Experimental Study

Antiproliferative effects of the GnRH antagonist cetrorelix and of GnRH-II on human endometrial and ovarian cancer cells are not mediated through the GnRH type I receptor

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Abstract

Background: The majority of human endometrial and ovarian cancer cell lines express receptors for GnRH. Their proliferation is time- and dose-dependently reduced by GnRH-I and its superagonistic analogues. Recently, we have demonstrated that, in human endometrial and ovarian cancer cell lines except for the ovarian cancer cell line EFO-27, the GnRH-I antagonist cetrorelix has antiproliferative effects comparable to those of GnRH-I agonists, indicating that the dichotomy between GnRH-I agonists and antagonists might not apply to the GnRH system in cancer cells. We were also able to show that the proliferation of human endometrial and ovarian cancer cells was dose- and time-dependently reduced by GnRH-II to a greater extent than by GnRH-I agonists.

Objective: In this study we have assessed whether or not the antiproliferative effects of the GnRH-I antagonist cetrorelix in endometrial and ovarian cancer cells are mediated through the GnRH-I receptor.

Methods: We analysed the antiproliferative effects of the GnRH-I agonist triptorelin, the GnRH-I antagonist cetrorelix and GnRH-II in a panel of endometrial and ovarian cancer cell lines expressing GnRH-I receptors, in the SK-OV-3 ovarian cancer cell line that does not express GnRH-I receptors, and in four GnRH-I receptor positive GnRH-I receptor knockout cell lines.

Results: We found that, after knockout of the GnRH-I receptor, the antiproliferative effects of the GnRH-I agonist triptorelin were abrogated, whereas those of the GnRH-I antagonist cetrorelix and of GnRH-II persisted.

Conclusions: These data suggest that, in endometrial and ovarian cancer cells, the antiproliferative effects of cetrorelix and of GnRH-II are not mediated through the GnRH-I receptor.

Introduction

The expression of gonadotropin-releasing hormone (GnRH, GnRH-I) and its receptor as a part of a negative autocrine/paracrine regulatory system of cell proliferation has been demonstrated in a number of human malignant tumours, including cancers of the endometrium, ovary and breast (1). In these cancers the in vitro proliferation can be inhibited by agonistic or antagonistic analogues of GnRH-I in a dose- and time-dependent manner (1–5). The dichotomy between GnRH-I agonists and antagonists as defined in pituitary gonadotrophs might not apply to the GnRH system in cancer cells. The mechanism by which GnRH-I antagonists work remains unknown.

Recently, we were able to show that GnRH type II (GnRH-II) has antiproliferative effects on these tumour cells that are significantly greater than those of the superactive GnRH-I agonist, triptorelin (6). In the ovarian cancer cell line SK-OV-3, which does not express GnRH-I receptors (7), triptorelin had no effects on cell proliferation (7), whereas the GnRH-I antagonist cetrorelix had strong antiproliferative effects. These findings suggest that the antiproliferative effects of the GnRH-I antagonist cetrorelix are not attributable to a cross reaction with the GnRH-I receptor. Choi et al. (8) have reported that GnRH-II, is expressed in normal neoplastic ovarian surface epithelial cells and in cancers derived from these cells. In addition, they showed that, in immortalized ovarian surface epithelial cells, GnRH-II had antiproliferative effects (8). It might be speculated that, in addition to the autocrine GnRH-I system, an additional autocrine system based on GnRH exists in human cancers. Millar et al. (9) have recently reported that the GnRH-I antagonist 135-18 has agonistic effects on the marmoset type II GnRH receptor transfected into COS-7 cells. As we have shown in human endometrial
and ovarian cancers that a putative GnRH-II receptor could be present (6), this might explain why the GnRH-I antagonist cetrorelix behaves like an agonist in reproductive tissue tumours (2–5).

In this study we have assessed whether or not the antiproliferative effects of the GnRH-I antagonist cetrorelix in endometrial and ovarian cancer cells are mediated through the GnRH-I receptor. For this purpose, we analysed the antiproliferative effects of the GnRH-I agonist triptorelin, the GnRH-I antagonist cetrorelix and of GnRH-II in a panel of endometrial and ovarian cancer cell lines expressing GnRH-I receptors, in the SK-OV-3 ovarian cancer cell line that does not express GnRH-I receptors, and in four GnRH-I receptor positive cell lines in which we knocked out the GnRH-I receptor.

Materials and methods

Cell lines and culture conditions

The human endometrial cancer cell lines Ishikawa, HEC-1A and Hec-1B and the ovarian cancer cell lines EFO-21, EFO-27, OVCAR-3, SK-OV-3 and BG-1 were obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA) or the sources detailed previously (2, 3). The cells were cultured at 37 °C in a humidified atmosphere of 5% CO2 in air as described previously (2–4).

GnRH analogues

The GnRH-I antagonist cetrorelix (SB-75; [Ac- d-Nal(2), d-Phe(4Cl)2, d-Pal(3)1, d-Cit6, d-Ala10]GnRH-I) was kindly provided by Zentaris (Frankfurt, Germany) and the GnRH-I agonist [d-Trp6]GnRH (trip-torelin; pGlu-His-Trp-Ser-Tyr-d-Trp-Leu-Arg-Pro-Gly-NH2) was kindly provided by Ferrering Pharmaceuticals (Copenhagen, Denmark). Human GnRH-II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH2) was purchased from Bachem (Heidelberg, Germany).

Isolation of mRNA and cDNA synthesis

Polyadenylated RNA [poly(A) mRNA] was prepared from cells grown in monolayer using the Oligotex direct mRNA kit (Qiagen, Hilden, Germany). The concentration of poly(A) mRNA in each sample was determined by photospectrometry. First-strand cDNA was generated by reverse transcription of 1 μg poly(A) mRNA using p(dT)15 primers (Roche Diagnostics, Mannheim, Germany) with MMLV-reverse transcriptase according to the instructions of the suppliers (Life Technologies, Karlsruhe, Germany).

PCR amplification

The cDNAs (2 ng) were amplified in a 50 μl reaction volume containing 10 mmol/l Tris–HCl (pH 8.3), 50 mmol/l potassium chloride, 1.5 mmol/l magnesium chloride, 200 μmol/l of each of the dNTPs, 1 μmol/l of primers specific for the human GnRH-I receptor mRNA (7) (forward primer: 5’-AGT CCA ATG GTA TGC TGG AG-3’, backward primer: 5’-ACC CGT GTC AGG GTG AAG AT-3’) or the human GnRH-II receptor-like mRNA (6) (forward primer: 5’-CTG GCT GTG GAC ATC GCA TGT-3’, backward primer: 5’-ATG GCA GTC AGT GGC AGC AGA-3’), and 1.25 U AmpliTaq Gold polymerase (Applied Biosystems, Weiterstadt, Germany) in a Applied Biosystems DNA thermal cycler 9600. Thirty-five cycles of amplification of PCR were carried out: denaturation at 94 °C for 30 s, annealing at 60 °C (GnRH-I receptor) or 58 °C (GnRH-II receptor) for 60 s, followed by extension at 72 °C for 60 s. The PCR products were separated by gel electrophoresis in 1.5% agarose and visualized by ethidium bromide staining on a u.v. transilluminator.

The complete and functional human GnRH-II receptor transcript still could not be identified, therefore the GnRH-II receptor mRNA was called ‘GnRH-II receptor-like mRNA’.

Restriction enzyme analysis

The GnRH-I receptor PCR products were digested with the restriction endonucleases BamHI and PstI, under conditions recommended by the manufacturer (Roche Diagnostics). The digested products, along with untreated aliquots of each PCR sample, were then fractionated on 1% agarose gel and stained.

Southern blot

The GnRH-I receptor PCR products were separated on 1.5% agarose gels, visualized as described, and then transferred to Hybond-N+ membranes (Amersham-Buchler, Braunschweig, Germany). The blots were hybridized with a full-length cDNA (7) for the human GnRH-I receptor (bp 1-1560) with the enhanced chemiluminescence–direct nucleic acid labelling and detection system (RPN 300; Amersham-Buchler) according to the instructions of the manufacturer.

Southern blot analysis of the GnRH-II receptor PCR products was performed using a biotin-3’-endlabelled GnRH-II receptor probe designed according to a region (transmembrane domain III) highly conserved from fish (Morone saxatilis) to primates (Macaca mulatta): 5’-CTG ATG TTC CTG AAA CTA ATG GCC AGC AGA TAT TCT GCA GCT TCC-3’ (6). The blots were prehybridized for 30 min at 50 °C with formamide, 5 × SSPE (containing 0.75 mol/l NaCl, 0.05 mol/l NaH2PO4, and 5 mmol/l EDTA at pH 7.4), 5 × Denhardt’s, 0.5% SDS and 100 μg/ml

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denatured herring sperm DNA] at 42 °C, followed by washing in high stringency conditions (0.1 × SSPE and 0.1% SDS at 65 °C for 10 min). Subsequently, the hybridized blots were visualized using the LightShift reagents according to the manufacturer’s instructions (Perbio Science, Rockfort, Illinois, USA).

### Proliferation assays

The time-course and dose–response proliferation experiments were performed as described in detail previously (2, 3). Briefly, 20,000 cells of each cell line were plated in four-well cluster dishes (Nunc, Roskilde, Denmark) and allowed to attach to the wells. After 24 h, the medium was changed and 20 µl PBS–BSA or appropriate dilutions of the GnRH-I agonist triptorelin or the GnRH-I antagonist cetrorelix were added, resulting in final concentrations of 10 pmol/l to 10 µmol/l. Every 12 h, fresh PBS–BSA or triptorelin or cetrorelix were added. After 24 h of incubation, the medium was changed. After 1, 2, 3 and 4 days, the cells were counted in a Neubauer haemocytometer (2, 3).

### GnRH-I receptor knockout experiments

A 43 bp fragment of the human GnRH-I receptor cDNA was cloned in antisense orientation (5′-CT AGA ACC ATG GAC TGT CCG ACT TTG CTG TTG CTT TTC-3′) into the Nhel/Sall sites of the eucaryotic expression vector, pIRES (Clontech, Palo Alto, California, USA), to produce the pGnRH-IR-antisense vector.

Cells were grown to approximately 50% confluence on Nunc two-well chamber slides (immune cytochemistry), in Nunc four-well cluster dishes (proliferation assay) or in Nunc 100 mm dishes (immunoblotting). Transfections were done using Superfect liposome reagents and following the manufacturer’s instructions (Qiagen). After 12 h, transfected cells and non-transfected control cells were treated with the GnRH-I agonist triptorelin (100 nmol/l) to induce GnRH-I receptor protein internalization. Twelve hours later, the medium was changed and 20 µl PBS–BSA or appropriate dilutions of triptorelin, the GnRH-I antagonist cetrorelix or GnRH-II were added, to a final concentration of 10 µmol/l. Every 12 h, fresh PBS–BSA or triptorelin or cetrorelix or GnRH-II was added. After 24 h of incubation, the medium was changed.

After 4 days of treatment, the cells grown on two-well chamber slides were fixed in 4% paraformaldehyde (PFA) at 4 °C overnight. The cells were washed in PBS and then treated with 1 mol/l glycine for blocking free aldehyde groups (30 min), washed in PBST (0.2% BSA, 0.1% Triton X-100 in PBS) for 2 × 15 min, and treated in PBSTN (5% FCS in PBST) for blocking further unspecific protein binding sites (10 min). The first antibody was a monoclonal mouse anti-human GnRH-I receptor (clone A9E4; Research Diagnostics, Flanders, New Jersey, USA), diluted 1:20 in PBSTN; the cells were incubated therein at 4 °C overnight. After three careful washes in PBST, the cells were incubated with PBSTN for 30 min and then treated with the Histostain SP kit for mouse primary antibody (Zymed, San Francisco, California, USA) according to the manufacturer’s instructions.

### Statistical analysis

All experiments were repeated three times with different passages of the respective cell lines. Data were tested for significant differences using the Mann–Whitney U-test. The data from the dose–response experiments were tested for significant differences by one-way analysis of variance followed by Student–Newman–Keuls’ test for comparison of individual groups, after a Bartlett test had shown that variances were homogenous.
Results

Expression of GnRH-I receptors and GnRH-II receptor-like mRNA in endometrial and ovarian cancer cells

PCR amplification of cDNA from the endometrial cancer cell lines Ishikawa, Hec-1A and Hec-1B and the ovarian cancer cell lines EFO-21, EFO-27, OVCAR-3 and BG-1 with the oligonucleotide primers for the human GnRH-I receptor yielded the expected 417 bp product (Fig. 1A). Cleavage of the 417 bp product with restriction enzymes yielded the expected fragments of 297 bp and 114 bp (PstI) and 232 bp and 179 bp (BamHI; data not shown). The sequence of the PCR products was further confirmed by Southern blot analysis using a full-length cDNA probe for the human GnRH-I receptor (Fig. 1B). Expression of GnRH-I receptor mRNA could not be detected in the ovarian cancer cell line SK-OV-3 (Fig. 1A, B).

Using oligonucleotide primers for a human GnRH-II receptor-like mRNA, the expected 337 bp product was detected (Fig. 2A). As the complete and functional human GnRH-II receptor transcript still could not be identified, the GnRH-II receptor mRNA was called ‘GnRH-II receptor-like mRNA’. Southern blot analysis using a biotin-labelled GnRH-II receptor-like probe confirmed the authenticity of the PCR products (Fig. 2A). All endometrial cancer cell lines and the ovarian cancer cell lines EFO-21, SK-OV-3, OVCAR-3 and BG-1 showed GnRH-II receptor-like mRNA expression (Fig. 2A, B). However, we found a stop codon in this sequence (data not shown). The GnRH-II receptor-like mRNA was therefore suspected to be non-functional. In the ovarian cancer cell line EFO-27, GnRH-II receptor-like mRNA expression was absent (Fig. 2A, B).

Effects of the GnRH-I agonist triptorelin, the GnRH-I antagonist cetrorelix and GnRH-II on cell proliferation

In the GnRH-I receptor-positive ovarian cancer cell line EFO-21, the proliferation was dose-dependently...
inhibited by 4 days of treatment with different concentrations (10 pmol/l, 1 nmol/l, 100 nmol/l, 10 μmol/l) of the GnRH-I agonist triptorelin (Fig. 3A). With 10 pmol/l triptorelin, a significant decrease in cell number to 84.1 ± 3.5% (P < 0.01) of the control ( = 100%) was observed. With 1 nmol/l triptorelin, the cell number was reduced to 78.0 ± 2.2% (P < 0.001) of control. At 100 nmol/l, triptorelin had still greater antiproliferative effects (70.8 ± 2.9% of control; P < 0.001), and its inhibitory effects were maximal at a concentration of 10 μmol/l (58.4 ± 2.5% of control: P < 0.001). Similar dose–response relationships for the antiproliferative effects were observed with the GnRH-I antagonist cetrorelix and with GnRH-II (Fig. 3A). With 10 μmol/l of the GnRH-I antagonist cetrorelix, the cell number was reduced to 66.1 ± 3.6% of control (P < 0.001) and with 10 μmol/l GnRH-II it was decreased to 47.8 ± 3.7% of control (P < 0.001).

In the GnRH-I receptor-positive ovarian cancer cell line EFO-27, the proliferation was dose-dependently inhibited by 4 days of treatment with different concentrations of the GnRH-I agonist triptorelin (Fig. 3B). With 10 pmol/l triptorelin, only a slight decrease in cell number, to 89.4 ± 3.5% (not significant) of the control (= 100%), was observed. With 1 nmol/l triptorelin, the cell number was 80.3 ± 2.8% of control (P < 0.001); with 100 nmol/l triptorelin it was reduced to 76.8 ± 2.9% of control (P < 0.001), and the inhibitory effects were maximal with a concentration of 10 μmol/l triptorelin (69.7 ± 3.1% of control; P < 0.001). In contrast to findings for the EFO-21 cancer cell line, no relevant antiproliferative effects were observed with the GnRH-I antagonist cetrorelix or with GnRH-II (Fig. 3B).

In the GnRH-I receptor-negative ovarian cancer cell line SK-OV-3, we did not observe antiproliferative effects of the GnRH-I agonist triptorelin (Fig. 3C). However, using the GnRH-I agonist cetrorelix or GnRH-II, the proliferation was dose-dependently inhibited by 4 days of treatment (Fig. 3C). With 10 pmol/l cetrorelix, only a slight decrease in cell number, to 91.3 ± 3.5% (not significant) of the control (= 100%), was observed. With 1 nmol/l cetrorelix, the cell number was reduced to 79.8 ± 3.9% of control (P < 0.001), with 100 nmol/l it was reduced to 67.5 ± 3.1% of control (P < 0.001), and the inhibitory effects were maximal with a concentration of 10 μmol/l cetrorelix (63.4 ± 3.7% of control; P < 0.001). With 10 μmol/l GnRH-II, the cell number was reduced to 48.8 ± 4.1% of control (P < 0.001).

The proliferation data for the ovarian cancer cell lines OVCAR-3 and BG-1 (Table 1) and the endometrial cancer cell lines Ishikawa, Hec-1A and Hec-1B (Table 1) were identical with the data obtained from the ovarian cancer cell line EFO-21 (Fig. 3A).

**GnRH-I receptor knockout**

A 43 bp cDNA encoding a fragment of the human GnRH-I receptor was cloned in antisense orientation into the Nhel/Sall sites of the eucaryotic expression vector pIRESV, to produce the pGnRH-I-R-antisense vector (Fig. 4).

Knockout of the GnRH-I receptor protein was controlled by immune cytochemistry using a monoclonal mouse anti-human GnRH-I receptor antibody (Fig. 5) and immunoblotting using a polyclonal rabbit anti-human GnRH-I receptor antiserum (Fig. 6). After internalization of the GnRH-I receptor induced by the GnRH-I agonist triptorelin, a high density of novel
Table 1  Antiproliferative effects of the GnRH-I agonist triptorelin (10 μmol/l), the GnRH-I antagonist cetrorelix (10 μmol/l) and GnRH-II (10 μmol/l) on Ishikawa, Hec-1A and Hec-1B human endometrial cancer cell lines and EFO-21, EFO-27, OVCAR-3, SK-OV-3 and BG-1 human ovarian cancer cell lines. Cell numbers are expressed as mean ± S.E.M. percentages of the controls (vehicle only: 100%).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Triptorelin</th>
<th>Cetrorelix</th>
<th>GnRH-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial cancer cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ishikawa</td>
<td>63.0 ± 1.8</td>
<td>69.7 ± 2.2</td>
<td>49.2 ± 5.7</td>
</tr>
<tr>
<td>Hec-1A</td>
<td>64.9 ± 4.4</td>
<td>70.1 ± 2.5</td>
<td>44.1 ± 4.3</td>
</tr>
<tr>
<td>Hec-1B</td>
<td>76.9 ± 16.9</td>
<td>59.2 ± 2.3</td>
<td>52.4 ± 6.5</td>
</tr>
<tr>
<td>Ovarian cancer cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFO-21</td>
<td>58.4 ± 2.5</td>
<td>66.1 ± 3.6</td>
<td>47.8 ± 3.7</td>
</tr>
<tr>
<td>EFO-27</td>
<td>69.7 ± 3.1</td>
<td>95.3 ± 2.0</td>
<td>97.0 ± 2.8</td>
</tr>
<tr>
<td>SK-OV-3</td>
<td>97.7 ± 3.5</td>
<td>63.4 ± 3.7</td>
<td>48.8 ± 4.1</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>71.6 ± 3.8</td>
<td>63.4 ± 1.8</td>
<td>38.5 ± 6.6</td>
</tr>
<tr>
<td>BG-1</td>
<td>70.8 ± 1.5</td>
<td>67.1 ± 3.2</td>
<td>51.2 ± 4.9</td>
</tr>
</tbody>
</table>

Values in bold italics indicate no antiproliferative effect; all other values represent significant (P < 0.001) antiproliferative effects.


After knockout of the GnRH-I receptor, the antiproliferative effects of the GnRH-I agonist triptorelin on endometrial and ovarian cancer cells were abrogated, whereas the growth inhibitory effects of the GnRH-I antagonist cetrorelix were still the same as observed in non-transfected cancer cells.

After 4 days of treatment of GnRH-I receptor knockout cell lines with cetrorelix (10 μmol/l) the observed cell numbers, expressed as % of control (= 100%) were: Hec-1A (Fig. 7A) 41.99 ± 7.17% of control (P < 0.001); Ishikawa (Fig. 7B) 33.20 ± 3.52% of control (P < 0.001); EFO-21 (Fig. 7C) 34.35 ± 3.44% of control (P < 0.001); OVCAR-3 (Fig. 7D) 30.92 ± 4.39% of control (P < 0.001).

Treatment of GnRH-I receptor knockout cell lines for 4 days with the GnRH-I agonist triptorelin (10 μmol/l) resulted in cell numbers, expressed as % of control (= 100%), as follows: Hec-1A (Fig. 7A) 94.14 ± 9.96% of control (not significant); Ishikawa (Fig. 7B) 84.50 ± 8.20% of control (not significant); EFO-21 (Fig. 7C) 83.97 ± 8.94% of control (not significant); OVCAR-3 (Fig. 7D) 89.12 ± 7.44% of control (not significant).

After 4 days of treatment of GnRH-I receptor knockout cell lines with GnRH–II (10 μmol/l) the observed cell numbers, expressed as % of control (= 100%), were: Hec-1A (Fig. 7A) 40.51 ± 5.06% of control (P < 0.001); Ishikawa (Fig. 7B) 30.81 ± 4.74% of control (P < 0.001); EFO-21 (Fig. 7C) 32.10 ± 3.58% of control (P < 0.001); OVCAR-3 (Fig. 7D) 31.61 ± 5.63% of control (P < 0.001).

Figure 5 Immune histochemical detection of GnRH-I receptor protein in EFO-21 human ovarian cancer cells grown on two-well chamber slides using a monoclonal mouse anti-human GnRH-I receptor antibody. (A) Non-transfected cells. (B) Cells transfected with pGnRH-I-R antisense expression vector. (C) Control performed by omission of the primary antibody. Experiments using the human endometrial cancer cell lines Ishikawa and Hec-1A and the human ovarian cancer cell line OVCAR-3 gave identical results.
Discussion

We have previously demonstrated that, in human endometrial and ovarian cancer cell lines, the GnRH-I antagonist cetrorelix has antiproliferative effects comparable to those of GnRH-I agonists, indicating that the dichotomy of GnRH-I agonists and antagonists might not apply to the GnRH-I system in these cells (2, 3). In endometrial cancer cell lines Hec-1A and Ishikawa, similar dose-dependent antiproliferative effects of both the GnRH-I agonist triptorelin and the GnRH-I antagonist cetrorelix were found (3). In the EFO-21 ovarian cancer cell line, the GnRH-I antagonists cetrorelix and ramorelix produced dose-dependent antiproliferative effects similar to those of the GnRH-I agonist triptorelin (2). In contrast, in the EFO-27 ovarian cancer cell line neither cetrorelix nor ramorelix had any effect on the proliferation of the cells, even at the high concentration of 10 μmol/l, whereas the GnRH-I agonist triptorelin exhibited dose-dependent antiproliferative effects (2). During simultaneous treatment, cetrorelix partly antagonized the antiproliferative effects of 1 nmol/l to 10 μmol/l concentrations of triptorelin, and thus acted like an antagonist in the EFO-27 cell line (2). Until now it has not been understood why GnRH-I antagonists had antiproliferative effects in endometrial and ovarian cancer cell lines, with the exception of the ovarian cancer cell line EFO-27.

In this study we were able to show that, in endometrial and ovarian cancer cells affected by both the GnRH-I agonist triptorelin and the GnRH-I antagonist cetrorelix (2, 3), GnRH-II also showed highly effective antiproliferative effects. In contrast, in the EFO-27 ovarian cancer cell line in which the GnRH-I antagonist cetrorelix had no such effects (2), GnRH-II was also not effective. In this cell line, cetrorelix had effects like those of a true GnRH-I antagonist: it partly antagonized the antiproliferative effects of the GnRH-I agonist triptorelin (2). In the ovarian cancer cell line SK-OV-3, which does not express GnRH-I receptors (7), the GnRH-I agonist triptorelin had no effects on cell proliferation (7), whereas the GnRH-I antagonist cetrorelix and GnRH-II (6) had strong antiproliferative effects.

In cell lines affected by both the GnRH-I agonist triptorelin and the GnRH-I antagonist cetrorelix, the effects of the former were abrogated after GnRH-I receptor knockout, whereas those of cetrorelix and of GnRH-II persisted. These findings suggest that, in endometrial and ovarian cancer cells, the antiproliferative effects of the GnRH-I antagonist cetrorelix and of GnRH-II are not mediated through the GnRH-I receptor.

Several groups have tried to find a functional human GnRH-II receptor (9–12). Until now, attempts to clone and sequence of a full-length human GnRH-II receptor have not been successful (13). Morgan et al. (14) found that the human GnRH-II receptor is expressed as a variety of splice variants and a functional human GnRH-II receptor transcript was not found. The GnRH-II receptor-like mRNA detected in this and in our previous study (6) is suspected to be non-functional because of the stop codon in the sequence. Thus further investigations are required to identify the receptor that mediates the activities of the GnRH-I antagonist.

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cetrorelix and of GnRH-II and to elucidate the entire mechanism of these effects.

Nevertheless, our above-mentioned data suggest that, in human endometrial and ovarian cancer cells in addition to the GnRH-I receptor, an additional functional receptor may be present, mediating the antiproliferative effects of the GnRH-I antagonist cetrorelix and GnRH-II. At present, the functional role of a putative additional receptor for GnRH co-expressed with GnRH-I receptor in human endometrial and ovarian cancer cells is unknown. As GnRH-II has strong antiproliferative effects without involving the induction of apoptosis (6), it could be speculated that the additional receptor interacts with pathways regulating the cell cycle. It is well established that GnRH-I and its receptor in human endometrial and ovarian cancers are parts of a negative autocrine mechanism of cell proliferation (1, 5, 15, 16) and that GnRH-I protects the cells against apoptosis (17). Co-expression of the GnRH-I receptor and an additional receptor for GnRH in human endometrial and ovarian cancer cells suggests a potential interaction between these two receptor types. Moreover, after knockout of the GnRH-I receptor, the antiproliferative effects of the GnRH-I antagonist cetrorelix seemed to be increased. We do not have any explanation for this effect at present, but it seems reasonable to speculate that, in cells in which the GnRH-I receptor and a putative additional receptor for GnRH are co-expressed, a cross-talk between the signalling of both receptors might take place. It would be very interesting to know whether or not the antiproliferative effects of the GnRH-I antagonist cetrorelix and GnRH-II would be abrogated after knockout of the putative additional receptor for GnRH. However, different knockout experiments using GnRH-II receptor antisense fragments resulted in apoptotic cell death (unpublished results). At present, therefore, this question cannot be answered.

In conclusion, our present data suggest that, in endometrial and ovarian cancer cells, the antiproliferative effects of the GnRH-I antagonist cetrorelix are not mediated through the GnRH-I receptor. If there is a functional additional receptor for GnRH, the GnRH-I antagonist cetrorelix may act as an agonist on this receptor. This could rationalize the paradox of similar effects of GnRH-I agonists and antagonists on tumour cell proliferation.

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