary gonadotroph and peripheral cancers remain unclear. Experimentally induced mutations of the GnRH receptor have altered GnRH binding, G-protein–receptor interactions, or correct membrane incorporation (90–96). However, in none of the analyzed breast, endometrial and ovarian cancer cell lines were we able to find any mutation in the coding region of the GnRH receptor gene transcripts (86). Therefore mutations or splice variants of the GnRH receptor cannot be responsible for the alternative GnRH signal transduction pathway in cancer cells (86). Conversely, some normal and neoplastic human tissues have been found to express differential splice variants of the GnRH receptor gene in a tissue-dependent manner (97). It is not clear yet whether these splice variants can be translated into active membrane receptors or not. In the tumor cell lines that we analyzed, however, no signs of alternative GnRH receptor splice variants were seen (86).

Figure 3 Quantification of tyrosine-phosphorylated EGF receptors (EGF-R) by quantitative Western blotting of membranes obtained from the ovarian cancer cell lines EFO-21 (A) and EFO-27 (B) and endometrial cancer cell lines Ishikawa (C) and Hec-1A (D). In the absence of EGF, no phosphorylated EGF-Rs were detectable. Relative changes in the amounts of phosphorylated EGF-Rs after treatment with EGF (100 nmol/l) are given in the absence (CON = 100%) or presence (TRP) of the GnRH agonist triptorelin (10 μ mol/l) with or without vanadate (VAN). Columns represent means $\pm$ s.e. of data obtained from three independent experiments run in duplicate in three different passages of each cell line. Significant differences ($P < 0.001$) compared with ^acontrol; ^bTRP. From Gründker *et al.* (86), with permission. © 2001 The Endocrine Society.

It might be possible that a different coupling to G-proteins is responsible for the distinct GnRH signal transduction in cancer cells as compared with pituitary gonadotrophs. We found that, in endometrial and ovarian cancer cells, the GnRH receptor couples to several G-proteins (G-protein α_q and G-protein α_i) (Fig. 5).

The antiproliferative signal transduction, however, is exclusively mediated through the pertussis toxin-sensitive G-protein α_i (86) (Fig. 6).

It has been speculated that GnRH activates a PTP and thus antagonizes growth-factor-induced tyrosine phosphorylation (60). It is likely that the antiproliferative

Figure 4 (A) PCR amplification of first-strand cDNA from the ovarian cancer cell line EFO-21. Oligonucleotide primers for human *c-fos* were used. Gel was stained with ethidium bromide, and bands were visualized with UV light. Upper bands represent *c-fos* amplification, lower bands represent internal standard amplification. Quiescent cells were incubated in the absence or presence (+Trp.) of the GnRH agonist triptorelin (100 nmol/l) for 15 min, followed by treatment with 100 nmol/l EGF for 10 min. (B) and (C) Immunoblotting of c-Fos protein in ovarian cancer cell lines EFO-21 (B) and SK-OV-3 (C) using a polyclonal rabbit anti human c-Fos antibody. Quiescent cells were kept for 15 min in the absence or presence of the GnRH agonist triptorelin (100 nmol/l) before being incubated for 10 min with 100 nmol/l EGF. The amount of c-Fos protein was very small in the serum-starved cells (lane 1), but increased strongly after EGF treatment (lane 2). In the ovarian cancer cell line EFO-21, EGF-induced c-Fos protein synthesis was inhibited by the GnRH agonist triptorelin (B, lanes 3 and 4). In the ovarian cancer cell line SK-OV-3, which does not express GnRH receptors, GnRH agonists or antagonists had no effects on the EGF-induced c-Fos protein synthesis (C, lanes 3 and 4). From Gründker *et al.* (88), with permission. © 2000 Academic Press.

effects of GnRH analogs might be directly mediated through inhibition of growth factor signaling on its first step, the auto-phosphorylation of tyrosine residues of growth factor receptors. Some indirect evidence that GnRH activates a PTP was found by Imai *et al.* (83) and by Emons *et al.* (76), showing that GnRH reduces the net tyrosine phosphorylation of membrane proteins. Direct evidence for the G-protein α -mediated activation of a PTP and a reduction in EGF receptor tyrosine

phosphorylation by GnRH had not been obtained to date. We recently found that, in endometrial and ovarian cancer cells, the GnRH receptor activates a PTP, counteracting EGF-induced tyrosine auto-phosphorylation of the EGF receptor, resulting in downregulation of mitogenic signal transduction and cell proliferation. This effect was completely blocked by pertussis toxin, indicating the involvement of pertussis toxin-sensitive G-protein α in GnRH-induced PTP activity (86) (Fig. 7).

Figure 5 Crosslinking of G-protein α subunits and GnRH receptor in the ovarian cancer cell lines EFO-21 (A) and EFO-27 (B). Left lanes: Detection of the GnRH receptor protein (62 kDa) and the crosslinked GnRH receptor-G-protein α complex using an anti-GnRH receptor antibody (anti GnRH-R) (116). Middle lanes: Detection of G-protein α_i and crosslinked GnRH receptor-G-protein α_i complex using an anti-G-protein α_i antibody (anti G α_i). Right lanes: Detection of G-protein α_q and crosslinked GnRH receptor-G-protein α_q complex using an anti-G-protein α_q antibody (anti G α_q). Assuming a stoichiometric binding of 1:1, subtraction of the molecular weight of G-protein α_i (41 kDa) or G-protein α_q (41 kDa) from the crosslinked complex (103 kDa) yielded a protein of about 62 kDa. This corresponds to the molecular weight of GnRH receptor. Both G-protein α_i and α_q were found to be crosslinked to GnRH receptor in the ovarian cancer cell line EFO-21 (A). In the EFO-27 cell line, which does not express G-protein α_q , only G-protein α_i was found to be crosslinked to GnRH receptor (B). The figure shown is representative of three independent experiments run in duplicate in three different passages of the cell lines that gave identical results. Experiments using endometrial cancer cell lines Hec-1A or Ishikawa gave results identical to those with the ovarian cancer cell line EFO-21. From Gründker *et al.* (86), with permission. © 2001 The Endocrine Society.

GnRH and apoptosis

In plasma membranes of specimens from ovarian and endometrial cancers, GnRH recently has been shown to increase the expression of Fas ligand (98), known to promote apoptotic cell death through binding to Fas-positive cells within tumors. GnRH analogs are able to induce Fas ligand production in some GnRH-receptor-positive ovarian and endometrial cancer cells (99, 100). Though we have tried to show induction of apoptosis by GnRH analogs, we have found it only in one ovarian cell line (Ca-Ov-3), among nine ovarian (EFO-21, EFO-27, NIH:OVCAR-3, AN-3-CA, Ca-Ov-3, SK-OV-3) and endometrial (HEC-1A, HEC-1B, Ishikawa) cell lines investigated (101 and unpublished results). In a study conducted to investigate whether GnRH affects apoptosis in ovarian cancer, we found that the GnRH agonist triptorelin reduced apoptosis induced by the cytotoxic agent, doxorubicin. As triptorelin-induced reduction of doxorubicin-induced apoptosis was blocked by inhibition of nuclear factor κ B (NF κ B) translocation into the nucleus, and triptorelin was shown to induce

Figure 6 Effects of pertussis toxin (PTX) on the GnRH agonist triptorelin (TRP)-induced inhibition of EGF-induced *c-fos* expression. *c-fos* expression of quiescent EFO-21 ovarian cancer cells (first bar = control) and after treatment with 100 nmol/l EGF (10 min) without (second bar) or with (third bar) previous treatment (15 min) with the GnRH agonist triptorelin (100 nmol/l) or with previous treatment with the GnRH agonist triptorelin and with 2 ng/ml PTX (fourth bar). After treatment with EGF, a significant increase in *c-fos* expression was observed ($P < 0.001$) (second bar). After treatment with triptorelin followed by EGF, no increase in *c-fos* expression was observed (third bar). After treatment with pertussis toxin, triptorelin-induced inhibition of EGF-induced *c-fos* expression was blocked ($P < 0.001$) (fourth bar). Columns represent means $\pm$ S.E. of data obtained from four independent experiments run in duplicate in four different passages of each cell line. Experiments using endometrial cancer cell lines Hec-1A or Ishikawa or the ovarian cancer cell line EFO-27 gave similar results. Significant differences ($P < 0.001$) compared with ^acontrol; ^bEGF; ^cEGF/TRP. From Gründker *et al.* (86), with permission. © 2001 The Endocrine Society.

NF κ B activation, we concluded that GnRH has an anti-apoptotic effect mediated through NF κ B activation in these human ovarian cancer cells (101) (Fig. 8). This possible protection of ovarian cancer cells from programmed cell death that this offers is a new and important feature in GnRH signaling in ovarian tumors, in addition to its inhibitory interference with the mitogenic pathway.

Additional signaling mechanisms

In human endometrial and ovarian cancer cells, the GnRH agonist triptorelin stimulates the activity of activator protein-1 (AP-1) mediated through the pertussis toxin-sensitive G-protein α_i . In addition, triptorelin activates c-Jun N-terminal kinase (JNK), which is known to activate AP-1 (102). In earlier investigations, we have shown that triptorelin does not activate PLC and PKC in endometrial and ovarian cancer cells (76). In addition, it has been demonstrated that triptorelin inhibits growth-factor-induced MAPK activity (76). Thus triptorelin-induced activation of the JNK/AP-1 pathway

Figure 7 Effects of the GnRH agonist triptorelin (TRP) on PTP activity of EFO-21 (A) and EFO-27 (B) ovarian cancer cells and of Ishikawa (C) and Hec-1A (D) endometrial cancer cells. Quiescent cells were incubated for 15 min in the absence (control, CON) or presence of triptorelin with or without pertussis toxin (PTX), before PTP activity was measured. PTP activity is expressed as a percentage of controls (control = no treatment = 100%). Columns represent means $\pm$ S.E. of data obtained from three independent experiments run in duplicate in three different passages of each cell line. Significant differences ($P < 0.001$) compared with ^acontrol; ^bTRP. From Gründker *et al.* (86), with permission. © 2001 The Endocrine Society.

in endometrial cancer cells is independent of the known AP-1 activators, PKC or MAPK.

In endometrial and ovarian cancer cells, GnRH analogs mediate antiproliferative actions via inhibition of growth-factor-induced mitogenic signal transduction. The GnRH agonist triptorelin protects the cancer cells from apoptosis via activation of NF κ B, and triptorelin stimulates AP-1 and JNK activity. Recently Yamauchi *et al.* (103) found that JNK is involved in inhibition of the cell proliferation that is induced by α 1B-adrenergic receptor in human embryonic kidney cells. In a study in rats, *c-jun* mRNA depression and endometrial epithelial cell proliferation were suggested to be linked (104). In

UT-OC-3 ovarian cancer cells, cytokines have inhibitory effects on cell proliferation and activate AP-1 and NF κ B (105). As the antiproliferative GnRH agonist triptorelin activates the JNK/*c-jun* pathway and JNK/*c-jun* was found to be involved in downregulation of cell proliferation in different systems, it seems reasonable to speculate that the JNK/*c-jun* pathway is involved in the antiproliferative actions of the GnRH agonist triptorelin. It might be interesting to investigate the basis of the cooperation of AP-1 with NF κ B, in addition to the molecular basis of the antiapoptotic functions. Further investigations are required to determine whether or not the triptorelin-induced increase in AP-1 activity is

Figure 8 Flow cytometric analysis of ovarian cancer cell lines EFO-21 (A) and EFO-27 (B). After 72 h of treatment with 1 nmol/l of the cytotoxic agent doxorubicin, characteristic apoptotic DNA degradation (31% or 34% respectively) was observed (column 1). After 72 h of treatment with 1 nmol/l doxorubicin in the presence of 100 nmol/l of the GnRH agonist triptorelin, a significantly lower amount of characteristic apoptotic DNA degradation (17% or 18% respectively) was observed ($P < 0.001$) (column 2). After 72 h of treatment with 1 nmol/l doxorubicin and 100 nmol/l triptorelin in the presence of 18 μ mol/l NF κ B nuclear translocation inhibitor SN50, the proportion of cells showing characteristic apoptotic DNA degradation was 28% or 31% respectively (column 3). Using the inactive SN50M control peptide instead of SN50, a significantly lower amount of characteristic apoptotic DNA degradation (16% or 15% respectively) was observed ($P < 0.001$) (column 4). From Gründker *et al.* (101), with permission. © 2000 The Endocrine Society.

involved in the antiproliferative action of GnRH analogs, possibly via increasing the percentage of cells in the G0/G1 phase of the cell cycle.

Imai *et al.* (106) recently found that, in membranes of ovarian cancer cells obtained from surgically removed ovarian tumors, lysophosphatidic acid was hydrolyzed by GnRH-induced activation of lysophosphatidic acid phosphatase.

GnRH-II and its receptor

In non-mammalian vertebrates, it became evident that three structural variants of GnRH were present in

individual species (107, 108). A similar situation seems to exist in mammals. One of these GnRH variants is GnRH-II (also called chicken GnRH-II), which is entirely conserved in structure in the evolution from fish to mammals (109, 110). In human granulosa-luteal cells, expression of GnRH-II has been found (111). In these cells, GnRH-I agonists exerted a biphasic effect on GnRH-I receptor density, whereas GnRH-II agonists just produced a downregulation of GnRH-I receptor expression and of GnRH-II (111). In human ovarian and endometrial tumor cells, we found that treatment with GnRH-II had much greater effects on the inhibition of tumor cell proliferation than GnRH-I and its agonists (unpublished results). In earlier studies, we were able to show that two types of GnRH binding sites exist in endometrial and ovarian cancer cells, one of low affinity and high capacity, the other of high affinity and low capacity. The latter is comparable to the pituitary GnRH receptor (4, 46, 51, 52). The low-affinity binding site (using GnRH-I agonists) may be the putative GnRH-II binding site, with GnRH-I cross-reaction. It could be speculated that a second GnRH receptor type would also be present in vertebrates. Neill *et al.* (112) cloned, from the rhesus monkey (*Macaca mulatta*), a type II GnRH receptor that is highly selective for GnRH-II and was found to be ubiquitously expressed in human tissues, using a 32 P-labeled riboprobe specific for GnRH-II receptor. As the type II receptor has a C-terminal cytoplasmatic tail, it is rapidly internalized, in contrast to the type I receptor, and has a distinctly different signaling pathway (113, 114). In addition, GnRH antagonists have agonistic effects on the type II receptor (115). This finding might explain earlier observations that GnRH antagonists behave like agonists in reproductive tissue tumors (60). The recent cloning of the primate type II GnRH receptor sets the stage for the development of new analogs with selective activities on either GnRH-I or GnRH-II systems.

Summary

In human cancers, the distinction between GnRH agonists and antagonists on the basis of pituitary responses may not be appropriate. In addition, the GnRH signal transduction pathway that operates in normal tissues seems not to be essential in cancer cells. The most important features of GnRH signaling in tumors are the inhibitory interference with the mitogenic pathway that results in antiproliferative actions, and the possibility of protecting the cells from apoptosis via activation of NF κ B. In addition, GnRH activates the AP-1 signaling pathway which is possibly involved in the antiapoptotic and antiproliferative functions. In endometrial and ovarian cancer cells, the GnRH receptor couples to G-protein α_q and G-protein α_i , but both the antiproliferative and the antiapoptotic signal transduction are mediated through G-protein α_i . In prostate cancers,

Figure 9 GnRH signaling in gynecological cancer cells. (A) GnRH activates a PTP, which inhibits the mitogenic signal transduction of growth factor receptors resulting in downregulation of cell proliferation. (B) GnRH downregulates EGF receptor (EGF-R) mRNA expression. (C) Activated GnRH receptor (GnRH-R) induces NFκB activation and nuclear translocation of activated NFκB. Activated NFκB now couples to κB DNA binding sites and induces expression of antiapoptotic mechanisms. (D) GnRH activates JNK and stimulates AP-1 activity. In gynecological cancer cells, GnRH analogs mediate antiproliferative actions via inhibition of growth-factor-induced mitogenic signal transduction. In addition, GnRH induces growth factor receptor downregulation. GnRH protects the cancer cells from apoptosis via activation of NFκB, and stimulates AP-1 and JNK activity. RPTK, Receptor protein tyrosine kinase; TCF, transcription factor; IκB, inhibitory κB; Gq, G-protein αq; Gi, G-protein αi; SN50, inhibitor of nuclear translocation of activated NFκB; p50, p65, NFκB subunits. For references and further details see text.

the GnRH receptors are linked to the G-protein αi-cAMP signal transduction pathway. Fig. 9 summarizes current knowledge on GnRH signal transduction in gynecological cancers.

The existence of the additional GnRH-II system in brain, pituitary and reproductive organs, and in tumors of these organs, together with knowledge of different mechanisms of action in these tissues, may contribute to the development of new GnRH agonists and antagonists having selective actions on these different GnRH receptors and systems. For example, new selective GnRH analogs could be developed that inhibit endometriosis without affecting pituitary gonadotrophs. Other selective GnRH analogs developed for assisted reproduction might downregulate pituitary gonadotroph function without having effects on the endometrium. The use of selective GnRH receptor modulators might improve the therapeutic efficacy of GnRH analogs.

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