Endocrine, *vol. 30, no. 1, 139–144, August 2006* 0969–711X/06/30:139–144/$30.00
ENDO (Online) ISSN 1559-0100

Regulation of GnRH I Receptor Gene Expression by the GnRH Agonist Triptorelin, Estradiol, and Progesterone in the Gonadotroph-Derived Cell Line αT3-1

J. M. Weiss,[1] S. Polack,[1] O. Treeck,[2] K. Diedrich,[1] and O. Ortmann[2]

[1]Department of Obstetrics and Gynaecology, Medical University Luebeck, 23538 Luebeck, Germany; and [2]Department of Obstetrics and Gynecology, Caritas St. Josef Hospital, University of Regensburg, Postfach 10 06 45, D-93006 Regensburg, Germany

The secretion of luteinizing hormone (LH) and the GnRH receptor (GnRH-R) concentration are modulated by ovarian steroids and GnRH. To elucidate whether this regulation is due to alterations at the transcriptional level, we examined the GnRH I-R mRNA expression in the gonadotroph-derived cell line αT3-1 treated with different estradiol and progesterone paradigms and the GnRH I agonist triptorelin. αT3-1 cells were treated with different steroid paradigms: 1 n*M* estradiol or 100 n*M* progesterone for 48 h alone or in combination. Cells were exposed to 10 n*M* or 100 p*M* triptorelin for 30 min, 3 h, 9 h, or, in pulsatile way, with a 5-min pulse per hour. The GnRH I-R mRNA was determined by Northern blot analysis. GnRH I-R mRNA from cells treated with continuous triptorelin decreased in a time- and concentration-dependent manner. Pulsatile triptorelin increased GnRH I-R gene expression. Progesterone alone further enhanced this effect, whereas estradiol and its combination with progesterone diminished it. Continuous combined treatment with estradiol and progesterone lead to a significant decrease of GnRH I-R mRNA by 30% and by 35% for estradiol alone. The addition of 10 n*M* triptorelin for 30 min or 3 h could not influence that steroid effect. In conclusion, estradiol and progesterone exclusively decreased GnRH I-R mRNA in αT3-1 cells no matter whether they are treated additionally with the GnRH I agonist triptorelin. The enhanced sensitivity of gonadotrophs and GnRH I-R upregulation by estradiol is not due to increased GnRH I gene expression because GnRH I-R mRNA is downregulated by estradiol and progesterone. Other pathways of the GnRH I-R signal transduction might be involved.

Received June 6, 2006; Revised June 28, 2006; Accepted July 6, 2006.
Author to whom all correspondence and reprint requests should be addressed: Dr. Juergen M. Weiss, PD, Department of Obstetrics and Gynecology, Medical University of Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany. E-mail: jmweiss1@hotmail.com

Key Words: GnRH receptor; GnRH agonist; estradiol; progesterone; αT3-1 cells.

Introduction

Pulsatile gonadotropin-releasing hormone (GnRH) secretion from hypothalamic nuclei stimulates the synthesis and secretion of the gonadotropin follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The gonadotropins consecutively adjust ovarian function. Thus, GnRH plays a major role in the regulation of female reproduction *(1)*. Pulsatile surges of GnRH are required for its function. Continuous administration of GnRH or its agonistic analogs like triptorelin, however, lead to a desensitization of gonadotrophs. After an initial rise of gonadotropins, called flare-up effect, the secretion of LH and FSH and sex steroid levels are suppressed. The mechanisms leading to desensitization are not yet fully elucidated yet *(2)*.

More than a decade ago the cloning of cDNAs encoding for the GnRH-R was successful in mice *(3)* and in humans *(4)*. The GnRH-R is characteristic of the family of G protein–coupled receptors and contains seven membrane spanning domains *(5)*. Two types of GnRH receptors have been demonstrated in humans and most vertebrates *(6–8)*. The function of the two different GnRH and GnRH receptor types are not fully understood yet. They seem to differ in respect to their distribution and even signal transduction *(9)*.

In the pituitary, GnRH-R has been found exclusively on gonadotrophs. Gonadotrophs are influenced by numerous paracrine and hormonal regulators. In vivo studies therefore could not separate between distinct effects of those modulators. Pituitary tissue and primary cultures of mixed pituitary cell populations have therefore been used to test, for example, the in vitro effects of steroids. Because primary cultures of rat pituitaries are composed of different cell types and contain only around 10% gonadotrophs *(10)*, the gonadotroph-derived cell line αT3-1, a pure gonodotropic population, provides compelling advantages for the study of direct effects of steroids on gonadotrophs and GnRH receptor gene expression. This monoclonal cell line was

derived from pituitary tumors in mice *(11)*. Two transcripts of GnRH I-R, approx 4.3 and 2 kb in size, have been found in the murine αT3-1 cell line. αT3-1 cells express α-subunit, but no LH- and FSH β-subunits. Therefore, this cell line has no known function. GnRH binding and expression of GnRH-R mRNA were shown *(12)*.

Ovarian steroids are known to modulate GnRH-induced gonadotropin secretion *(13,14)*. To understand the biology of reproduction it is important to elucidate the actions of ovarian steroids on GnRH-R and its gene expression. Our steroid paradigms mimic the two distinct phases of the menstrual cycle. Estradiol alone stands for the late follicular phase and combined long-term treatment of estradiol and progesterone for the luteal phase.

It might be possible that the actions of these steroids are also affected by GnRH and its analogs. Pulsatile GnRH enhances GnRH-R amount and gene expression *(15)*. Controversy exists on how continuous GnRH affects GnRH-R gene expression. Some found no effect *(15)*, others found an increase of GnRH-R mRNA by continuous GnRH application *(16)*. Increasing estradiol from developing preovulatory follicles might induce a higher amount of GnRH-R mRNA preceding the maximal number of GnRH-R before LH surge *(17)*. Contradictory to that, neither estradiol nor progesterone did affect GnRH-R mRNA levels in primary female rat pituitary cultures *(18)*.

To test the hypothesis that the altered sensitivity of gonadotrophs by estradiol and progesterone is reflected by changes of GnRH I-R mRNA levels and that triptorelin modulates them, we treated gonadotrophs with different paradigms of estradiol, progesterone and the GnRH I agonist triptorelin.

Results

Effects of GnRH Agonist on GnRH I-R mRNA Level

To discriminate the effects of the GnRH I agonist alone, cells were treated with 10 n*M* or 100 p*M* triptorelin, a GnRH I agonist, for 30 min or 9 h. Continuous treatment with 10 n*M* triptorelin for 30 min decreased GnRH I receptor mRNA significantly compared with controls. Triptorelin in low concentrations (100 p*M*) did not affect GnRH I-R mRNA levels; 10 n*M* triptorelin was therefore used for all other experiments.

Extension of treatment time with triptorelin to 9 h led to lower amounts of GnRH I-R mRNA levels compared to 30 min treatment, but again only 10 n*M* triptorelin diminshed the amount of GnRH I-R mRNA significantly (Fig. 1).

Effects of Estradiol and its Combination with Progesterone on GnRH I-R mRNA Levels

Cells were treated with different steroid paradigms as follows: 1 n*M* estradiol alone or in combination with 100 n*M* progesterone for 48 h. Both steroid paradigms significantly decreased GnRH I receptor mRNA levels. The lowest amount of GnRH I-R mRNA was observed in cells treated exclusively with estradiol (65%). The addition of progesterone attenuates this effect slightly, but still GnRH I-R mRNA levels remained significantly decreased compared to controls (70%) (Fig. 2).

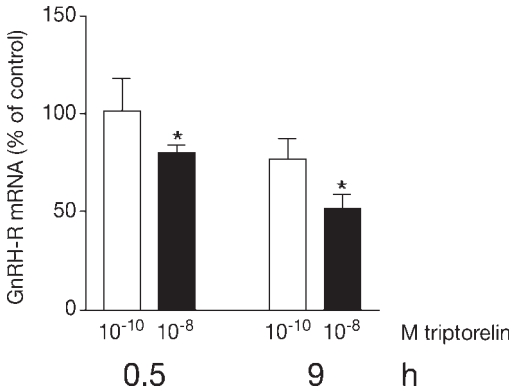

Fig. 1. Effects of continuous GnRH I agonist on GnRH I-R mRNA level. Cells were treated with 100 p*M* or 10 n*M* triptorelin for 0.5 h and 9 h. Cells were harvested after the indicated period and the isolated total RNA was subjected to a Northern blot analysis using a GnRH I-R probe. Autoradiogram bands were quantified by densitometry and were expressed in percentage of control as means ± SD. Vehicle controls did not affect GnRH I-R mRNA levels ($n = 5$, $^*p < 0.05$ vs control).

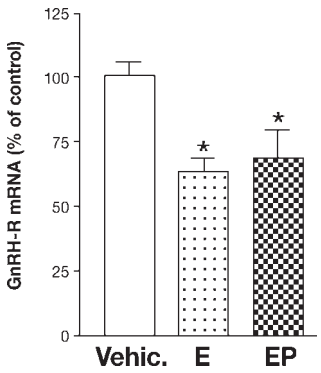

Fig. 2. Effects of estradiol alone and in combination with progesterone on GnRH I-R mRNA level. Cells were treated with 1 n*M* estradiol for 48 h (E) and with a combination of 100 n*M* progesterone for the same period (EP). Cells were harvested after the indicated period, the isolated total RNA was subjected to a Northern blot analysis using a GnRH I-R probe. Autoradiogram bands were quantified by densitometry and were expressed in percentage of control as means ± SD ($n = 3$, $^*p < 0.05$ vs control).

Effects of a Continuous GnRH I Agonist Treatment and Steroid Paradigms on GnRH I-R mRNA Levels

Cells were treated with different steroid paradigms and stimulated with continuous triptorelin for 30 min (Fig. 3A) or 3 h (Fig. 3B). All steroid treatments significantly decreased GnRH I receptor mRNA to less than half compared with control. No differences were observed between different steroid treatments or whether cells were stimulated with triptorelin for 30 min or 3 h.

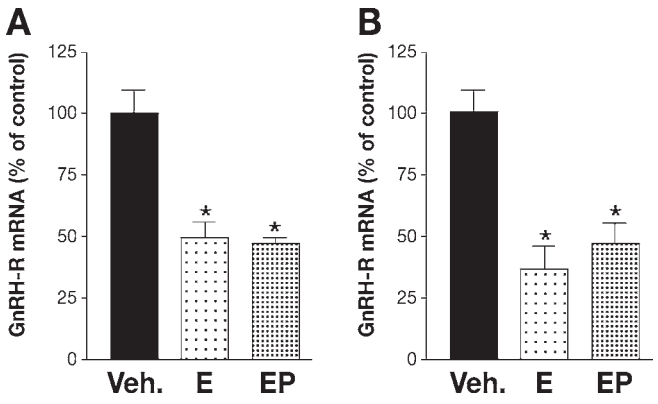

Fig. 3. Effects of GnRH I agonist on GnRH I-R mRNA levels in cells pretreated with estrogen and progesterone. Cells were cultured in medium containing estradiol for 48 h (E) or a combination of both substances for the same period (EP). 10^{-8} *M* triptorelin was added (**A**) 30 min or (**B**) 3 h before harvesting of the cells. The isolated total RNA was subjected to a Northern blot analysis using a GnRH-R probe. Autoradiogram bands were quantified by densitometry and were expressed in percentage of control as means ± SD ($n = 3$, $*p < 0.05$ vs control).

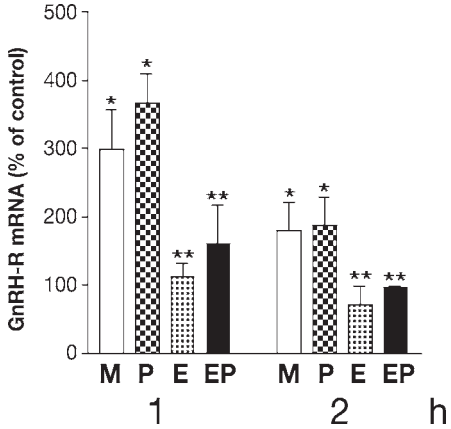

Fig. 4. Effects of a pulsatile GnRH I agonist treatment on GnRH I-R mRNA level. Cells cultured in medium (M) containing estradiol (E), progesterone (P), or a combination of both substances (EP) were treated with a 5 min pulse of 10^{-8} *M* triptorelin each hour. Cells were harvested after the indicated period, the isolated total RNA was subjected to a Northern blot analysis using a GnRH-R probe. Autoradiogram bands were quantified by densitometry and were expressed in percentage of control as means ± SD. Vehicle controls did not affect GnRH I-R mRNA levels [$n = 4$, $*p < 0.05$ vs control (without pulse) $**p < 0.05$ vs M].

Effects of a Pulsatile GnRH I Agonist Treatment and Steroid Paradigms on GnRH I-R mRNA Levels

Cells were treated with estradiol or progesterone alone or in combination. Triptorelin was administered by a 5 min pulse each hour. After 1 h incubation GnRH I receptor gene expression was upregulated by one pulse of 10 n*M* triptorelin close to 300% compared to control. In cells pretreated with progesterone alone this effect was even more pronounced, increasing GnRH I-R mRNA up to 380%. Treatment with estradiol alone or combined treatment with progesterone and estradiol significantly decreased GnRH I-R mRNA levels compared to triptorelin alone or triptorelin and progesterone alone (100% and 140%, respectively). Two pulses of 5 min triptorelin in 2 h incubation with the same steroid treatments lead to lower levels of GnRH I-R mRNA. But still triptorelin alone and progesterone enhanced GnRH I-R mRNA up to 180%, whereas treatment with estradiol or estradiol and progesterone again decreased GnRH I receptor gene expression significantly to 70% or 90%, respectively. Progesterone attenuated the effect of estradiol alone (Fig. 4).

Discussion

Here we found that long-term treatment with estradiol and the combination of estradiol and progesterone decreased GnRH I receptor transcripts in αT3-1 gonadotrope cells. Pulsatile triptorelin increased, whereas continuous triptorelin in nanomolar doses decreased, GnRH I-R gene expression. The addition of pulsatile or continuous triptorelin to the steroid treatments did not abrogate the negative effects of the steroids.

It is widely accepted that GnRH regulates its own receptor and its gene expression in rats in vivo *(19,20)* and in vitro in primary cultures of pituitary cells *(21,22)*. Pulsatile GnRH enhances GnRH-R amount and gene expression *(15, 22)*. Debate exists on how continuous GnRH influences the gene expression of GnRH-R. Here we demonstrated that GnRH-I R mRNA is time and dose dependently decreased by continuous GnRH I agonist treatment, whereas 5 min pulses of triptorelin increased GnRH I receptor gene expression. Cheon and co-workers *(16)* found an increase of GnRH-R mRNA by continuous GnRH application, others found no effect *(15,22)*. On the other hand, continuous administration of triptorelin suppressed the GnRH-R gene expression in castrated and intact male rats *(23)* and in sheep *(24)*. These differences might be due to the dosage of GnRH. Low concentrations of triptorelin upregulated GnRH receptor gene expression of pre- and peripubertal rats, whereas GnRH receptor mRNA levels were not upregulated by high-dose triptorelin (100 μg/d) *(25)*. The exposure of pituitary cells in vitro to 0.1 n*M* triptorelin for 5 h increased the GnRH-R mRNA level by 77–88% *(26)*. In contrast, 25 μg/d triptorelin reduced GnRH-R mRNA expression in adult female pituitaries to 41% and 56–65% on d 10 and 30, respectively *(27)*.

Conflicting data exist on the homologous regulation of GnRH-R mRNA by GnRH in αT3-1 cells as well. Twenty minute exposure of αT3-1 cells to GnRH in nanomolar doses enhanced the number of GnRH-R *(28)*. Mason et al. noted a homologous time- and dose-dependent downregulation of messenger ribonucleic acid transcripts in αT3-1 cells after the treatment with GnRH and the agonist analog

(29), whereas Alarid and Mellon and Tsutsumi et al. found no change in GnRH mRNA levels in αT3-1 cells treated continuously with a GnRH agonist *(28,30)*.

A larger decline in receptor number than in mRNA levels was noted. This is consistent with the involvement of additional mechanisms, including endocytosis and degradation, in downregulation of the GnRH receptor *(29)*. These findings, however, agree with the view that GnRH receptor number and its gene expression are differentially regulated and that the posttranslational efficacy is affected *(31)*.

Our findings that long-term estradiol and progesterone decreased GnRH I-R mRNA even when pulsatile triptorelin was added are in contrast with the view that estradiol positively affects GnRH-R gene expression. Estradiol is known as a positive regulator of GnRH receptor gene expression in the preovulatory period. An increased serum concentration of estradiol from developing preovulatory follicles most likely causes an elevation of pituitary GnRH receptor mRNA, which precedes maximal numbers of GnRH receptors before the LH surge *(17)*. The effect of estradiol on GnRH-R number could be dependent on treatment time. Short-term incubation of primary rat pituitary cells with estradiol leads to a decrease, and long-term treatment to an increase, in GnRH-R *(32)*. In accordance with this, 12 h treatment with estradiol enhanced GnRH-R numbers in ovine pituitary cells *(33)*. Quinones-Jenab et al. observed an increase of GnRH-R mRNA in pituitary tissue after long-term estradiol treatment *(34)*. In L beta T2 cells, another gonadotrope cell line, estradiol enhanced GnRH-R mRNA levels *(35)*. Another study noted an estrogen-induced increase and progesterone-induced decrease of GnRH-R and GnRH-R mRNA *(36)*. Wu et al. also demonstrated an increase in GnRH-R mRNA by estradiol and a decrease by progesterone in sheep *(24)*. GnRH-R mRNA levels in anterior pituitaries are regulated during the rat estrous cycle with the highest amount at diestrus/proestrus and lowest at estrus, indicating that they positively correlate with estradiol levels *(37,38)*.

Neither estradiol nor progesterone affected GnRH I-R mRNA levels in primary female rat pituitary cultures. Estradiol did not modulate homologous upregulation of transcripts of GnRH I-R. Progesterone at 100 n*M* attenuated GnRH-induced increase in GnRH-R mRNA and estradiol potentiates this effect *(18)*. Estradiol acts on αT3-1 cells exclusively by decreasing GnRH-R levels *(39)*. Concordantly, Harris et al. found a decrease of GnRH-R mRNA advancing the GnRH surge at the time of the estradiol-induced LH surge in ewes *(40)*. In female rats, ovariectomy increased and estrogen-replacement in vivo decreased GnRH-R mRNA levels *(22)*.

Our results are consistent with the latter findings. Here we found that estradiol and its combination with progesterone decreased GnRH I-R mRNA levels of pituitary gonadotrophs. The differences between mixed populations of primary pituitary cells and the αT3-1 cell line might be due to different homologous regulation of the GnRH-R, different Ca-signaling and indirect effects of steroids on other pituitary subpopulations *(41)*.

The combination of estradiol and pulsatile GnRH given to female rats in vivo increased GnRH-R mRNA even more than GnRH or estradiol alone *(17)*. Other than expected here, we found that pulsatile triptorelin could not extenuate the negative estradiol effect.

The in vivo studies in sheep and ewes dealing with the effects of estradiol, progesterone, and GnRH delivered fairly consistent results. The triggering event could be the decreasing progesterone level after luteolysis. It has been suggested that progesterone acts as a negative regulator of the GnRH-R gene expression *(42)*. Progesterone attenuated the stimulatory effect of estradiol on GnRH-R gene expression in vivo in ewes *(43)*. Ovarectomized ewes were treated with GnRH continuously for 6 d and at the last day additionally with estradiol. GnRH treatment decreased, whereas estradiol increased, GnRH-R and its gene expression *(43)*. Kirkpatrick et al. suggested that both estradiol and pulsatile GnRH are needed for GnRH-R expression and numbers *(44)*. The same group noted that increased amounts of GnRH-R mRNA require removal of progesterone and presence of estradiol in vivo in ovarectomized ewes *(44)*. In orchidectomized sheep estradiol induced an increase in the concentration of mRNA encoding for the GnRH-R even in absence of GnRH achieved by immunization with GnRH antisera *(45)*.

In contrast, castration induced a 3.8-fold elevation in the amounts of GnRH-R mRNA after 3 wk, compared with corresponding values in intact animals. Administration of the GnRH agonist readily prevented, for as long as 3 wk, the stimulatory effects of castration on the GnRH-R mRNA *(23)*. This suggests that estradiol and continuous GnRH agonist treatment diminish GnRH-R gene expression.

The latter is in accordance with our results. Here we showed that the positive effect of pulsatile GnRH I agonist on GnRH I-R mRNA was alleviated by estradiol and its combination with progesterone, whereas progesterone alone even aggravated the positive effect of GnRH pulses. Adding continuous triptorelin to the steroid treatments could not burst the negative effects of long-term estradiol and estradiol/progesterone on GnRH I-R gene expression.

Our present results reveal that the modification of GnRH I-R gene expression by the GnRH I agonists depends not only on the regimen of administration and concentration of the agonists, but also on the actual level and hormonal environment of the receptors. The increased sensitivity of gonadotrophs and the upregulation of GnRH I receptors by estradiol might be due to alterations of other pathways of the GnRH receptor signal transduction rather than an increased GnRH I-R gene expression, because GnRH I-R mRNA is downregulated by estradiol and progesterone.

Materials and Methods

αT3-1 Cell Culture

Because mixed populations of pituitary cells contain only about 10% of gonadotrophs, we used αT3-1 cells, an immortalized gonadotroph-derived cell line kindly provided by Dr. P. L. Mellon (San Diego), to save rats and to attribute effects exclusively to gonadotrophs. Cells were cultured on 6-well dishes (500,000 cells/well) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Life Technologies, Karlsruhe, Germany) and 100 mg/L gentamycin (Biochrom KG, Berlin, Germany). To allow cell attachment, cells were kept for 36 h in humidified incubators at 37°C in an atmosphere of 5% CO_2 and 95% air. At 70–80% confluency, cells were harvested after detachment with accutase (GiBCO, Wiesbaden, Germany) and treated promptly with steroids in serum-free medium for 48 h.

Steroid and Triptorelin Treatments

To determine the influence of steroids on the actions of GnRH analogs, cells were treated with different steroid paradigms mimicking different phases of the estrous cycle. Estradiol alone stands for the late follicular phase, estradiol and combined long-term treatment of estradiol and progesterone for the luteal phase. Steroid treatments were commenced 36–48 h after cell preparation. Cells were incubated for 48 h with 1 n*M* estradiol (E) alone or with combinations of 100 n*M* progesterone (P) (Sigma, St. Louis, MO) for 48 h. These concentrations of steroids are in the physiological range. P and E were dissolved in ethanol and the final concentration of ethanol in the culture media was 0.2%. Triptorelin (Ferring, Kiel, Germany) was dissolved in 0.1% acetic acid solution. Cells were treated with 100 p*M* or 10 n*M* triptorelin for 0.5 h, 3 h, 9 h or with 5 min pulses every hour for 2 h. Controls received media containing 0.2% ethanol alone or 0.1% acetic acid solution, respectively (vehicle).

Preparation of Total RNA and Northern Blot Analysis

Total cellular RNA was isolated using the standard trizol method (Gibco BRL). Cells from approx 80% confluent cultures were harvested by trypsin–EDTA treatment and lysed in trizol. After preparation of total RNA according to the manufacturer's protocol, 10 μg of the RNA were separated on a 0.9 agarose gel containing 2.2 *M* formaldehyde. The RNA was then vacuum blotted onto Hybond$^+$ nylon membranes (Amersham, Germany) and cross-linked by UV-irradiation. The blots were hybridized with 1.5×10^6 cpm/mL of a ^{32}P-dCTP-labeled GnRH-R cDNA probe, which was prepared after RT-PCR and sequencing using a random primer labeling kit (Dianova, Germany). As internal standard, a probe for GAPDH was used. Autoradiogram bands were quantified after measurement of the optical density using EasyWin software (Herolab, Wiesloch, Germany) and were expressed in percentage of control as means ± SEM.

Statistical Analysis

Data from three to four experiments run in triplicates were pooled. The results were expressed as means ± SEM. One factor analysis of variance (ANOVA) followed by Dunnett's posttest was used for statistical comparison among groups using Prism 2.0 Software (Graph Pad, San Diego, USA). Statistical significance was accepted at $p < 0.05$.

References

1. Knobil, E. (1980). *Recent Prog. Horm. Res.* **36,** 53–88.
2. Ortmann, O., Weiss, J. M., and Diedrich, K. (2002). *Reprod. Biomed. Online* **5(Suppl. 1),** 1–7.
3. Reinhart, J., Mertz, L. M., and Catt, K. J. (1992). *J. Biol. Chem.* **267,** 21281–21284.
4. Kakar, S. S., Musgrove, L. C., Devor, D. C., Sellers, J. C., and Neill, J. D. (1992). *Biochem. Biophys. Res. Commun.* **189,** 289–295.
5. Probst, W. C., Snyder, L. A., Schuster, D. I., Brosius, J., and Sealfon, S. C. (1992). *DNA Cell Biol.* **11,** 1–20.
6. Leung, P. C. and Peng, C. (1996). *Biol. Signals* **5,** 63–69.
7. Millar, R., Lowe, S., Conklin, D., et al. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 9636–9641.
8. Neill, J. D., Duck, L. W., Sellers, J. C., and Musgrove, L. C. (2001). *Biochem. Biophys. Res. Commun.* **282,** 1012–1018.
9. Millar, R. P., Lu, Z. L., Pawson, A. J., Flanagan, C. A., Morgan, K., and Maudsley, S. R. (2004). *Endocr. Rev.* **25,** 235–275.
10. Ibrahim, S. N., Moussa, S. M., and Childs, G. V. (1986). *Endocrinology* **119,** 629–637.
11. Windle, J. J., Weiner, R. I., and Mellon, P. L. (1990). *Mol. Endocrinol.* **4,** 597–603.
12. Horn, F., Bilezikjian, L. M., Perrin, M. H., et al. (1991). *Mol. Endocrinol.* **5,** 347–355.
13. Brann, D. W. and Mahesh, V. B. (1991). *Front. Neuroendocrinol.* **12,** 165–170.
14. Ortmann, O., Merelli, F., Stojilkovic, S. S., Schulz, K. D., Emons, G., and Catt, K. J. (1994). *J. Steroid Biochem. Mol. Biol.* **48,** 47–54.
15. Vizcarra, J. A., Wettemann, R. P., Braden, T. D., Turzillo, A. M., and Nett, T. M. (1997). *Endocrinology* **138,** 594–601.
16. Cheon, M., Park, D., Kim, K., Park, S. D., and Ryu, K. (1999). *Endocrine* **11,** 49–55.
17. Yasin, M., Dalkin, A. C., Haisenleder, D. J., Kerrigan, J. R., and Marshall, J. C. (1995). *Endocrinology* **136,** 1559–1564.
18. Cheon, M., Park, D., Park, Y., Kam, K., Park, S. D., and Ryu, K. (2001). *Endocrine* **15,** 131–136.
19. Clayton, R. N. and Catt, K. J. (1981). *Endocr. Rev.* **2,** 186–209.
20. Frager, M. S., Pieper, D. R., Tonetta, S. A., Duncan, J. A., and Marshall, J. C. (1981). *J. Clin. Invest.* **67,** 615–623.
21. Loumaye, E. and Catt, K. J. (1982). *Science* **215,** 983–985.
22. Kaiser, U. B., Jakubowiak, A., Steinberger, A., and Chin, W. W. (1993). *Endocrinology* **133,** 931–934.
23. Lerrant, Y., Kottler, M. L., Bergametti, F., Moumni, M., Blumberg-Tick, J., and Counis, R. (1995). *Endocrinology* **136,** 2803–2808.
24. Wu, J. C., Sealfon, S. C., and Miller, W. L. (1994). *Endocrinology* **134,** 1846–1850.
25. Roth, C., Schricker, M., Lakomek, M., et al. (2001). *J. Endocrinol.* **169,** 361–371.
26. Kovacs, M. and Schally, A. V. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 12197–12202.
27. Horvath, J. E., Bajo, A. M., Schally, A. V., Kovacs, M., Herbert, F., and Groot, K. (2002). *Proc. Natl. Acad. Sci. USA* **99,** 15048–15053.

28. Tsutsumi, M., Laws, S. C., and Sealfon, S. C. (1993). *Mol. Endocrinol.* **7,** 1625–1633.
29. Mason, D. R., Arora, K. K., Mertz, L. M., and Catt, K. J. (1994). *Endocrinology* **135,** 1165–1170.
30. Alarid, E. T. and Mellon, P. L. (1995). *Endocrinology* **136,** 1361–1366.
31. Tsutsumi, M., Laws, S. C., Rodic, V., and Sealfon, S. C. (1995). *Endocrinology* **136,** 1128–1136.
32. Emons, G., Hoffmann, H. G., Brack, C., et al. (1988). *J. Steroid Biochem.* **31,** 751–756.
33. Gregg, D. W., Allen, M. C., and Nett, T. M. (1990). *Biol. Reprod.* **43,** 1032–1036.
34. Quinones-Jenab, V., Jenab, S., Ogawa, S., Funabashi, T., Weesner, G. D., and Pfaff, D. W. (1996). *Brain Res. Mol. Brain Res.* **38,** 243–250.
35. Turgeon, J. L., Kimura, Y., Waring, D. W., and Mellon, P. L. (1996). *Mol. Endocrinol.* **10,** 439–450.
36. Sakurai, H., Adams, B. M., and Adams, T. E. (1997). *J. Endocrinol.* **152,** 91–98.
37. Bauer-Dantoin, A. C., Hollenberg, A. N., and Jameson, J. L. (1993). *Endocrinology* **133,** 1911–1914.
38. Kakar, S. S., Grantham, K., Musgrove, L. C., Devor, D., Sellers, J. C., and Neill, J. D. (1994). *Mol. Cell. Endocrinol.* **101,** 151–157.
39. McArdle, C. A., Schomerus, E., Groner, I., and Poch, A. (1992). *Mol. Cell. Endocrinol.* **87,** 95–103.
40. Harris, T. G., Robinson, J. E., Evans, N. P., Skinner, D. C., and Herbison, A. E. (1998). *Endocrinology* **139,** 57–64.
41. Kaiser, U. B., Conn, P. M., and Chin, W. W. (1997). *Endocr. Rev.* **18,** 46–70.
42. Turzillo, A. M., and Nett, T. M. (1999). *J. Reprod. Fertil. Suppl.* **54,** 75–86.
43. Turzillo, A. M., Clapper, J. A., Moss, G. E., and Nett, T. M. (1998). *J. Reprod. Fertil.* **113,** 251–256.
44. Kirkpatrick, B. L., Esquivel, E., Gentry, P. C., Moss, G. E., Wise, M. E., and Hamernik, D. L. (1998). *Endocrine* **8,** 93–99.
45. Adams, B. M., Sakurai, H., and Adams, T. E. (1997). *J. Reprod. Fertil.* **111,** 207–212.