0013-7227/99/$03.00/0
Endocrinology

Vol. 140, No. 8
Printed in U.S.A.

Absence of Gonadotropin Surges and Gonadotropin-Releasing Hormone Self-Priming in Ovariectomized (OVX), Estrogen (E_2)-Treated, Progesterone Receptor Knockout (PRKO) Mice*

PATRICK E. CHAPPELL, JOHANNA S. SCHNEIDER, PAULINE KIM, MING XU, JOHN P. LYDON, BERT W. O'MALLEY, AND JON E. LEVINE

Department of Neurobiology and Physiology (P.E.C., J.S.S., P.K., M.X., J.E.L.), Northwestern University, Evanston, Illinois 60208; and Department of Cell Biology (J.P.L., B.W.O.), Baylor College of Medicine, Houston, Texas 77030

ABSTRACT

It is well known that estrogen (E_2) stimulates expression of progesterone receptors (PRs), thereby inducing responsiveness of several tissues to the actions of progesterone (P). Recent studies have also suggested, however, that biological actions previously ascribed to E_2 alone may also be mediated by activation of E_2-induced PRs, even independently of signal changes in P concentrations. In the present experiments, the progesterone receptor knockout (PRKO) mice were used to assess the role of PR activation in the positive feedback actions of E_2 on gonadotropin release. Ovariectomized (OVX) PRKO mice were tested for their capacity to mount primary gonadotropin surges in response to exogenous E_2, and to exhibit a GnRH self-priming effect in response to sequential injections of the decapeptide. Wild-type (WT) and PRKO mice were OVX, treated with both 17β-estradiol and estradiol benzoate (EB), and then killed at 1900 h on day 7 postOVX. Plasma LH RIA revealed that WT mice exhibited surges in response to the E_2 treatment; the PRKO mice, however, showed no elevation in plasma LH above untreated controls. Instead, plasma LH levels in E_2-treated, OVX PRKO mice decreased significantly in comparison to untreated OVX PRKO mice, suggesting that E_2 can exert a negative feedback influence on LH release in PRKO mice, despite the absence of positive feedback effects. A slight but significant rise in plasma FSH was observed in E_2-treated OVX WT mice in comparison to untreated controls: an effect not seen in E_2-treated OVX PRKO mice, reinforcing the observation that estrogen's positive feedback effects are compromised in PRKO mice. In a second experiment, E_2-treated OVX WT and PRKO mice were given either one or two pulses of GnRH 60 min apart, and killed 10 min later. The WT mice were found to exhibit a robust GnRH self-priming effect, as WT mice receiving two GnRH pulses displayed LH responses approximately 2-fold greater than those receiving only one pulse. By contrast, PRKO mice receiving two GnRH pulses exhibited no additional increase in plasma LH levels. We conclude that PR activation is obligatory for expression of the GnRH self-priming effect as well as for generation of E_2-induced LH and FSH surges. The extent to which failure of LH surge secretion in PRKO mice is due to the absence of GnRH self-priming, lack of hypothalamic GnRH surges, and/or defects in other processes remains to be determined. These observations clearly demonstrate, however, that the presence of PR is an absolute requirement for the transmission of E_2-induced signals leading to gonadotropin surges. (*Endocrinology* **140:** 3653–3658, 1999)

PREOVULATORY gonadotropin surges are dependent upon the ability of estrogen (E_2) to both stimulate hypothalamic GnRH surges (1) and greatly augment pituitary responsiveness to the decapeptide (2). Exogenous progesterone (P) has been shown to enhance both processes, as demonstrated by the finding that P administered to proestrous rats can amplify as well as temporally advance the LH surge (3) and that acute P administration can increase pituitary LH secretion in response to a GnRH challenge both *in vitro* (4) and *in vivo* (5). Additionally, pharmacological blockade of PRs with the antiprogestins RU486 (6, 7) or ZK98299 (8) can block the LH and primary FSH surges in intact rats, likewise suggesting the importance of PR in the initiation of gonadotropin surges.

Generation of LH surges may depend, in part, upon the process of GnRH self-priming in gonadotropes, and a role for PR in this process has also been proposed (9). The GnRH self-priming mechanism occurs as a net increase in LH response magnitude to a series of GnRH stimuli and is expressed only in gonadotropes that have been exposed to high preovulatory concentrations of E_2 (5, 10). Moreover, it can be blocked by pharmacological antagonists of PR, thus suggesting that PR activation is obligatory for this response (9). Recent evidence has more specifically implicated ligand-independent activation of PRs via the cAMP/PKA pathway in the manifestation of GnRH self-priming *in vitro* (11).

We used progesterone receptor knockout (PRKO) mice to directly test whether PR activation is an absolute requirement for GnRH self-priming and in the generation of LH and primary FSH surges in response to exogenous E_2. The PRKO mice, generated using targeted gene disruption techniques, exhibit many endocrine abnormalities, including female infertility associated with anovulation (12) and a lack of endogenous preovulatory gonadotropin surges evoked by male mouse odor (13). We reasoned that if PR activation is critical for GnRH self-priming in gonadotropes, this mech-

Received February 17, 1999.
Address all correspondence and requests for reprints to: Jon E. Levine, Ph.D., Department of Neurobiology & Physiology, Northwestern University, Evanston, Illinois 60208. E-mail: jlevine@nwu.edu.
* This work was supported in part by NIH Grants R01-HD-20677, P01-HD-21921, and P30-HD-28048.

anism should therefore be absent in PRKO mice *vs.* wild-type (WT) counterparts, and this defect may be at least partially responsible for any decrement in LH release in response to exogenous estrogen. Moreover, any self-priming deficit observed in PRKO mice should be distinguishable from any other general alteration of LH responses to GnRH, such that the initial pituitary responsiveness to the decapeptide is not compromised. Our immediate aims were therefore to determine if E_2 treatment is capable of inducing gonadotropin surges in ovariectomized (OVX) PRKO mice and to determine if any disrupted or absent LH surge is accompanied by a decrement in the GnRH self-priming response in these animals.

Materials and Methods

Steroid treatment and hormone measurements

All animal and surgical experimental procedures were conducted in accordance with the policies of Northwestern University's Animal Care and Use Committee. WT (C57/BL/6; Charles River Laboratories, Inc., Wilmington, MA) and PRKO (C57/BL/6X129SvEv) mice were housed four or five to a cage under a 14:10 (0500 h–1900 h) light cycle and fed lab chow *ad libitum*. Estrogen treatment was adapted from a protocol described previously (14). Briefly, mice were ovariectomized (OVX) at 0900 h under methoxyflurane anesthesia (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ), and 1 cm SILASTIC (Dow Corning Corp., Midland, MI) brand capsules (0.04" ID, 0.085" OD; American Scientific Products, McGaw Park, IL) containing 2.5 μg of 17β-estradiol (17β-E_2, Sigma Chemical Co., St. Louis, MO) mixed into Silicone Type A Medical Adhesive (Dow Corning Corp.) were placed sc under one flank. Controls were implanted with empty capsules. Following 6 days, mice were administered an sc injection of estradiol benzoate (EB, 1 μg in 0.1 ml sesame oil) or vehicle at 0900 h. On the evening of the seventh day postOVX at 1900, animals were deeply anesthetized with Metofane, and 2 cm horizontal incisions were made with sharp scissors just below the xiphoid process. The rib cage was bisected, exposing the heart. A 21 g needle was inserted into the right ventricle, and 0.5–1.0 ml of blood was withdrawn. Blood was centrifuged and plasma was frozen at −20 C for later LH and FSH RIA.

GnRH dose response and LH temporal secretory profile

WT (n = 48) and PRKO (n = 25) mice were OVX and treated with 17β-E_2 as above. On the sixth day postOVX, mice received 1 μg EB or oil vehicle injected sc at 0900 h. On the seventh day postOVX beginning at 0700, WT mice were injected sc with varying concentrations (50, 100, 200, or 400 ng/kg) of GnRH (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml saline or vehicle. PRKO mice received only the 200 ng/kg dose. All animals were then killed via cardiac puncture, as described above, at 10, 20, 30, and 40 min following GnRH or saline injection. Plasma was stored at −20 C for later LH RIA.

GnRH self-priming

WT (n = 40) and PRKO (n = 24) mice were treated with 17β-E_2 as described above. Control mice of both genotypes were implanted with empty capsules. On the sixth day postOVX, mice were given sc injections of EB (1 μg) at 0900 h; controls were given sesame oil. On day 7 postOVX between 0700 and 0900 h, all mice were given one pulse of GnRH (200 ng/kg) or saline vehicle; one cohort of mice from both genotypes was killed, as described above, 10 min following injections. Remaining WT and PRKO mice were given a second pulse of GnRH or saline vehicle 60 min following the first injection, and killed 10 min later. Plasma was stored for later LH RIA.

RIA and statistical analysis

LH standard, RP-3, and FSH standard, RP-2, were generously provided by NIDDK. The sensitivity of LH and FSH RIA was 40 pg/tube. Intraassay coefficients of variance (CV) for LH and FSH were 16.4% and 15.6%, respectively. LH and FSH data are presented as means ± SEM. For all experiments, groups were compared using two-way ANOVA and Tukey's and Newman-Keuls' multiple comparison posthoc tests, with $P < 0.05$ being considered significant.

Results

Estrogen-treated gonadotropin levels

OVX WT mice treated with E_2 exhibited significantly ($P < 0.05$) increased levels of plasma LH in comparison to untreated OVX WT controls (n = 8 in each group; E_2-treated: 6.11 ± 1.68 ng LH/ml, untreated: 1.78 ± 0.43 ng LH/ml). By contrast, plasma LH levels of E_2-treated OVX PRKO mice did not increase above untreated OVX PRKO plasma LH levels, but instead exhibited a significant ($P < 0.05$) decrement in plasma LH below untreated OVX controls (n = 8 in each group; E_2-treated: 0.70 ± 0.27 ng LH/ml, untreated: 1.97 ± 0.21 ng LH/ml) (Fig. 1).

E_2-treated OVX WT mice exhibited small but significant ($P < 0.05$) increases in plasma FSH levels in comparison to untreated OVX WT mice (n = 10 in each group; E_2-treated: 41.6 ± 2.28 ng FSH/ml, untreated: 35.12 ± 1.50 ng FSH/ml), whereas no elevation in plasma FSH was observed in E_2-treated OVX PRKO mice *vs.* untreated OVX PRKO mice (n = 6 in each group; E_2-treated: 25.87 ± 5.67 ng FSH/ml, untreated: 32.68 ± 1.07 ng FSH/ml) (Fig. 2). No elevation in either plasma LH or FSH, in comparison to OVX controls, was observed in mice that were implanted with 17β-E_2 capsules and injected with oil, as well as in mice implanted with empty capsules and receiving an injection of EB (data not shown).

GnRH self-priming

To assess GnRH self-priming in WT and PRKO mice, dose-response studies were first conducted in WT mice to identify an appropriate GnRH treatment regimen. Groups of OVX, E_2-treated WT mice were administered 50, 100, 200, or 400 ng/kg GnRH, and killed at 10, 20, 30, or 40 min after sc injections. The dose of 200 ng/kg GnRH was selected for subsequent experiments, as this GnRH stimulus produced a significant but submaximal LH response that peaked at 10

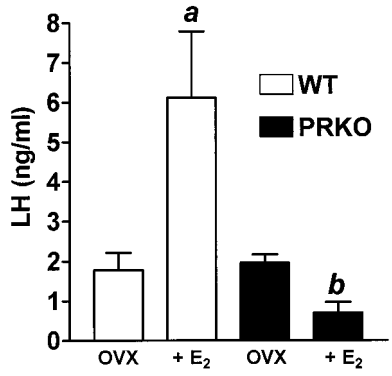

FIG. 1. Effect of E_2-treatment on plasma LH levels of OVX WT and PRKO mice. E_2-treated (+E_2) OVX WT mice exhibited an LH surge (a, $P < 0.05$, n = 8) at 1900 h in comparison to untreated OVX WT mice (OVX; n = 8). Plasma LH levels in untreated OVX PRKO mice (OVX; n = 8) were similar to their WT counterparts, whereas E_2-treatment (+E_2; n = 8) led to a significant (b, $P < 0.05$ below PRKO OVX) decrement in LH release at 1900 h.

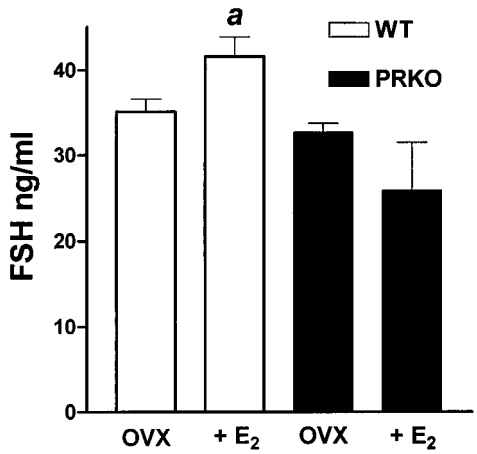

FIG. 2. Effect of E_2 treatment on plasma FSH levels of OVX WT and PRKa, mice. FSH levels were elevated in E_2-treated OVX WT mice (+E_2; *a*, $P < 0.05$, n = 8) in comparison to untreated OVX WT mice (OVX; n = 8). Untreated OVX PRKO mice (OVX; n = 8) exhibited plasma FSH levels similar to untreated OVX WT mice; additionally, no elevation was observed in E_2-treated OVX PRKO mice (+E_2; n = 8) *vs.* untreated OVX PRKO controls.

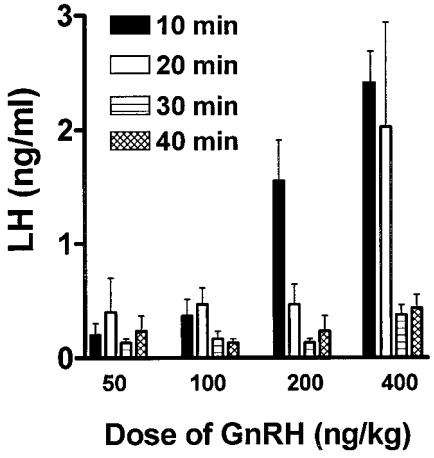

FIG. 3. Effect of varying doses of sc GnRH (50, 100, 200, and 400 ng/kg) on plasma LH levels in WT mice. Groups of OVX, E_2-treated WT mice (n = 4 per group) were killed at 10, 20, 30, or 40 min following injections of one of the GnRH doses indicated. Values are shown as mean ± SEM for groups of animals at each time, and for each dose.

min and returned to baseline levels by 30 min following injections (Fig. 3).

E_2-treated, OVX WT and PRKO mice receiving a single pulse of 200 ng/kg GnRH exhibited significant ($P < 0.001$) increases in plasma LH 10 min following injections in comparison to corresponding vehicle treated, E_2-treated OVX WT and PRKO controls (WT controls: 0.43 ± 0.09 ng LH/ml, n = 9; PRKO controls: 0.25 ± 0.05 ng LH/ml, n = 4; WT given one GnRH pulse: 1.80 ± 0.17 ng LH/ml, n = 9; PRKO given one GnRH pulse: 1.48 ± 0.29 ng LH/ml, n = 4). However, the plasma LH levels of E_2-treated, OVX WT mice receiving a second pulse of GnRH 60 min after the first were increased significantly ($P < 0.001$) above E_2-treated OVX WT mice receiving only one GnRH pulse, whereas E_2-treated, OVX PRKO mice exhibited no additional elevation following two pulses of GnRH (WT given two GnRH pulses: 3.37 ± 0.30 ng LH/ml, n = 7; PRKO given two GnRH pulses: 1.85 ± 0.23 ng LH/ml, n = 4) (Fig. 4). Neither WT (n = 5) nor PRKO (n = 4) mice given two saline pulses exhibited any increases in plasma LH above controls (data not shown). Additionally, no significant increases in plasma LH were observed in OVX WT mice not treated with E_2 following either one or two GnRH pulses in comparison to untreated controls receiving pulses of saline (n = 4 in each group; saline controls: 2.50 ± 0.97 ng LH/ml; one GnRH pulse: 2.8 ± 0.30 ng LH/ml; two GnRH pulses: 3.95 ± 0.73). This lack of a significant plasma LH increase in response to GnRH in untreated mice most likely reflects the large postOVX increase in gonadotropins in animals lacking E_2; thus, no direct comparison with E_2-treated animals was possible.

It was considered possible that the lack of a GnRH self-priming response in PRKO mice may be due to an abnormally prolonged response to the initial GnRH pulse; this may have depleted the releasable LH pool before exposure to the second pulse. To assess this possibility, a temporal secretory response was repeated in E_2-treated, OVX PRKO mice, using only the optimal 200 ng/kg dose of GnRH, which were subsequently killed at 20, 30, and 40 min following GnRH injection. For a clearer comparison of LH secretory profiles between genotypic groups, data from Figs. 2 and 4 are reproduced in Fig. 5. As seen in Fig. 5B, plasma LH levels in PRKO mice killed 10 min following a GnRH pulse were significantly ($P < 0.001$) elevated in comparison with those levels observed following longer intervals (10 min: 1.48 ± 0.29 ng LH/ml, n = 4; 20 min: 0.51 ± 0.18 ng LH/ml, n = 3; 30 min: 0.28 ± 0.12 ng LH/ml, n = 3; 40 min: 0.15 ± 0.01 ng LH/ml, n = 3). This LH secretory profile is similar in nature to that of E_2-treated, OVX WT mice receiving 200 ng/kg GnRH (10 min: 1.55 ± 0.36 ng LH/ml, n = 4; 20 min: 0.47 ± 0.17 ng LH/ml, n = 4; 30 min: 0.13 ± 0.03 ng LH/ml,

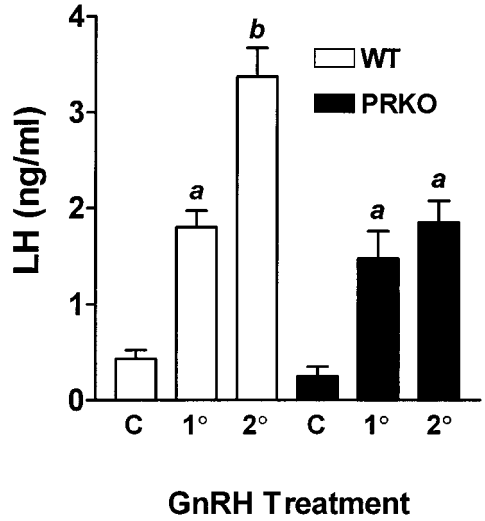

FIG. 4. Plasma LH levels in WT and PRKO mice given either one or two pulses of exogenous GnRH. One pulse of GnRH (1°) given to E_2-treated OVX WT and PRKO mice elicited significant (a, $P < 0.001$) increases in LH above OVX E_2-treated controls in both genotypes. E_2-treated, OVX WT mice given two pulses of GnRH 60 min apart (2°) exhibited a significant (b, $P < 0.001$) additional increase in LH, whereas no further elevation in LH was observed in E_2-treated OVX PRKO mice.

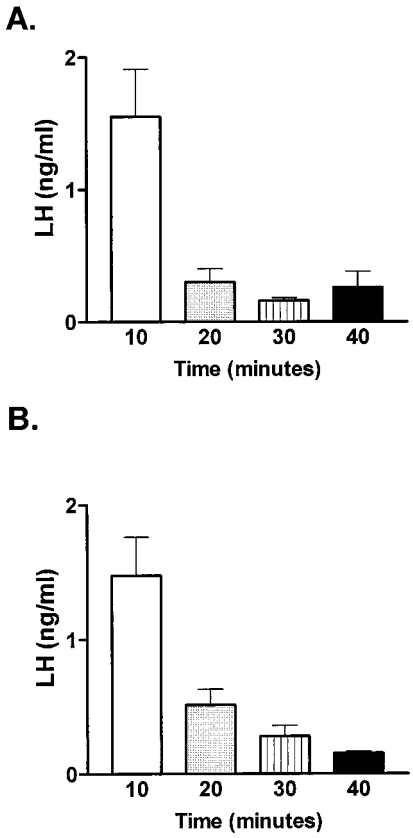

FIG. 5. Effect of one pulse of 200 ng/kg GnRH on plasma LH levels of E_2-treated OVX WT (A) and PRKO (B) mice killed 10, 20, 30, or 40 min following GnRH injection. One-way ANOVA revealed a significant ($P < 0.001$) difference in plasma LH release at 10 min in comparison to 20, 30, and 40 min post injection in both WT and PRKO mice. There was no significant difference between LH levels at 20, 30, or 40 min time points in either genotype.

n = 4; 40 min: 0.23 ± 0.13 ng LH/ml, n = 4). No significant difference in LH responsiveness to a single GnRH pulse exists between WT and PRKO mice at any of the above time points.

Discussion

We have previously shown that PRKO mice do not exhibit estrous cyclicity, nor do they release preovulatory gonadotropin surges following exposure to male mouse odor (13). In these studies, we have attempted to determine whether the lack of surge production in PRKO mice is due to an intrinsic defect in the ability of the hypothalamus and/or pituitary to respond to E_2. Our findings clearly demonstrate that the hypothalamus and/or pituitary gland of PRKO mice are refractory to the positive feedback effects of E_2. Our results additionally suggest that the absence of the GnRH self-priming response in these animals may account for at least part of this neuroendocrine deficiency.

Ovariectomy of both WT and PRKO mice results in a complete disappearance of assayable progesterone in these animals (data not shown). It is therefore logical to assume that the gonadotropin surges that we observed in E_2-treated, OVX WT mice were evoked in the absence of any signal changes in circulating P. We found, nevertheless, that the ability of E_2 to induce LH surges in mice lacking PRs was completely abolished. Thus, it is clear that the ability of E_2 to induce LH surges is dependent upon the presence, and most likely the activation, of PRs, even in the absence of any changes in circulating P concentrations. Ligand-independent activation of PRs has been implicated in the neural regulation of lordosis behavior in female rats (15) and mice (16), and in the GnRH self-priming mechanism in enriched gonadotrope populations (11). We hypothesize that the induction of LH surges by E_2 is similarly dependent upon ligand-independent activation of PRs by intracellular second messengers, which could convey signals through hypothalamic PRs leading ultimately to production of GnRH surges; in the pituitary gland, activation of unliganded PRs by intracellular activators downstream from the GnRH receptor would lead to GnRH self-priming. The ligand-independent activation of PRs at both the hypothalamic and pituitary levels may contribute to an integrated mechanism which mediates initiation of the LH surge. It should be noted, however, that our observations are not in conflict with studies demonstrating that P of ovarian and possibly adrenal origin may act to augment the E_2-induced LH surge, but suggest that neither ovarian nor adrenal P is required for the *initiation* of LH surges.

Interestingly, E_2 continues to exert a negative feedback influence on LH release in PRKO mice despite the absence of a positive feedback response, suggesting that the negative and positive feedback effects of E_2 are mediated through distinct mechanisms. These observations are in agreement with earlier work in this laboratory revealing that gonadotropin levels of PRKO mice are increased following OVX, demonstrating that estrogen's negative feedback influence is present in intact PRKO mice (13). Previous studies have attempted to determine if estrogen's positive and negative feedback actions are exerted at different anatomical loci (17) or via mechanisms that are differentially sensitive to protein synthesis inhibition (18). Our findings indicate that at least one point of divergence must occur in the signaling pathways that mediate the two effects; estrogen's positive feedback effects are conveyed through an obligatory step involving PR activation, whereas its negative feedback influence can be transmitted through a PR-independent pathway. The point of divergence leading to negative feedback could occur as early as receptor binding, *e.g.* binding to a nonclassical membrane receptor (19), or farther downstream from activation of the intracellular ERα and/or ERβ receptors.

In agreement with earlier observations, FSH levels in OVX mice from both genotypes were considerably elevated (13), and this elevation was not reduced by E_2-treatment, consistent with studies in the rat (20) demonstrating that E_2 alone is unable to produce a sufficient negative feedback signal upon FSH release in the absence of ovarian inhibin. Due to the lack of negative feedback, therefore, the usual characteristics of an FSH surge in WT mice were obscured in this study. Nevertheless, a slight but significant FSH increase was observed in E_2-treated, OVX WT mice, in contrast with PRKO mice, consistent with the observation of an E_2-treated LH surge in WT but not PRKO animals. Although it is conceivable that the timing of gonadotropin surges in PRKO mice could be altered such that the sampling schedule used in these studies was insufficient to detect peak release, previous

studies (14) have indicated that LH surges occur over a 4–6 h time frame with mean peak values occurring at 1900 h, suggesting that at least a measurable elevation above untreated controls should be evident in the case of all but the most drastic advances or delays in LH surge release.

These studies demonstrate for the first time that the GnRH self-priming effect can be induced in the mouse, and more importantly, that the GnRH self-priming response is absent in PRKO mice. The latter result implicates a role for hypophyseal PR in increasing pituitary responsiveness to GnRH. A GnRH self-priming effect, defined as an increase in the magnitude of LH responses to sequential GnRH stimuli, has been characterized in rats (21), sheep (22), and humans (23). The GnRH self-priming response is manifest within a relatively short period of time (~60 min) and has been shown to be dependent on both RNA and protein synthesis (24). We have found that this effect is remarkably similar in mice compared with these other species, both in its magnitude, dependency upon E_2 treatment, and time course (21). This response is absent in PRKO mice, even though the magnitude and time course of initial LH responses to GnRH were not different in these mice *vs.* WT controls. Our observations are thus consistent with the idea that PR activation is a requisite step in the physiological manifestation of the GnRH self-priming response. Dependency of the effect on E_2 treatment likely reflects the involvement of E_2-induced PRs, which may thereafter be activated as a part of the self-priming mechanism (11). Indeed, PR protein levels increase in pituitary and reach peak levels about 24 h following E_2 treatment (25, 26); a temporal induction which mirrors the rise in ovarian E_2 beginning on diestrus II in intact rats. Compatible with the above hypothesis is the additional observation that OVX WT mice that were not exposed to sufficient E_2 also did not exhibit a GnRH self-priming effect, ostensibly due to a lack of E_2-inducible PR, although it remains possible that any GnRH self-priming could have been masked by previously elevated plasma LH levels in OVX mice without the presence of an ovarian E_2 negative feedback signal.

The PR activation in GnRH self-priming also appears to be a ligand-independent process. Turgeon and Waring (11) have demonstrated that that successive GnRH stimuli, as well as cAMP, can increase PR-mediated transcriptional activity in transiently transfected pituitary gonadotropes. This effect required E_2 treatment was mimicked by P and could be blocked by the PR antagonist, RU486, in the absence of P (9, 11). In our WT animals, GnRH self-priming occurred in animals with no detectable circulating P, yet this effect was absent in PRKO animals which are devoid of PRs. We favor the most straightforward explanation of these findings, based on the previous *in vitro* results, that GnRH self-priming in normal animals is dependent upon P-independent activation of PRs.

It cannot be determined from these studies if the absence of LH surges in PRKO mice is solely or partially resultant from the GnRH self-priming deficiency, or whether it may even be independent of this defect. The initial LH responses observed in PRKO mice were no different from those produced in WT mice, indicating that basal pituitary responsiveness to GnRH is not compromised in these animals. It therefore appears likely that a generalized defect in pituitary function is not responsible for refractoriness to E_2. It still remains unclear, however, if GnRH self-priming is a necessary component of the LH surge, as it has not been possible to independently block this process to assess its impact on LH surge generation. Current work in this laboratory is aimed at determining if activation of unliganded hypothalamic PRs is an obligatory step in the processes leading to GnRH surges; if so, then the absence of LH surges in PRKO mice may also be explained as a lack of GnRH surges in these animals. Nevertheless, our data clearly demonstrate that PR activation somewhere in either hypothalamus, anterior pituitary, or both tissues is obligatory in the manifestation of estrogen's positive feedback actions.

Acknowledgments

The authors wish to thank Brigitte Mann and Stephanie Kluge for their expert technical assistance with hormone measurements.

References

1. **Levine JE, Ramirez VD** 1980 *In vivo* release of luteinizing hormone-releasing hormone estimated with push-pull cannulae from mediobasal hypothalami of ovariectomized, steroid-primed rats. Endocrinology 107:1782–1790
2. **Fink G, Aiyer MS** 1974 The role of steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinizing hormone releasing factor in the female rat. J Endocrinol 62:553–572
3. **Krey LC, Tyrey L, Everett JW** 1973 The estrogen-induced advance in the cyclic LH surge in the rat: dependency of ovarian progesterone secretion. Endocrinology 93:385–389
4. **Turgeon JL, Waring DW** 1990 Rapid augmentation by progesterone of agonist-stimulated luteinizing hormone secretion by cultured pituitary cells. Endocrinology 127:773–780
5. **Fink G** 1988 Oestrogen and progesterone interactions in the control of gonadotrophin and prolactin secretion. J Steroid Biochem 30:169–178
6. **Rao IM, Mahesh VB** 1986 Role of progesterone in the modulation of the preovulatory surge of gonadotropins and ovulation in the pregnant mare's plasma gonadotropin-primed immature rat and the adult rat. Biol Reprod 35:1154–1161
7. **Szabo M, Knox KL, Ringstrom SJ, Perlyn CA, Sutandi S, Schwartz NB** 1996 Mechanism of the inhibitory action of RU486 on the secondary follicle-stimulating hormone surge. Endocrinology 137:85–89
8. **Ringstrom SJ, Szabo M, Kilen SM, Saberi S, Knox KL, Schwartz NB** 1997 The antiprogestins RU486 and ZK98299 affect follicle-stimulating hormone secretion differentially on estrus but not on proestrus. Endocrinology 138:2286–2290
9. **Waring DW, Turgeon JL** 1992 A pathway for luteinizing hormone releasing-hormone self-potentiation: cross-talk with the progesterone receptor. Endocrinology 130:3275–3282
10. **Waring DW, Turgeon JL** 1980 Luteinizing hormone-releasing hormone-induced luteinizing hormone secretion *in vitro*: cyclic changes in responsiveness and self-priming. Endocrinology 106:1430–1436
11. **Turgeon JL, Waring DW** 1994 Activation of the progesterone receptor by the gonadotropin-releasing hormone self-priming signaling pathway. Mol Endocrinol 8:860–869
12. **Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery Jr CA, Shyamala G, Conneely OM, O'Malley BW** 1995 Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev 9:2266–2278
13. **Chappell PE, Lydon JP, Conneely OM, O'Malley BW, Levine JE** 1997 Endocrine defects in mice carrying a null mutation for the progesterone receptor gene. Endocrinology 138:4147–4152
14. **Bronson FH, Vom Saal FS** 1979 Control of the preovulatory release of luteinizing hormone by steroids in the mouse. Endocrinology 104:1247–1255
15. **Auger AP, Moffatt CA, Blaustein JD** 1997 Progesterone-independent activation of rat brain progestin receptors by reproductive stimuli. Endocrinology 138:511–514
16. **Mani SK, Allen JMC, Lydon JP, Mulac-Jericevic B, Blaustein JD, DeMayo FJ, Conneely O, O'Malley BW** 1996 Dopamine requires the unoccupied progesterone receptor to induce sexual behavior in mice. Mol Endocrinol 10:1728–1737
17. **Blake CA, Norman RL, Sawyer CH** 1974 Localization of the inhibitory actions

of estrogen and nicotine on release of luteinizing hormone in rats. Neuroendocrinology 16:22–35
18. **Kalra SP** 1976 Tissue levels of LHRH in the preoptic area and hypothalamus, and plasma concentrations of gonadotropins following anterior hypothalamic deafferentation and estrogen treatment of the female rat. Endocrinology 99:101–107
19. **Lagrange AH, Wagner EJ, Ronnekleiv OK, Kelly MJ** 1996 Estrogen rapidly attenuates a $GABA_B$ response in hypothalamic neurons. Neuroendocrinology 64:114–123
20. **Luderer U, Schwartz NB** 1994 Acute changes in pulsatile LH and FSH secretion after ovariectomy in rats: treatment with oestradiol for 24 h suppresses LH, but not FSH, for at least 48 h. J Reprod Fertil 100:613–621
21. **Aiyer MS, Fink G, Greig F** 1974 Changes in the sensitivity of the pituitary gland to luteinizing hormone releasing factor during the oestrous cycle in of the rat. J Endocrinol 60:47–54
22. **Phogat JB, Smith RF, Dobson H** 1997 Effect of ACTH on gonadotrophin releasing hormone-induced luteinizing hormone secretion *in vitro*. Anim Reprod Sci 48:53–65
23. **Urban RJ, Veldhuis JD, Dufau ML** 1991 Estrogen regulates the gonadotropin-releasing hormone-stimulated secretion of biologically active luteinizing hormone. J Clin Endocrinol Metab 72:660–668
24. **Turgeon JL, Waring DW** 1991 The timing of progesterone-induced ribonucleic acid and protein synthesis for augmentation of luteinizing hormone secretion. Endocrinology 129:3234–3239
25. **MacLusky NJ, McEwen BS** 1978 Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. Nature 274:276–278
26. **Parsons B, MacLusky NJ, Krey L, Pfaff DW, McEwen BS** 1980 The temporal relationship between estrogen-inducible progestin receptors in the female rat brain and the time course of estrogen activation of mating behavior. Endocrinology 107:774–779