Down-regulation and change in subcellular distribution of receptors for luteinizing hormone-releasing hormone in OV-1063 human epithelial ovarian cancers during therapy with LH-RH antagonist Cetrorelix

GABOR HALMOS, ANDREW V. SCHALLY and ZSUZSANNA KAHAN

Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical Center and Department of Medicine, Tulane UniversitySchool of Medicine, New Orleans, LA 70112-2699, USA

Received May 3, 2000; Accepted May 18, 2000

Abstract. The inhibition of growth of various hormone-dependent cancers by analogs of luteinizing hormone-releasing hormone (LH-RH) may be exerted in part through receptors for LH-RH present on tumor cells, but the direct mode of action of LH-RH agonists and antagonists is still not completely understood. The aim of this study was to investigate the effects of agonist [D-TrpP]LH-RH and antagonist Cetrorelix, administered s.c. at a dose of 100 μg/day for 3 weeks on the binding characteristics and subcellular localization of receptors for LH-RH in OV-1063 human epithelial ovarian cancers xenografted into nude mice. Using radioligand binding studies, following in vitro desaturation, we demonstrated the presence of specific, high affinity binding sites for LH-RH in both cell membrane and nuclear fraction of OV-1063 tumors. Treatment with Cetrorelix, but not [D-TrpP]LH-RH, caused about 60% reduction (p<0.01) in tumor volume and weight. [D-TrpP]LH-RH decreased the number of LH-RH receptors on OV-1063 tumor membranes by 44% after 14 days (p<0.01), and the concentration of receptors remained at that level on day 21. The maximal binding capacity of receptors for LH-RH in the nuclei was significantly higher (p<0.05) after 3 weeks of treatment with [D-TrpP]LH-RH. Cetrorelix decreased the concentration of membrane receptors for LH-RH by 53% (p<0.01) after 14 days and the levels on day 21 were even lower, showing a 70% reduction (p<0.01). In contrast, the number of LH-RH binding sites in the nuclear pellet was significantly increased (p<0.01) by Cetrorelix at that time. Our results demonstrate for the first time that the down-regulation of LH-RH receptors on the cell membranes of OV-1063 human ovarian cancers after therapy with antagonist Cetrorelix or agonist [D-TrpP]LH-RH is associated with an increase in receptor concentration in the nuclei. These phenomena could be related to the internalization and subcellular translocation of receptors in these tumor cells.

Introduction

The hormonal actions of luteinizing hormone-releasing hormone (LH-RH) and its analogs are mediated by high affinity receptors for LH-RH found on pituitary gonadotrophs (1-3). In addition, specific receptors for LH-RH are also found on breast, ovarian, endometrial and prostatic cancers (1-12). The function of these receptors is not completely elucidated, but it has been suggested that locally produced LH-RH may be involved in the proliferation of LH-RH receptor-positive cancers (1-3,9,12-16). The existence of an autocrine/paracrine loop based on LH-RH in prostatic, endometrial, breast and ovarian cancers has been proposed (9,12-15). Various studies demonstrated that LH-RH receptors on tumor cells can mediate direct antiproliferative effects of LH-RH agonists, antagonists and cytotoxic LH-RH analogs in vitro and in vivo (1,3,7-9,11-20). In the case of ovarian cancers, 78% of surgically removed tumor specimens (6,8) as well as EFO-21, EFO-27 and OV-1063 human ovarian cancer cell lines express LH-RH receptors (7,11,12). The expression of mRNA for LH-RH and LH-RH receptors in these cell lines was also reported (9,12,16,21). Studies in vitro indicate that the agonist [D-TrpP] LH-RH and antagonist Cetrorelix can reduce proliferation of ovarian cancer cell lines in culture (7,9,11,16). It was likewise shown that Cetrorelix binds to LH-RH receptors on OV-1063 and EFO-21 cells in vitro (7,11). Various experimental results demonstrated that LH-RH analogs and their cytotoxic conjugates inhibit growth of OV-1063 human epithelial ovarian cancers in nude mice (2,3,7,17-19). Recent studies revealed that treatment with LH-RH antagonist Cetrorelix induces a down-regulation of pituitary LH-RH receptors, which was previously thought to occur only with LH-RH agonists (22,23). Molecular biology analyses also showed a major reduction in the levels of mRNA for pituitary LH-RH receptors after administration of Cetrorelix (23). On the basis of these observations, it was important to determine whether chronic treatment with LH-RH agonists and antagonists could also decrease the concentration of LH-RH receptors on tumors.

Correspondence to: Professor Andrew V. Schally, Veterans Administration Medical Center, 1601 Perdido Street, New Orleans, LA 70112-1262, USA

Key words: LH-RH receptors, ovarian cancer, subcellular localization of receptors
Such a modulation of binding sites for LH-RH and in the expression of the LH-RH receptor gene in human ovarian cancer and other tumors produced by LH-RH analogs might be related to tumor inhibition.

Thus, in this study we investigated the presence and subcellular localization of binding sites for LH-RH in OV-1063 human epithelial ovarian cancers xenografted into nude mice. The purpose of this study was also to examine whether agonist [D-Trp³]LH-RH and antagonist Cetrorelix administered s.c. at a dose of 100 μg/day per animal for three weeks would affect the binding characteristics of receptors for LH-RH on cell membrane and in nuclei of OV-1063 tumors. The pattern of changes in the levels and localization of LH-RH receptors might provide further insight into the mechanism of action of these analogs on ovarian and other cancers.

Materials and methods

Peptides and chemicals. Agonistic analog [D-Trp³]LH-RH (1,2) was supplied by Debiopharm S.A. (Lausanne, Switzerland). LH-RH antagonist Cetrorelix (SB-75) (1,2) was made by ASFA Medica (Frankfurt am Main, Germany) as Cetrorelix acetate (D-20761). Radiotracer [¹²⁵I]Labeled sodium was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). All other peptides and chemicals, unless otherwise mentioned, were obtained from Sigma (St. Louis, MO), Bachem (Torrance, CA), R&D Systems (Minneapolis, MN) or California Peptide Research, Inc. (Napa, CA).

Animals. Female athymic nude mice (Ncr nu/nu), 5-6 weeks old on arrival, were obtained from the Frederick Cancer Research Facility of the National Cancer Institute (Frederick, MD) and housed and fed as described previously (18,19). All animal studies were conducted in accord with institutional ethical guidelines for the care and use of experimental animals.

Cell line and tumors. Human ovarian epithelial cancer cell line OV-1063, which originated from a metastatic papillary serous adenocarcinoma of the ovary in a 57-year-old woman (17,18), was obtained from the American Type Culture Collection (ATCC, Manassas, VA). It was grown in RPMI 1640 medium (Gibco, Grand Island, NY) with supplements (18,19). Xenografts were initiated by s.c. (subcutaneous) injection of 1x10⁶ cells into the right flanks of several nude mice.

In vivo studies. Tumors resulting after 4 weeks were aseptically dissected and mechanically minced. 1 mm³ pieces of tumor tissue were transplanted s.c. by trocar needle into female mice as described (18,19). The experiments were started one week after transplantation with mice bearing 20-30 mm³ tumors. In experiment 1 the mice were divided into 2 groups that received the following treatment: group 1 (controls), saline only (10 animals); group 2, agonist [D-Trp³]LH-RH at a dose of 100 ng/day/animal s.c. (15 animals). In experiment 2 the mice were also divided into 2 groups that received the following treatment: group 1 (controls), saline only (9 animals); group 2, antagonist Cetrorelix at a dose of 100 μg/day/animal s.c. (17 animals). Tumors were measured once a week with microlipsticks, and tumor volume was calculated as length x width x height x 0.5236. Body weight was measured weekly.

In both experiments the treatment was continued for 3 weeks, however 4-5 mice in each experiment were sacrificed before (day 9) and 14 days after the initiation of treatment to collect tumor samples. Thus, during and at the end of the experiments, for tumor removal, animals were anaesthetized with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL) and sacrificed by decapitation. Tumors were removed, cleaned and frozen on dry ice and then stored at -70°C until analyses of LH-RH receptors.

Preparation of tumor cell membranes and nuclei. Preparation of membranes for receptor studies was performed as described previously (10,22). Briefly, the tumors were thawed and cleaned, then each sample was cut in half. One half was homogenized in 50 mM Tris-HCl buffer (pH 7.4) supplemented with protease inhibitors using an Ultra-Turrax tissue homogenizer (IKA Works, Wilmington, NC) on ice. The homogenate was centrifuged at 500 x g for 10 min at 4°C to remove nuclear debris and lipid layer. The supernatant containing the crude membrane fraction was ultracentrifuged (Beckman L8-80M) twice at 70,000 x g for 50 min at 4°C after resuspending in fresh buffer. The final pellet was resuspended in homogenization buffer and stored at -80°C until assayed. Protein concentrations were determined by the method of Bradford (24) using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). The crude nuclear pellets were prepared from the other half of the tumor samples as described (25) with some modifications. Briefly, the samples were homogenized in 50 mM Tris-HCl buffer (pH 7.4) using a teflon glass homogenizer (Glas-Col, Terre Haute, IN). The homogenate was filtered through 2 layers of nylon gauze and centrifuged at 800 x g for 10 min at 4°C. The pellet was resuspended and washed twice with fresh homogenization buffer then stored at -70°C until assayed. DNA content in each tumor nuclear preparation was determined by the method of Labara and Pajger (26).

Radioligand binding studies. Radiolabeled derivatives of [D-Trp³]LH-RH were prepared by the chloramine-T method and purified by reverse-phase HPLC in our laboratory (22). LH-RH receptor binding assays were carried out as reported (10,22) using in vitro ligand competition assays based on binding of [¹²⁵I]D-Trp³LH-RH as radioligand to tumor membranes and nuclear fractions. This radioligand shows high-affinity binding to rat pituitary and human breast, prostate, and other cancers and was well-characterized previously (4-6,10,11,22). Since certain portion of LH-RH receptors may remain occupied by the LH-RH analogs after an in vivo administration, desaturation of LH-RH receptors in vitro, was also performed using 0.2 M MoCl₃ as chaotropic agent following the preparation of tumor fractions (22). After in vitro desaturation, membrane and nuclear fractions were incubated in duplicate or triplicate with 60-90,000 cpm [¹²⁵I]D-Trp³LH-RH and increasing concentrations (10⁻⁵ to 10⁻¹ M) of nonradioactive peptides as competitors in a total volume of 150 μl of binding buffer. At the end of the incubations, 125 μl aliquots of suspension were transferred onto the top of 1 ml of ice-cold binding buffer containing 1.5% bovine serum albumin in siliconized polypropylene microcentrifuge tubes (Sigma). The tubes were centrifuged at 12,000 x g for 3 min
Table 1. Effect of treatment with agonist [D-Trp³]LH-RH and antagonist Cetrorelix on the weight and volume of OV-1063 human epithelial ovarian cancers and body weights of tumor bearing nude mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor volume, mm³</th>
<th>Tumor weight (mg)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31.3±0.3</td>
<td>1877.9±325.1</td>
<td>2609.4±411.7</td>
</tr>
<tr>
<td>[D-Trp³]LH-RH</td>
<td>30.8±0.5</td>
<td>1285.3±345.1</td>
<td>1987.9±597.2</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.7±0.3</td>
<td>1600.3±257.8</td>
<td>2512.4±348.7</td>
</tr>
<tr>
<td>Cetrorelix</td>
<td>21.1±0.6</td>
<td>584.7±197.7</td>
<td>1044.9±287.4</td>
</tr>
</tbody>
</table>

The results are mean ± SEM. *p<0.01 vs. control by Duncan's new multiple range test.

Figure 1: Tumor volumes in nude mice bearing OV-1063 human epithelial ovarian cancer (●, control) during the treatment with LH-RH agonist [D-Trp³]LH-RH (●), administered s.c. at a dose of 100 µg/day per animal. Vertical bars represent SE.

Figure 2: Tumor volumes in nude mice bearing OV-1063 human epithelial ovarian cancer (●, control) during the treatment with LH-RH antagonist Cetrorelix (●), administered s.c. at a dose of 100 µg/day per animal. Vertical bars represent SE. *p<0.01 vs. control.

at 4°C (Beckman J2-21M). Supernatants were aspirated and the bottoms of the tubes containing the pellet were cut off and counted in a gamma counter (Micromedic System, Huntsville, AL).

Analysis of experimental data. Specific ligand-binding capacities and affinities were calculated by the Ligand-PC computerized curve-fitting program of Munson and Rodbard (27). To determine the types of receptor binding, equilibrium dissociation constants (Kᵦ values); and the maximal binding capacity of receptors (Bₘₐₓ), LH-RH binding data were also analyzed by the Scatchard method (28). Statistical analyses were performed using a computer software (SigmaStat, Jandel, San Rafael, CA). p<0.05 was accepted as a statistically significant difference. SigmaPlot graphing program (Jandel, San Rafael, CA) was used to visualize experimental data and to prepare figures.

Results

Effect of LH-RH analogs on the growth of OV-1063 human epithelial ovarian cancer in nude mice. The effects of treatment with LH-RH agonist [D-Trp³]LH-RH and antagonist Cetrorelix on body and tumor weights in nude mice implanted s.c. with OV-1063 human epithelial ovarian cancer cells as well as initial and final tumor volumes, are shown in Table 1. Changes in tumor volumes in experiments 1 and 2 are illustrated in Figs. 1 and 2, respectively. In experiment 1, the average tumor weights and volumes in the group receiving [D-Trp³]LH-RH were reduced, but the differences from
Table II. Effect of daily injections of [D-Trip]LH-RH (100 μg per animal, s.c.) on binding characteristics of LH-RH receptors in the membrane and nuclei of OV-1063 human epithelial ovarian cancers.

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Membrane receptors</th>
<th></th>
<th>Nuclear receptors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$ (nM)</td>
<td>$B_{max}$ (fmol/mg protein)</td>
<td>$K_d$ (nM)</td>
<td>$B_{max}$ (fmol/mg DNA)</td>
</tr>
<tr>
<td>Control (Day 0)</td>
<td>9.14±2.19</td>
<td>673.3±37.4</td>
<td>4.24±0.99</td>
<td>68.2±12.1</td>
</tr>
<tr>
<td>Treated Day 14</td>
<td>8.34±2.03</td>
<td>378.3±60.9</td>
<td>3.08±1.87</td>
<td>79.4±23.5</td>
</tr>
<tr>
<td>Day 21</td>
<td>6.95±2.27</td>
<td>412.7±22.3</td>
<td>5.02±2.01</td>
<td>98.7±9.12</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.05; **p<0.01.

The controls were not significant statistically (Table I). In experiment II the growth of OV-1063 human epithelial ovarian cancer in animals treated daily with Cetrorelix was significantly (p<0.01) inhibited within 7 days from the start of the experiment (Fig. 2). After 3 weeks, the average tumor weights and volumes in the group receiving Cetrorelix were reduced by about 60% (p<0.01) compared to the control group (Table I) (Fig. 2). The effects were not significant differences in blood weights between treated and control groups at the end of the experiments.

Characteristics of receptors for LH-RH after treatment with agonist [D-Trip]LH-RH. The characteristics of binding of [125I]-LH-RH to the membrane receptors on OV-1063 tumor cells following in vitro denaturation were determined using ligand competition assays. The equilibrium dissociation curve fitting and the Scatchard plot analysis of the binding data indicated that in membranes of untreated OV-1063 tumors, labeled peptide was bound to a single class of high affinity, low capacity binding sites ($K_d=9.14±2.19$ nM; $B_{max}=673.3±37.4$ fmol/mg protein) (Table II). Daily injections of [D-Trip]LH-RH produced a significant (p<0.01) decrease in the number of LH-RH binding sites 14 days after initiation of treatment (Table II). The reduction in the levels of membrane receptors was in the same range on day 21 (Fig. 3). High affinity binding sites for LH-RH were also found in the crude nuclear pellet of OV-1063 cells. Before the initiation of treatment (day 0), tumor nuclear receptors exhibited a mean dissociation constant ($K_d$) of 4.22±0.99 nM and a mean maximal binding capacity ($B_{max}$) of 65.2±13.1 fmol/mg DNA (Table II), analyzed by complete displacement. Treatment with [D-Trip]LH-RH significantly (p<0.05) increased the concentration of nuclear LH-RH receptors after 3 weeks (Fig. 3), indicating a marked shift in the subcellular distribution.

A daily administration of agonistic analog [D-Trip]LH-RH did not significantly influence the affinity of LH-RH receptors.
Table III. Effect of daily injections of Cetrorelix (100 µg per animal; s.c.) on binding characteristics of LH-RH receptors in the membrane and nuclei of OV-1063 human epithelial ovarian cancers.

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Membrane receptors</th>
<th>Nuclear receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$ (nM)</td>
<td>$B_{max}$ (fmol/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>(Day 0)</td>
<td>8.84±1.4</td>
</tr>
<tr>
<td>Treated</td>
<td>Day 14</td>
<td>6.43±1.1</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>4.87±0.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *p<0.05; **p<0.01

The binding affinity of receptors for LH-RH in the membrane or nuclei of OV-1063 tumors was not altered after treatment with Cetrorelix (Table III). There was no difference in the affinity and capacity of receptors for LH-RH in the membrane or nuclei of untreated OV-1063 tumors between day 0 and day 21 (data not shown).

Discussion

LH-RH receptors belong to the family of G protein-coupled receptor proteins and have been localized in the anterior pituitary, brain, kidney and reproductive organs as well as in many set steroid-dependent and -independent tumor tissues (1-3). Various studies have shown that LH-RH agonists and antagonists exert their main therapeutic effects through inhibition of release of LH, FSH, and sex steroids which result from desensitization of gonadotropes, down-regulation of LH-RH receptors, and reduction in the mRNA for LH-RH receptors in the pituitary (1-3,8). In addition, inhibition of cell growth and ['H]thymidine incorporation into cancer cells in culture by LH-RH analogs and the presence of high affinity receptors for LH-RH in these cancer cell lines suggest a direct effect of LH-RH agonists and antagonists on tumor cell proliferation (1-15).

Experimental findings indicate that LH-RH agonists and antagonists inhibit the growth of epithelial ovarian cancers in vitro and in vivo (1-3,7-9,11,16-19). The inhibitory action of LH-RH analogs on ovarian cancer growth was thought to be mediated mainly by suppression of pituitary secretion of LH and FSH, but some of the inhibitory effects could be direct, since human ovarian epithelial cancers express receptors for LH-RH (2,6,9,11,12,18,19,21). Approximately 80% of human epithelial ovarian cancer specimens and OV-1063, EFO-21, EFO-27, and ES-2 ovarian cancer cell lines have been found to be LH-RH receptor positive (6,9,11,12,16,21). The expression of mRNA for LH-RH and LH-RH receptors in ovarian cancers was also reported (12,6,21). Experimental oncological studies indicate that LH-RH antagonist Cetrorelix inhibits growth of human OV-1063 and ES-2 epithelial ovarian cancers in vitro better than agonist [D-Trp<sup>8</sup>]-LH-RH therefore it may be also more efficacious clinically (1,2,11,13,17). Although it was initially reported that chronic treatment with agonists of LH-RH can induce the regression of advanced ovarian cancer, reviewed in refs. (1-3,8) no beneficial effects of therapy with [D-Trp<sup>8</sup>]-LH-RH could be found in a large controlled trial comprising 200 patients with advanced ovarian cancer who received surgical cytoreduction and chemotherapy (29). However, in phase II clinical trials in patients with advanced ovarian cancer after first line chemotherapy, some patients showed a partial remission and others a stabilization of disease following therapy with Cetrorelix (30).

Our previous studies showed that administration of the LH-RH antagonist Cetrorelix to rats also caused a clear down-regulation of membrane receptors for LH-RH in the pituitaries of rats and not merely an occupancy of these binding sites (22,23). A major decrease in the expression of mRNA for pituitary LH-RH receptors was also found following treatment with Cetrorelix (23). The down-regulation of LH-RH receptors paralleled the decrease in mRNA levels for these receptors (23). These findings indicate that the loss of receptors might occur in part at the pretranslational level and could be the result of down-regulation of gene transcription.

The present study confirms and extends our previous findings (17) demonstrating that Cetrorelix, but not [D-Trp<sup>8</sup>]-LH-RH, inhibits the growth of OV-1063 human epithelial ovarian cancer in nude mice. Cetrorelix administered s.c. at a dose of 100 µg/day per animal for 3 weeks caused approximately 60% reduction in tumor volume and weight, while the same doses of [D-Trp<sup>8</sup>]-LH-RH showed only marginal effect. However, the most important findings of our investigation were on LH-RH receptors on tumors. Using ligand competition assays, following in vitro desaturation of LH-RH receptors (22), we were able to demonstrate that daily administration of agonist [D-Trp<sup>8</sup>]-LH-RH or LH-RH antagonist Cetrorelix to nude mice bearing human ovarian...
OV-1063 tumors resulted in a significant fall in the number of receptors for LH-RH on tumor membranes by day 14, as compared to controls. Cetrorelix induced a more rapid decrease (76%) in the number of total LH-RH receptors by day 21, than [D-Trp]6-LH-RH which caused only a 39% reduction.

In the present study, we also demonstrated the presence of specific, high affinity binding sites for LH-RH in the nuclear fraction and not only on membranes of OV-1063 tumors. Millar et al. (64th Meeting of the Endocrine Society, San Francisco, CA, USA, abstr. 48, pp91, 1982) were the first to report the presence of specific LH-RH binding sites in isolated nuclei from rat anterior pituitary. These results are consistent with the subsequent findings of Marian and Comri (251), indicating subcellular localization of the receptors for LH-RH in rat anterior pituitary and ovarian tissue. The nuclear and membrane binding sites differed in their affinity for an early LH-RH antagonist. Later, Szende et al. (31) investigated the localization of LH-RH receptors in pancreatic and mammary cancers. Using radioreceptor assay and electron microscopic immunohistochemistry, they demonstrated the presence of high affinity binding sites in the nuclear pellet of DOP-induced pancreatic cancer in hamsters, while low affinity receptors were found in the membrane fraction (31). The same group also reported that [D-Trp]6-LH-RH treatment resulted in a marked increase in the concentration of LH-RH binding sites in the nuclei of chemically induced pancreatic cancers (32). Immunogold labeling indicated the presence of LH-RH receptors in the pancreatic tumor cells, whereas no labeling was found in the acinar or ductal pancreatic epithelial cells of normal, tumor-free hamsters (32).

This study is the first to report the down-regulation of LH-RH binding sites in the nuclear fraction of OV-1063 human epithelial ovarian cancers after therapy with agonist [D-Trp]6-LH-RH or LH-RH antagonist Cetrorelix. This was accompanied by a significant increase in the concentration of LH-RH receptors in the tumor cell nuclei. We also demonstrated that a stronger antitumor effect of Cetrorelix as compared to the agonist [D-Trp]6-LH-RH is associated with a greater decrease in the level of receptor binding and a larger increase in the concentration of nuclear receptors. On the basis of this data, it appears that the effect of powerful LH-RH antagonists like Cetrorelix on human ovarian cancers may be further increased by the use of an agonist (LHRH) binding sites and cause a simple inhibition of the binding of the natural ligand. The binding of LH-RH antagonist Cetrorelix to high affinity receptors on tumor cell membrane may initiate intracellular changes that may be similar to or different from those induced by LH-RH agonists. Emirz et al. have reported that classical signal transduction mechanisms for hormone appear to be different from those for the pituitary (81). The down-regulation of LH-RH receptors on the tumor membranes and the parallel increase in the receptor concentration is the nuclei after therapy with Cetrorelix or [D-Trp]6-LH-RH are probably linked to the internalization and translocation of receptors in ovarian cancer cells. Further studies are needed to elucidate the exact events that follow the binding of LH-RH analogs in human ovarian cancers, but our findings suggest that nuclear receptors for LH-RH could be involved in mediating the antitumor action of LH-RH agonists and antagonists.

Acknowledgments

We thank Dr. Masahiro Miyazaki for providing tumor xenografts, Dr. I. Hoffman for her valuable experimental assistance and Dr. Attila Nagy for advice in the preparation of the manuscript. The work described in this paper was supported by the Medical Research Service of the Veterans Affairs Department and a grant from ASTA Medica (Frankfurt am Main, Germany) to Tulane University (all to A.S.V.).

References


