BIOLOGY OF REPRODUCTION **71,** 464–469 (2004)
Published online before print 7 April 2004.
DOI 10.1095/biolreprod.104.027342

Evidence for Different Gonadotropin-Releasing Hormone Response Sites in Rat Ovarian and Pituitary Cells[1]

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ABSTRACT

The participation of type I GnRH receptor (GnRH-R) on GnRH-II-induced gonadotropin secretion in rat pituitary cells was investigated. Furthermore, we extended the study of GnRH-II action to ovarian cells. The GnRH-II was able to mobilize inositol triphosphate (IP_3) and to induce LH and FSH release in a dose-dependent manner in pituitary cells and in a GnRH-I-like manner. The GnRH-analog 135-18 (agonist for type II GnRH-R and antagonist for type I GnRH-R) was unable to elicit any cellular response tested in these pituitary cells. The GnRH-II responses were blocked by the type I GnRH-R-antagonists CRX or 135-18, suggesting that these effects were mediated by the type I GnRH-R. In contrast to pituitary cells, GnRH-I, but not GnRH-II, elicited an IP_3 response in superovulated ovarian cells; 135-18 also had no effect. However, GnRH-II as well as GnRH-I presented antiproliferative effects on these cells. Surprisingly, 135-18 had stronger antiproliferative effects than either GnRH peptide. The 135-18 analog, but not GnRH-I or GnRH-II, increased progesterone secretion in superovulated ovarian cells. These results strongly suggest that GnRH-II is able to stimulate rat pituitary cells through the type I GnRH-R, with no evidence for the presence of type II GnRH-R. On the other hand, our results indicate a putative GnRH-R in superovulated ovarian cells with response characteristics that differ from those of the GnRH-R in the pituitary.

gonadotropin-releasing hormone, gonadotropin-releasing hormone receptor, neuroendocrinology, ovary, pituitary

INTRODUCTION

A decapeptide, GnRH plays a fundamental role as the physiologic regulator of reproduction by stimulating the synthesis and secretion of pituitary gonadotropins. Originally, this peptide was isolated from ovine and porcine brain, and its structure is conserved in all mammals [1, 2]. Therefore, it was named mGnRH. Today, 16 GnRH variants isolated from vertebrates have been described [3]. The second isoform isolated from chicken (cGnRH-II) is phylogenetically the most ubiquitous GnRH peptide, being present from jawed fish to humans. In a phylogenetic study based on DNA sequences and brain distribution, Fernald and White [4] classified GnRH subtypes in different branches. Following these suggestions, mGnRH is currently designated GnRH-I, and cGnRH-II is called GnRH-II.

Two kinds of GnRH receptors (GnRH-Rs) are reported: type I and type II. Type I GnRH-R is present in all the mammals studied, and type II GnRH-R was first described in nonmammal vertebrates. Recently, however, the expression of a type II GnRH-R together with the type I GnRH-R was reported in primates (marmoset, rhesus, and green monkeys) [5, 6]. By transfecting COS-7 cells with human type I GnRH-R or marmoset type II GnRH-R, it was shown that GnRH-II has approximately 10% of the activity of GnRH-I when binding to the type I GnRH-R, whereas GnRH-I has approximately 2% of the GnRH-II activity at the type II GnRH-R. On the other hand, both receptor types are coupled to $G_{q/11}$ and are able to stimulate production of inositol triphosphate (IP_3) [5].

For primates in which the presence of type II GnRH-R was demonstrated, this receptor was shown to be more widely distributed than the type I receptor, with the type II receptor being expressed throughout the brain, in the majority of gonadotropes, and in diverse nonneural and reproductive tissues [5, 6]. Reverse transcription-polymerase chain reaction and immunocytochemical evidence shows the type II GnRH-R being expressed ubiquitously in human tissues [7, 8]; however, to date, direct evidence for a full-length functional type II GnRH-R in human tissues is lacking [9].

The GnRH peptides are expressed not only in the hypothalamus but also in several areas of the brain and a variety of peripheral tissues. In different species, the expression of GnRH-I and of the type I receptor has been reported in a diversity of reproductive organs, such as the ovary, testes, myometrium, placenta, and endometrium [10–14]. In addition, GnRH-I and GnRH-II have been demonstrated in a range of human ovarian cells, and a variety of functional studies have established that both peptides regulate steroidogenesis, inhibit cell proliferation, and play a role in apoptosis induction [15]. However, the expression of their mRNAs is differentially regulated [16].

In rodents, using reverse-phase high-performance liquid chromatography/RIA, immunocytochemistry, and molecular biology techniques, the presence of GnRH-II in the brain of mouse and rat was suggested [17–19]. Additionally, GnRH-II expression in the rat brain was supported by evidence from our laboratory [20]. We also reported that GnRH-II is able to release LH and FSH from rat pituitary cells with similar potency to that of GnRH-I [20]. To our knowledge, little information is available regarding the

[1]Supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT, BID 1201/OC AR PICT 2000, 05 08664 to C.L.), Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 02231 to V.A.L.-L. and PIP 02282 to C.L.), Universidad de Buenos Aires (M045 to C.L.), and Ministerio de Salud de la Nación (to V.A.L.-L.).
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Received: 20 January 2004.
First decision: 11 February 2004.
Accepted: 18 March 2004.

ISSN: 0006-3363. http://www.biolreprod.org

presence or activity of GnRH-II/type II GnRH-R in rat ovary.

The aims of the present study were to examine participation of the type I GnRH-R or a putative type II receptor in the LH and FSH stimulation raised by GnRH-II in rat pituitary cells and to investigate the effects of GnRH-II in luteinized ovarian rat cells. For this purpose, we evaluated different cellular responses after stimulating cell cultures with GnRH-I, GnRH-II, 135-18 (agonist for type II GnRH-R and antagonist for type I GnRH-R [5, 21]), and Cetrorelix (CRX; antagonist for type I GnRH-R, devoid of effect on type II GnRH-R [7]).

MATERIALS AND METHODS

Animals

Sprague-Dawley female rats from the Instituto de Biología y Medicina Experimental (IBYME) colony were used; the animals were housed in groups in an air-conditioned room, with lights-on from 0700 to 1900 h. Rats were given free access to laboratory chow and tap water. Animals were killed by decapitation according to protocols for animal use approved by the institutional Animal Care and Use Committee (IBYME-CONICET) and following NIH guidelines (*Guide for Care and Use of Laboratory Animals*). For pituitary cell cultures, 15-day-old females were used. For superovulated ovarian (SPO) cells, 23- to 25-day-old female rats were used, and the animals were injected with 25 IU of eCG (Novormon-Syntex, Buenos Aires, Argentina) and then 25 IU of hCG (Endocorion-Elea, Buenos Aires, Argentina) 48 h later. These animals were killed 5 days after the hCG injection, and highly luteinized ovaries were obtained for cell culture.

GnRH Isoforms and Analogs

Both GnRH-I and GnRH-II were purchased from Bachem (Torrance, CA). The CRX was a gift from Serono (Buenos Aires, Argentina). The 135-18 was kindly provided by Dr. Roger Roeske (Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN), and the buserelin was a gift from Hoechst (Buenos Aires, Argentina).

Monolayer Cultures of SPO Cells

To determine the effects of GnRH isoforms in rat ovary, SPO cells were selected, both because this system provides a very high percentage of luteinized cells, rendering it a more homogeneous cell source, and because it is a well-characterized model in our laboratory.

Cells from 23- to 25-day-old SPO rat ovaries were obtained by dispersion with collagenase, as described previously [22, 23]. Briefly, cells were plated in plastic culture plates, coated with 0.5 mg/ml of rat tail collagen, and incubated in medium 1 (Dulbecco modified Eagle medium [DMEM] with high glucose-F12 [Invitrogen Argentina S.A., Buenos Aires, Argentina] supplemented with 2.2 g/L of sodium bicarbonate, 10% fetal calf serum, fungizone [Invitrogen Argentina S.A.], and gentamicin [Invitrogen Argentina S.A.]). After 24 h of incubation, culture media were replaced to remove cellular debris. Cells were maintained in the incubator for a further 48 h for the cell proliferation assays and for 5 extra days for IP_3 determinations.

Monolayer Cultures of Adenohypophyseal Cells

For pituitary cell cultures, 12-day-old female rat pituitaries were used, because this is a highly sensitive model for GnRH stimulation. Anterior pituitary cells were obtained as previously described [20, 24]. Briefly, anterior pituitaries were rapidly removed and placed in freshly prepared Krebs-Ringer bicarbonate buffer without Ca^{2+} or Mg^{2+}. Pituitaries were cut into small pieces and incubated in 0.2% trypsin for 30 min. After addition of DNase and lima bean trypsin inhibitor, the fragments were dispersed into individual cells and filtered through Nytex mesh (Nytex 50; Nytex, Geneva, Switzerland). Pituitary cells were plated in DMEM with low glucose, supplemented with 10% horse serum, 2.5% fetal calf serum, 1% minimum essential medium Eagle nonessential amino acids, fungizone, and gentamicin (medium 2). Cells were maintained in the incubator for 4 days for IP_3 determination and for gonadotropin RIAs.

IP_3 Determination

Levels of IP_3 were measured as described previously [25] with minor modifications. Briefly, 500 000 pituitary cells and 750 000 SPO ovarian cells were plated as described previously. After 4 days in culture for pituitary cells and 5 days for SPO cells, the wells were washed twice with medium 1 for SPO cells and with medium 2 for adenohypophyseal cells, then incubated for 24 h with 4 μCi ml^{-1} $well^{-1}$ of [2-^{3}H(N)]-myo-inositol in the corresponding media. The next day, cells were washed twice and incubated for 15 min in the presence of 20 mM LiCl in DMEM-high glucose-F12 supplemented with 2.2 g/L of sodium bicarbonate, 0.1% bovine serum albumin, fungizone, and gentamicin. Thereafter, these cells were further incubated for 30 min in the presence of the corresponding stimuli. Media were collected for hormone secretion measurement by RIA. Cells were scraped in 0.5% perchloric acid, and lysates were transferred to microfuge tubes and centrifuged for 10 min at 850 $\times$ *g*. Pellets were kept for DNA measurement. The supernatants were neutralized with 0.72 M KOH and 0.6 M $HKCO_3$ and then centrifuged at 850 $\times$ *g* for 10 min. Supernatants were chromatographed in Dowex ionic exchange columns (Dowex AG1-XP Resin, 200-400 mesh, formate form; Bio-Rad Laboratories, Hercules, CA) to elute inositol phosphate (IP_1), inositol biphosphate (IP_2), and IP_3. Two-milliliter aliquots of the eluates were mixed with 6 ml of OptiPhase ''Hisafe'' 3 (Perkin-Elmer Life Sciences, Wallac Oy, Turku, Finland) and counted in a liquid scintillation counter.

Radioimmunoassays

Both LH and FSH were determined in media samples by RIA using kits obtained through the National Hormone and Peptide Program; National Institute of Diabetes, Digestive, and Kidney Diseases; and Dr. A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA. Results were expressed in terms of RP3 rat LH and RP2 rat FSH standards. Assay sensitivities were 0.6 ng/ml for LH and 1.5 ng/ml for FSH. Intra- and interassay coefficients of variation were 7.2% and 11.4%, respectively, for LH and 8.0% and 13.2%, respectively, for FSH.

Progesterone was determined in culture media of the SPO cells after 48 h of stimulation. Progesterone was determined by RIA using a specific antiserum kindly provided by Dr. G.D. Niswender (Colorado State University, Fort Collins, CO). Assay sensitivity for progesterone was 6.25 pg/ml, and intra- and interassay coefficients of variation were 7.5% and 11.9%, respectively. Each sample was measured in duplicate.

Cell Proliferation Assay

Cell viability was measured using the Cell Titer 96® Non Radioactive Cell Proliferation Assay (Promega Corp., Madison, WI). Incubation of 50 000 SPO cells was carried out at 37°C for 72 h in the presence of respective stimuli. After this time, media were aspirated (stored for progesterone RIA), 115 μl of media containing 15 μl of tetrazolium salt solution were added to each well, and incubation was performed for an additional 2 h. The color reaction was developed for 1 h by adding 100 μl of the Solubilization/Sop solution. The assay was read at A_{580nm} using a 96-well plate reader. Cell number was calculated after interpolation in a cell number curve performed using 12 500, 25 000, 50 000, 100 000, and 200 000 cells. Parameters of the linear regression were as follows: $y = 2.79 \cdot 10^{-6} \cdot x - 0.0034097$ ($r^2 = 0.9906$).

Statistical Analysis

All experiments were run in replicates and were repeated three to four times. Results are expressed as the mean $\pm$ SEM. The data were analyzed by one-way analysis of variance followed by Student-Newman-Keuls multiple-comparisons test or Tukey honestly significant difference for unequal N.

RESULTS

Anterior Pituitary Cells

To elucidate participation of the type I GnRH-R or the putative type II receptor in the LH and FSH secretion raised by GnRH-II in rat pituitary cells, we investigated IP_3 levels produced after stimulation with GnRH-I, GnRH-II, and 135-18 (agonist for type II GnRH-R and antagonist for type I GnRH-R). After 30 min of stimulation, GnRH-I and GnRH-II (at both 10^{-6} and 10^{-8} M) increased IP_3 levels in

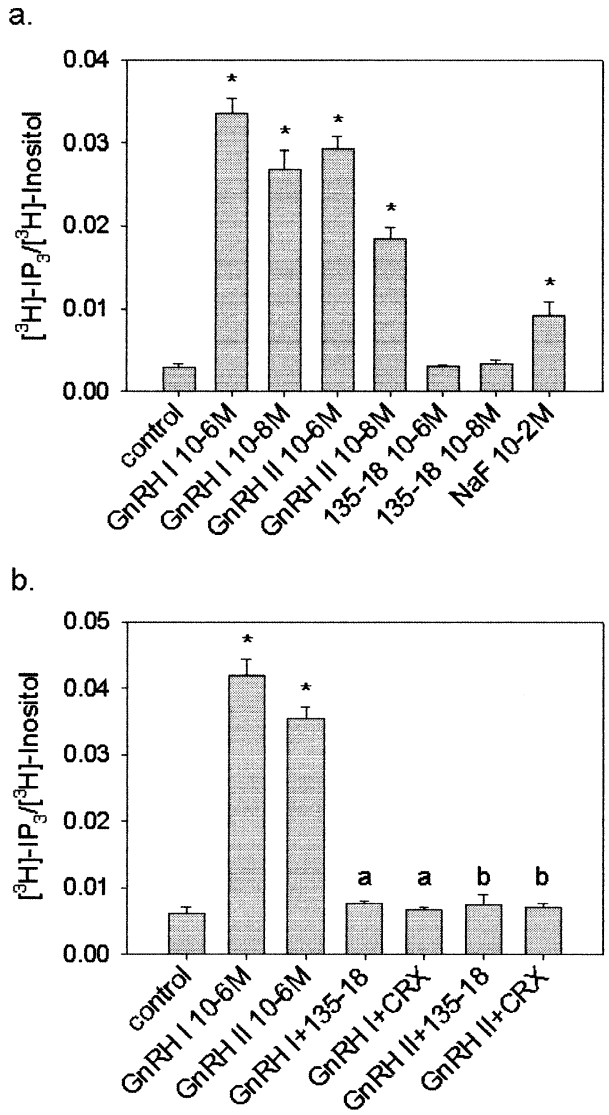

FIG. 1. Inositol triphosphate release in 12-day-old female rat pituitary cells. **a**) Effect of GnRH-I, GnRH-II, and 135-18 on IP_3 production after 30 min of stimulation in anterior pituitary cells. Sodium fluoride (NaF) is an unspecific stimulus for IP mobilization. Values are expressed as $[H^3]IP_3$ (cpm) relative to $[H^3]$inositol incorporated by cells (n = 4). $^*P < 0.01$ vs. control. **b**) Effects of type I GnRH-R-antagonists CRX and 135-18 in the response elicited by GnRH-I and GnRH-II. All stimuli were applied at 10^{-6} M (n = 3). $^*P < 0.01$ vs. control, $^aP < 0.01$ vs. GnRH-I, $^bP < 0.01$ vs. GnRH-II).

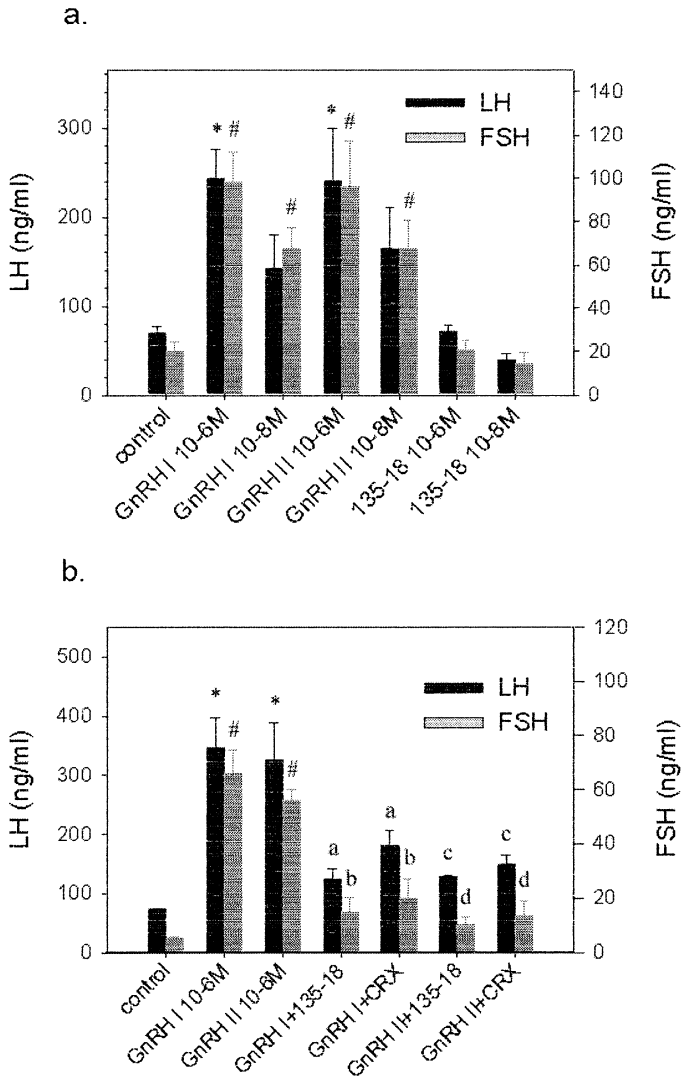

FIG. 2. Gonadotropin secretion from 12-day-old female rat pituitary cells. **a**) Levels of LH and FSH secreted to the culture media after 30 min of stimulation (n = 3 for LH, n = 4 for FSH). $^*P < 0.05$ vs. LH-control, $\#P < 0.05$ vs. FSH-control. **b**) Effect of 135-18 and CRX on the LH and FSH levels stimulated by GnRH-I or GnRH-II. Stimuli were applied at 10^{-6} M (n = 3). $^*P < 0.001$ vs. LH-control, $\#P < 0.001$ vs. FSH-control, $^aP < 0.01$ vs. LH-GnRH-I, $^bP < 0.01$ vs. FSH-GnRH-I, $^cP < 0.01$ vs. LH-GnRH-II, $^dP < 0.01$ vs. FSH-GnRH-II.

rat adenohypophyseal cells (Fig. 1a). The GnRH-I seemed to be more potent than GnRH-II in this response, but this difference did not achieve statistical significance. On the other hand, 135-18 (at 10^{-6} and 10^{-8} M) was unable to stimulate IP_3 production in these cells, suggesting that both GnRH isoforms were acting through the type I GnRH-R. To further test this hypothesis, we decided to block the increase in IP_3 levels using the type I GnRH-R-antagonists CRX and 135-18, because the last one was not able to elicit an agonistic response for IP_3 production. Figure 1b shows that CRX as well as 135-18 (at 10^{-6} M) blocked the increase in IP_3 triggered by GnRH-I or by GnRH-II (at 10^{-6} M). Moreover, in all the experiments, IP_1 and IP_2 profiles showed patterns similar to those of IP_3 (data not shown). These results are supported by comparable response profiles for LH and FSH release (Fig. 2a) evaluated after stimulation with GnRH-I, GnRH-II, and 135-18. In these experiments, GnRH-I and GnRH-II increased LH and FSH secretion in a dose-response fashion, whereas 135-18 was not able to modify either gonadotropin. Moreover, after coincubation of type I GnRH-R antagonists (135-18 or CRX) with GnRH-I or GnRH-II, the LH/FSH stimulation raised by the GnRH peptides was abolished (Fig. 2b). These experiments were repeated in a different endocrine model (cell cultures from adult male pituitaries), and IP_3 profiles as well as LH and FSH patterns in this model were similar to those of 12-day-old female cell cultures (data not shown).

SPO Cells

In SPO cells, GnRH-I at 10^{-6} M stimulated IP_3 increase with respect to control levels (Fig. 3). In contrast to pituitary cells, GnRH-II at 10^{-6} M was unable to induce a significant IP_3 rise in SPO cells. The analog 135-18 did not show agonistic properties, and it inhibited the GnRH-I-in-

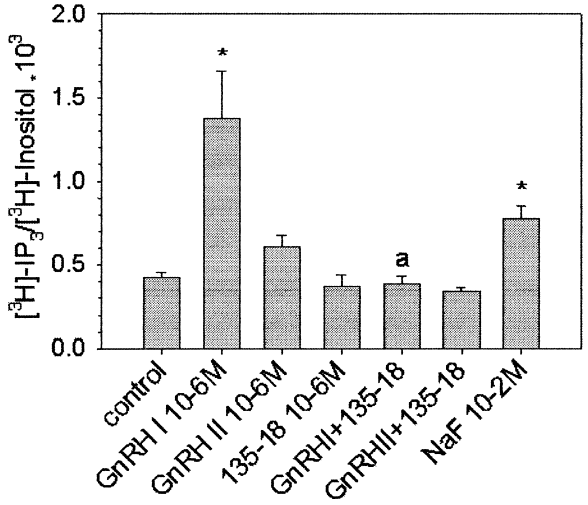

FIG. 3. Inositol triphosphate release in SPO cells. Stimuli were applied for 30 min, and the production of IP_3 was determined. Values are expressed as $[H^3]IP_3$ (cpm) relative to $[H^3]$inositol incorporated by cells (n = 3). $^*P < 0.05$ vs. control, $^aP < 0.01$ vs. GnRH-I at 10^{-6} M.

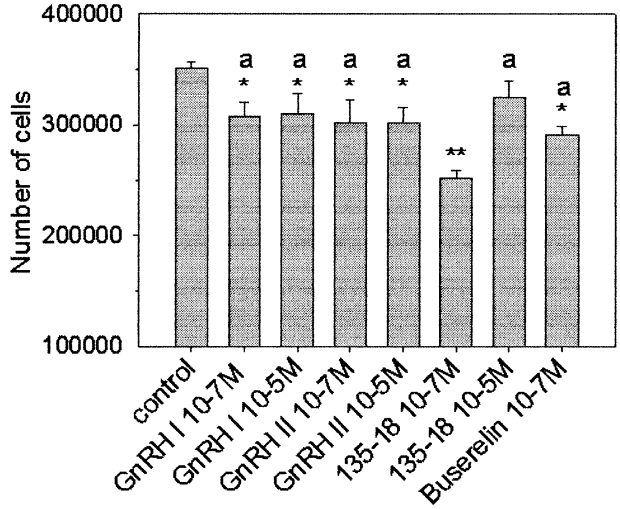

FIG. 4. Cell proliferation assay in SPO cells. Cell viability was measured after 72 h in culture with the respective stimuli (n = 4). $^*P < 0.05$, $^{**}P < 0.001$ vs. control, $^aP < 0.05$ vs. 135-18 at 10^{-7} M.

duced stimulation (Fig. 3). The IP_1 and IP_2 profiles for these experiments were similar to those of IP_3 (data not shown).

Because it was reported that both GnRH-I and GnRH-II had antiproliferative effects in different ovarian cells systems, we investigated this response in our model. In SPO cells, GnRH-I as well as GnRH-II led to a decrease in cell proliferation with respect to control values when either peptide was applied at 10^{-7} or 10^{-5} M (Fig. 4). Moreover, buserelin, a superactive GnRH-I analog, at 10^{-7} M showed similar effects to those of GnRH-I. Interestingly, 135-18 at 10^{-7} M had a significantly stronger antiproliferative action than the GnRH peptides. However, this effect had a biphasic profile, because 135-18 at 10^{-5} M was unable to modify the cell number with respect to control values.

In addition, because previous reports involved GnRH in ovarian steroidogenesis modulation, progesterone secretion was evaluated in the last 48-h media from the cells subjected to the proliferation assay. Because we had observed that our treatments modified the number of viable cells, we normalized the progesterone levels to cell numbers. Neither GnRH-I, GnRH-II, nor buserelin (Fig. 5) significantly modified progesterone secretion at the concentrations tested (10^{-7} and 10^{-5} M). In contrast, 135-18 at 10^{-7} M substantially increased progesterone secretion; this effect was biphasic, showing no effect at 10^{-5} M.

DISCUSSION

The existence of two GnRH systems in several mammalian species suggests that both GnRH-I and GnRH-II may play important roles as endocrine/autocrine/paracrine regulators of reproductive functions. In the rhesus monkey, both systems (GnRH-I/type I GnRH-R and GnRH-II/type II GnRH-R) are present in several tissues [6]. Moreover, type II GnRH-R has been found in gonadotropes, and GnRH-II has been detected in the hypothalamic median eminence, implicating a possible participation of GnRH-II in the hypothalamus-pituitary axis. The type II GnRH-R has also been cloned and characterized from another primate, the marmoset [5]. Furthermore, GnRH-I and GnRH-II are expressed in the human brain and a variety of peripheral tissues. To our knowledge, however, no evidence suggests a full-length functional human type II GnRH-R [9]. Additionally, in the musk shrew, the first placental mammal shown to have GnRH-I and GnRH-II, Temple et al. [26] demonstrated that GnRH-II, but not GnRH-I, is a neurotransmitter/modulator for reproductive behavior. Complete cDNA from both GnRH-I and GnRH-II has also been sequenced from the insectivore tree shrew [27]. However, in rodents, such as the rat or mouse, no clear evidence regarding the existence of a GnRH system different from the GnRH-I/type I GnRH-R has been reported.

Previously, we demonstrated that GnRH-II stimulated rat pituitaries, inducing LH and FSH secretion similar to GnRH-I [20]. In those experiments, both peptides were ineffective at 10^{-11} M; GnRH-I, but not GnRH-II, elicited a significant LH response at 10^{-9} M. Additionally, the two were equipotent gonadotropin stimulators at 10^{-7} M. These results persuaded us to test if this effect was mediated by the type I GnRH-R or by a different one. For this purpose, we selected relatively high GnRH concentrations that would significantly increase gonadotropin secretion and IP_3 production. In the present study, we show that GnRH-II

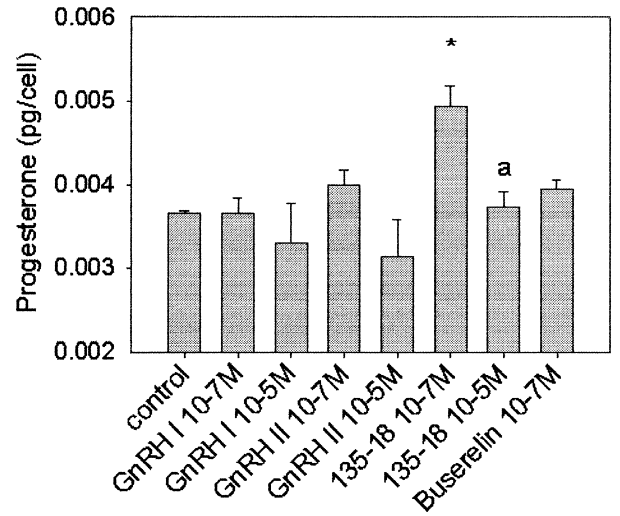

FIG. 5. Progesterone secretion from SPO cells. Progesterone levels were evaluated in the culture media after 48 h of stimulation. Data were normalized by cell number (n = 4). $^*P < 0.05$ vs. control, $^aP < 0.05$ vs. 135-18 at 10^{-7} M.

was able to mobilize IP_3 and to stimulate LH and FSH release in rat anterior pituitary cells in a concentration-response way and in a GnRH-I-like manner. In this model, the GnRH-analog 135-18 (an agonist for type II GnRH-R) was unable to elicit any cellular response tested. On the other hand, that GnRH-II responses were blocked by type I GnRH-R antagonists such as CRX or 135-18 strongly suggests that these effects were mediated by the type I GnRH-R. These results show no evidence of a pituitary type II GnRH-R. This conclusion is supported by recent experiments performed in the rhesus monkey [28] and sheep [29], which showed that the physiological regulator of gonadotropin secretions in these models is the GnRH-I/type I GnRH-R system and that exogenously administered GnRH II acts through the type I receptor. However, in our rat pituitary cells, we did not detect the potency differences between GnRH-I and GnRH-II observed in COS-7 cells transfected with the human type I GnRH-R. Lack of differences in the potency of both peptides for gonadotropin secretion has also been observed in monkey pituitary cultures and in rat pituitary [30, 31]. In addition, structural differences between the rodent type I GnRH-R and the receptor in other mammals have been described [32].

Ovarian GnRH peptides have been implicated in the control of normal and malignant reproductive tissues [10–15]. Recently, the presence of GnRH-II together with GnRH-I in different models of human ovarian cells has been reported [16, 33], and potential roles for GnRH-I and GnRH-II in human ovary have been reviewed [15]. In baboon, a role for the GnRH-II system in the regulation ovarian functions has also been demonstrated [34].

Because a variety of studies have demonstrated the presence of the GnRH-I/type I GnRH-R system in the rat ovary [15, 25, 35, 36], and because GnRH-II ovarian actions have been reported in other species, we decided to investigate the effects of GnRH-II in rat ovarian cells. For this purpose, we evaluated IP_3 production in SPO cells, and surprisingly, we found that GnRH-I, but not GnRH-II, induced an increase in IP_3 with respect to controls. Moreover, 135-18 had no effect by itself but blocked the GnRH-I-induced IP_3 increase. These data involve the type I GnRH-R in the GnRH-I-induced IP_3 ovarian response, and they show differences in GnRH-II action with regard to the adenohypophyseal cells. In vitro studies reported proteolytic inactivation of GnRH in rat ovary [37]; however, in baboon granulosa cells, GnRH-I analogs were more susceptible then GnRH-II or its analogs to degradation by peptidases. Therefore, proteolytic inactivation does not seem to be the cause for the lack of GnRH-II action on mobilization of inositol phosphates.

Because the antiproliferative actions of GnRH-I and GnRH-II are well documented in several normal and pathogenic human ovarian cell models [15, 38], we evaluated cell viability after 72 h of incubation in the presence of diverse stimuli. At the concentrations tested, GnRH-I, GnRH-II, and buserelin decreased cell viability in a similar way. In contrast, 135-18 presented a biphasic response, being the most potent antiproliferative stimulus assayed when applied at 10^{-7} M but losing its effect at 10^{-5} M, suggesting an agonistic downregulation of a putative different binding site. Besides being an agonist for the type II receptor, 135-18 is also an antagonist for the type I GnRH-R; unpublished results from our laboratory indicate that classical type I GnRH-R-antagonists CRX and Antide are also able to induce a decrease in cell viability in SPO cells after 72 h of incubation. Furthermore, in ovarian cancer cell lines, CRX showed an antiproliferative effect comparable to that of GnRH-I agonists [8]. In addition, after type I GnRH-R knockdown in EFO-21 and OVCAR-3 human ovarian cancer cells, the antiproliferative effect of GnRH-I agonists was abrogated, but the actions of CRX or GnRH-II were still present, suggesting that this effect was not mediated by the type I GnRH-R [38]. Additionally, we studied the capacity of GnRH variants to modify progesterone secretion after 48 h of incubation. In these experiments, GnRH-I, buserelin, and GnRH-II did not induce changes in progesterone levels. Nevertheless, 135-18 presented again a biphasic response, because it produced an increase in progesterone secretion at 10^{-7} M and did not modify the secretion at 10^{-5} M. In these luteinized ovarian cells, we had previously reported that buserelin did not modify basal progesterone production [23]. However, studies carried out in baboon granulosa cells revealed a decrease in progesterone secretion induced by buserelin or GnRH-II at concentrations comparable to those used in the present study [34]. Despite disparities in the cell models, the main difference may be explained by the way in which results were expressed. Here, we normalized the progesterone produced to the number of remnant cells, because GnRH-I, GnRH-II, and the analogs used affected the cell viability. These data propose that 138-18-induced release of progesterone and decrease of cell viability share, at some point, the same signaling pathways, which differ from the inositol phosphates pathway.

Taken together, these results suggest that pituitary gonadotropin secretion is mediated by the type I GnRH receptor in the rat. On the other hand, summarizing the results in rat ovarian luteinized cells, 135-18 does not mobilize IP_3, in contrast to GnRH-I, and is a more potent antiproliferative stimulus than GnRH-I, showing a downregulation response at high doses. Additionally, 135-18 is able to stimulate progesterone production when GnRH-I is not. Taking these data into consideration, we suggest that in these cells, at least one GnRH response site is different from the type I GnRH-R. Further studies will elucidate the nature of this putative ovarian GnRH-R, which may be activated by type I GnRH-R antagonists.

ACKNOWLEDGMENT

We are very grateful to Dr. Roger Roeske (Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN) for the generous gift of the 135-18 compound.

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