Effect of oestradiol on mRNA encoding GnRH receptor in pituitary tissue of orchidectomized sheep passively immunized against GnRH

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The effect of oestradiol on tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor was assessed in orchidectomized sheep (wethers) made deficient in GnRH by passive immunization. Wethers were assigned to one of four groups ($n = 6$ animals per group). Animals in groups 2 and 4 received ovine anti-GnRH sera (200 ml; i.v.) at passive immunization, while antisera against the carrier protein was administered to wethers in groups 1 and 3. Oestradiol was delivered as a continuous infusion ($2 \mu\text{g h}^{-1}$) to wethers in groups 1 and 2. Animals in groups 3 and 4 were infused with vehicle alone. Anterior pituitary tissue was collected at the end of the 48 h infusion. Anti-GnRH sera induced a rapid reduction in the serum concentration of LH. Continuous delivery of oestradiol resulted in a twofold increase ($P < 0.05$) in tissue concentration of GnRH receptor. This oestradiol-induced response was manifest even in wethers in which endogenous GnRH had been neutralized by passive immunization. Conversely, infusion of oestradiol increased ($P < 0.05$), and intravenous administration of anti-GnRH sera decreased ($P < 0.05$), concentrations of mRNA encoding GnRH receptor in pituitary tissue. When delivered in combination, anti-GnRH sera reduced ($P < 0.05$), but did not eliminate, the oestradiol-induced augmentation of steady-state concentrations of mRNA encoding GnRH receptor. These data demonstrate that the basal concentration of mRNA encoding GnRH receptor is dependent on continued GnRH stimulation. In contrast, the oestradiol-induced increase in steady-state concentration of mRNA encoding GnRH receptor is manifest even in the absence of GnRH.

Introduction

Steady-state concentrations of GnRH receptor and mRNA encoding GnRH receptor in pituitary tissue are increased during the preovulatory period in sheep (Padmanabhan *et al.*, 1995) and rodents (Bauer-Dantoin *et al.*, 1993). Oestrogenic input during the preovulatory period appears to be involved in this process (Brooks *et al.*, 1993). Indeed, oestrogenic stimulation increases steady-state concentrations of GnRH receptor and mRNA encoding GnRH receptor in ovariectomized sheep (Turzillo *et al.*, 1994; Hamernik *et al.*, 1995) and rats (Kaiser *et al.*, 1993). Similarly, tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor rapidly decay after withdrawal of the oestrogenic stimulus (Adams *et al.*, 1996).

Pituitary tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor are also dependent on hypothalamic stimulation. Indeed, the concentration of GnRH receptor in pituitary tissue of ovariectomized sheep is decreased by hypothalamo-pituitary disconnection (Hamernik and Nett, 1988). Surprisingly, the concentration of mRNA encoding GnRH receptor in pituitary tissue of ovariectomized sheep is not significantly affected by hypothalamo-pituitary disconnec-

tion (Turzillo *et al.*, 1995a). Conversely, pulsatile GnRH stimulation induces a marked increase in the concentrations of mRNA encoding GnRH receptor in pituitary cells of rats (Kaiser *et al.*, 1993). Similarly, the concentration of mRNA encoding GnRH receptor is reduced in the pituitary tissue of rats in which GnRH secretion is blocked by administration of pentobarbital (Bauer-Dantoin *et al.*, 1995). Concentrations of GnRH receptor and mRNA encoding GnRH receptor are also reduced in sheep made insensitive to GnRH by administration of a GnRH antagonist or prolonged treatment with a potent GnRH agonist (Brooks and McNeilly, 1994).

Bauer-Dantoin *et al.* (1995) suggested that oestrogenic induction of tissue concentrations of mRNA encoding GnRH receptor reflects oestrogenic actions primarily at hypothalamic loci. However, oestrogen-induced increases in the tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor are evident in ovariectomized sheep with hypothalamo-pituitary disconnection (Turzillo *et al.*, 1995a). The oestrogenic response is also evident in sheep made resistant to GnRH by prolonged administration of a potent GnRH agonist (Brooks and McNeilly, 1994). In the studies reported here, orchidectomized sheep (wethers) were used to assess the interaction between physiological concentrations of oestradiol and GnRH on expression of GnRH receptor and mRNA encoding GnRH receptor. We hypothesized that oestrogenic stimulation would increase

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tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor even in wethers made deficient in GnRH by passive immunization.

Materials and Methods

Animals

Crossbred whiteface sheep were castrated within 2 weeks of birth. The orchidectomized lambs (wethers) were housed in an open-sided barn under natural lighting and were afforded free access to water and alfalfa pellets supplemented with cereal grains and a vitamin and mineral premix. The studies described here were conducted during the early summer, a period of seasonal anoestrus in female sheep at this latitude (38°N). The wethers were 6–8 months of age (bodyweight = 45–50 kg) at the time of experimentation. All experimental procedures involving the use of animals were conducted in accordance with National Institutes of Health (NIH) Guidelines and were reviewed and approved by the Animal Use and Care Committee of the University of California.

Cannulation

Before experimentation, two polyethylene cannulae (Intra-medec PE 190, Clay Adams, Parsippany, NJ) were inserted into the left jugular vein. These cannulae were used for intravenous delivery of antisera and continuous infusion of oestradiol or vehicle. A third cannula, inserted into the contralateral vein, was used for blood collection. All cannulae were passed through a protective Tygon tubing sheath to the exterior of the animal holding area. Animals were freely mobile at the end of a 1 m lead.

Hormone delivery

The oestradiol delivery cannulae were connected to syringes placed in Harvard infusion pumps (Model 2265, Harvard Bioscience, South Natick, MA). Oestradiol ($2 \mu\text{g ml}^{-1}$; Sigma Chemical Co., St Louis, MO) in 10% ethanol–saline (vehicle), or vehicle alone, was continuously infused (1 ml h^{-1}).

Active immunization of donor sheep

Antibodies directed against GnRH were generated in wethers by active immunization with a GnRH–keyhole limpet haemocyanin (GnRH–KLH) conjugate using procedures described by Adams and Adams (1986). A 1:40 000 dilution of the pool of anti-GnRH sera used in this study bound 41.7% of ^{125}I -labelled GnRH added. Anti-KLH sera was obtained from wethers actively immunized against KLH alone.

Immunoneutralization of GnRH and concurrent oestradiol infusion

Twenty-four wethers were assigned at random to one of four groups ($n=6$ animals per group) in a 2×2 factorial experiment designed to assess the effect of oestradiol and immunoneutralization of endogenous GnRH on steady-state

concentrations of GnRH receptor and mRNA encoding GnRH receptor. At passive immunization, 200 ml of ovine anti-KLH (groups 1 and 3) or anti-GnRH (groups 2 and 4) sera was infused (i.v.) over 30 min. Oestradiol was delivered as a continuous infusion ($2 \mu\text{g h}^{-1}$) to wethers in groups 1 (KLH/oestradiol) and 2 (GnRH/oestradiol). Animals in groups 3 (KLH/V) and 4 (GnRH/V) were infused with vehicle alone. Infusion of oestradiol or vehicle was initiated at passive immunization and continued for 48 h. Blood samples were collected at passive immunization and at 3, 6 or 12 h intervals thereafter. Serum was harvested and stored frozen at -20°C for later analysis of anti-GnRH titre and concentrations of LH, FSH and oestradiol. At the end of the 48 h infusion, animals were stunned by means of a captive bolt pistol and killed by exsanguination at the UC Davis Slaughter Facility. Anterior pituitary tissue was quickly excised, halved by a midsagittal cut and each half was immediately frozen in liquid nitrogen and stored at -80°C for later analysis.

Endocrine analysis

Serum concentrations of LH, FSH and oestradiol were determined using previously validated procedures (Adams *et al.*, 1975, 1988; Sakurai *et al.*, 1992). The LH and FSH reference standards (NIAMDD-oLH-25 and NIAMDD RP-1, respectively) were gifts of the National Hormone and Pituitary Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD; Baltimore, MD). In all cases, intra- and interassay coefficients of variation were $<10\%$. Anti-GnRH titre was determined as described by Sakurai *et al.* (1992). Briefly, 8 fmol ^{125}I -labelled GnRH (20 000 c.p.m.) in 0.1 ml of 0.1 mol PBS l^{-1} containing 0.1% (w/v) gelatin (Gel–PBS) was combined with 0.1 ml of a 1:100 or 1:1000 serum:Gel–PBS dilution. After incubation for 24 h at 4°C , the radiolabelled GnRH not bound to antibody was separated by precipitation with 95% ethanol. Anti-GnRH titre in sheep passively immunized with anti-GnRH sera is expressed as percentage of total ^{125}I -labelled GnRH bound by 0.1 ml of a 1:1000 dilution of serum.

The affinity and tissue concentration of GnRH receptor were quantified by means of the procedure described by Sakurai and Adams (1991). Tissue concentrations of mRNA encoding GnRH receptor were determined using the solution hybridization–RNase protection assay described by Adams *et al.* (1996). A plasmid containing the cDNA insert for the ovine GnRH receptor (Brooks *et al.*, 1993) was kindly provided by J. Brooks (MRC Reproductive Biology Unit, Edinburgh). The sense and anti-sense cRNAs were generated from linearized cDNA by *in vitro* transcription using either T7 or SP6 RNA polymerase and the Riboprobe Gemini System II reagent system (Promega Corp., Madison, WI).

Statistical analysis

Wethers were assigned to one of four treatment groups in a 2×2 factorial experiment, with the factors of interest being oestradiol infusion and administration of anti-GnRH sera. The significance of treatments was assessed by two-way ANOVA (Gill, 1978). Where significant treatment effects were noted,

comparison between means was made using Duncan's multiple range test. Data are presented in the text and figures as means $\pm$ SEM.

Results

Anti-GnRH titre and serum concentrations of oestradiol

Administration of 200 ml of ovine anti-GnRH sera increased anti-GnRH titre in recipient wethers to $76.7 \pm 0.5\%$ (percentage ^{125}I -labelled GnRH bound by a 1:1000 dilution of serum; $n = 12$) within 3 h of passive immunization. Anti-GnRH titre at tissue collection, 48 h after passive immunization, was $73.5 \pm 0.7\%$ ($n = 12$). Anti-GnRH titre was not evident ($< 1\%$ ^{125}I -labelled GnRH bound by a 1:100 dilution of serum; $n = 12$) in wethers passively immunized against KLH.

Delivery of oestradiol at a rate of $2 \mu\text{g h}^{-1}$ established a serum concentration of oestradiol of $11.8 \pm 0.6 \text{ pg ml}^{-1}$ ($n = 12$) within 3 h of the start of infusion. Serum oestradiol was maintained at this concentration throughout the remainder of the infusion period. At tissue collection, the mean serum concentration of oestradiol in wethers receiving exogenous oestradiol was $12.1 \pm 0.5 \text{ pg ml}^{-1}$ ($n = 12$). The concentration of oestradiol in serum of wethers receiving vehicle was below the sensitivity of the assay ($< 0.6 \text{ pg ml}^{-1}$).

Serum concentrations of LH and FSH

Passive immunization with anti-KLH sera led to a modest and short-lived decrease in serum concentrations of LH in control wethers receiving vehicle alone (Fig. 1). Serum concentrations of LH in control animals returned to pretreatment concentrations ($15.4 \pm 1.9 \text{ ng ml}^{-1}$) within 12 h of passive immunization and continued at this concentration for the remainder of the experiment. Conversely, passive immunization with anti-GnRH sera induced a rapid and persistent reduction in the serum concentration of LH. Continuous infusion of oestradiol alone, or in combination with administration of anti-GnRH sera, had a similar effect. At the end of the 48 h infusion, serum concentrations of LH (6.0 ± 1.4 and $5.3 \pm 1.2 \text{ ng ml}^{-1}$) in wethers receiving oestradiol (KLH/oestradiol and GnRH/oestradiol, respectively) were significantly lower than LH concentrations ($16.2 \pm 0.8 \text{ ng ml}^{-1}$) in wethers receiving anti-KLH and vehicle (KLH/vehicle). The final serum concentration of LH ($2.6 \pm 0.4 \text{ ng ml}^{-1}$) in wethers passively immunized against GnRH and receiving vehicle alone (GnRH/vehicle) was significantly lower than the final concentration of LH in all other treatment groups.

Similarly, when expressed relative to pretreatment concentrations of FSH ($18.5 \pm 2.5 \text{ ng ml}^{-1}$), infusion of oestradiol (KLH/oestradiol; $11.3 \pm 1.4 \text{ ng ml}^{-1}$) or passive immunization against GnRH (GnRH/vehicle; $12.9 \pm 1.3 \text{ ng ml}^{-1}$) alone, or in combination (GnRH/oestradiol; $11.8 \pm 1.5 \text{ ng ml}^{-1}$), significantly reduced serum concentrations of FSH by the end of the 48 h infusion (Fig. 1). Conversely, passive immunization with anti-KLH sera did not significantly affect serum concentrations of FSH (KLH/vehicle; $19.9 \pm 2.4 \text{ ng ml}^{-1}$) 48 h after passive immunization.

Fig. 1. Serum concentrations of (a) LH and (b) FSH in orchidectomized sheep (wethers; $n = 6$ wethers per group) receiving 200 ml ovine anti-GnRH ($\circ$, $\bullet$) or ovine anti-KLH ($\triangle$, $\blacktriangle$) sera i.v. at time 0 (passive immunization). Oestradiol ($\bullet$, $\blacktriangle$; $2 \mu\text{g h}^{-1}$ in 10% ethanol-saline vehicle) or vehicle alone ($\circ$, $\triangle$) was delivered by continuous infusion during the 48 h period after passive immunization. At certain time points, mean FSH concentrations are depicted without error bars to improve the clarity of graphical presentation.

Steady-state concentrations of GnRH receptor and mRNA encoding GnRH receptor

In comparison with control animals (KLH/vehicle = $5.8 \pm 0.8 \text{ fmol mg}^{-1}$ fresh tissue equivalent (FTE)), the concentration of GnRH receptors in pituitary tissue was significantly increased by infusion of oestradiol (KLH/oestradiol = $11.4 \pm 1.2 \text{ fmol mg}^{-1}$ FTE) for 48 h (Fig. 2). Concurrent administration of anti-GnRH did not significantly affect the concentration of GnRH receptor in wethers receiving vehicle (GnRH/vehicle = $5.0 \pm 1.2 \text{ fmol mg}^{-1}$ FTE) or oestradiol (GnRH/oestradiol = $10.4 \pm 0.7 \text{ fmol mg}^{-1}$ FTE). Conversely, infusion of oestradiol and administration of anti-GnRH sera had divergent effects on steady-state concentrations of mRNA encoding GnRH receptor (Fig. 2). Although no significant interaction ($P > 0.05$) among treatments was noted, infusion of oestradiol increased ($P < 0.001$), and administration of anti-GnRH decreased ($P < 0.005$), tissue concentrations of mRNA encoding GnRH receptor. Administration of anti-GnRH sera decreased ($P < 0.05$) the concentration of mRNA encoding GnRH receptor (GnRH/vehicle = $0.27 \pm 0.03 \text{ pg } \mu\text{g}^{-1}$ total RNA), relative to the concentration noted in control wethers (KLH/vehicle = $0.50 \pm 0.05 \text{ pg } \mu\text{g}^{-1}$ total

Fig. 2. Steady-state concentrations of (a) mRNA encoding GnRH receptor and (b) GnRH receptor in pituitary tissue of orchidectomized sheep (wethers; $n = 6$ wethers per group). Pituitary tissue was collected 48 h after passive immunization with ovine anti-GnRH or ovine anti-keyhole limpet haemocyanin (anti-KLH) sera (200 ml, i.v.). Wethers received oestradiol (□; $2 \mu\text{g h}^{-1}$ in 10% ethanol-saline vehicle) or vehicle alone (■) by continuous infusion during the 48 h after passive immunization. FTE, fresh tissue equivalent.

RNA). Conversely, infusion of oestradiol increased ($P < 0.05$) the tissue concentration of mRNA encoding GnRH receptor (KLH/oestradiol = $1.11 \pm 0.11 \text{ pg } \mu\text{g}^{-1}$ total RNA) in wethers receiving anti-KLH sera. When delivered in combination, anti-GnRH sera reduced ($P < 0.05$), but did not eliminate, the oestradiol-induced augmentation of steady-state concentrations of mRNA encoding GnRH receptor (GnRH/oestradiol = $0.71 \pm 0.07 \text{ pg } \mu\text{g}^{-1}$ total RNA). Indeed, the magnitude of the oestradiol-induced increase in steady-state concentration of mRNA encoding GnRH receptor above basal concentrations did not differ ($P > 0.05$) in wethers receiving anti-KLH or anti-GnRH sera (0.61 ± 0.12 and $0.44 \pm 0.08 \text{ pg } \mu\text{g}^{-1}$ total RNA, respectively).

Discussion

Tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor are increased by oestradiol and GnRH itself (Turzillo *et al.*, 1995b; Adams *et al.*, 1996). However, the extent to which change in tissue concentration of GnRH receptor and mRNA encoding GnRH receptor is dependent on interaction between oestradiol and GnRH has not been precisely defined. In the study presented here, orchidectomized

sheep made deficient in GnRH by passive immunization were used to examine the hypothesis that the oestradiol-induced increase in tissue concentration of mRNA encoding GnRH receptor is independent of GnRH. In this animal model, serum concentrations of oestradiol comparable with those noted during the periovulatory period in female sheep ($10\text{--}12 \text{ pg ml}^{-1}$) are established by continuous infusion of exogenous oestradiol.

As demonstrated here and in previous studies (Sakurai and Adams, 1991; Sakurai *et al.*, 1997), intravenous administration of ovine anti-GnRH sera leads to a rapid and persistent reduction in the serum concentration of LH. Passive immunization against GnRH also dampens the episodic pattern of LH secretion (Herman and Adams, 1990). These observations indicate that passive immunization establishes an immunological barrier between the hypothalamus and anterior pituitary that markedly reduces the passage of endogenous GnRH. Similar patterns of change in the serum concentration of LH are noted in ovariectomized sheep after withdrawal of GnRH stimulation effected by hypothalamo-pituitary disconnection (Hamernik *et al.*, 1986; Hamernik and Nett, 1988), pentobarbital-induced anaesthesia (Wright and Clarke, 1988), or administration of a GnRH antagonist (Sanchez *et al.*, 1994).

Continuous infusion of oestradiol also decreases the serum concentration of LH. This effect of oestradiol is likely to reflect oestrogenic action at both hypothalamic and pituitary loci (Lubbers *et al.*, 1996). Our recent observation (Sakurai and Adams, 1991; Sakurai *et al.*, 1996) that the acute oestradiol-dependent decrease in serum LH is not affected by concurrent pulsatile delivery of exogenous GnRH suggests that oestradiol acts directly at the pituitary to reduce gonadotrope responsiveness. Conversely, the decrease in serum LH noted during chronic oestrogen stimulation is reversed by episodic administration of GnRH (Sakurai and Adams, 1991; Sakurai *et al.*, 1996), indicating that the effect of more extended oestrogenic stimulation is manifest primarily at hypothalamic sites.

Although the serum concentration of LH was significantly depressed by either infusion of oestradiol or immunoneutralization of GnRH, the extent of reduction in serum LH was greater in animals deprived of endogenous GnRH. The final serum concentration of LH in wethers receiving both anti-GnRH sera and oestradiol was significantly greater than the serum concentration of LH in animals receiving anti-GnRH sera alone. This apparent potentiation of basal LH secretion by oestradiol is consistent with previous observations (Sakurai and Adams, 1991). Similar oestradiol-induced augmentation of basal LH secretion has been noted *in vitro* using ovine or rat pituitary cells (Huang and Miller, 1980; Liu and Jackson, 1988; Liu *et al.*, 1993). Although the mechanism of this oestrogenic response has not been fully resolved, several reports indicate that oestradiol enhances the activity of second messenger systems involved in LH secretion (Liu and Jackson, 1988; Fahmy *et al.*, 1989; Ortmann *et al.*, 1995).

Withdrawal of endogenous GnRH by passive immunization also reduced tissue concentrations of mRNA encoding GnRH receptor. This is consistent with previous observations (Sakurai *et al.*, 1997) and indicates that GnRH stimulation plays an essential role in maintenance of tissue concentrations of mRNA encoding GnRH receptor. Although Turzillo *et al.* (1995a) have reported that tissue concentrations of mRNA encoding GnRH

receptor were not acutely affected by withdrawal of hypothalamic support, most other work suggests that maintenance of tissue concentrations of mRNA encoding GnRH receptor is critically dependent on continued GnRH stimulation. Indeed, tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor are markedly reduced in sheep treated with a GnRH antagonist (Brooks and McNeilly, 1994). Similarly, episodic GnRH stimulation increased the concentration of GnRH receptor and mRNA encoding GnRH receptor in pituitary tissue of sheep (Hamernik and Nett, 1988; Turzillo *et al.*, 1995b). Pulsatile delivery of GnRH also increased the concentration of mRNA encoding GnRH receptor in perfused rat pituitary cells (Kaiser *et al.*, 1993; Bauer-Dantoin *et al.*, 1995). Moreover, suppression of GnRH secretion by pentobarbital administration leads to rapid reduction in the concentration of mRNA encoding GnRH receptor in the pituitary tissue of ovariectomized rats (Bauer-Dantoin *et al.*, 1995). Collectively, these data support the postulate that maintenance of the steady-state concentration of mRNA encoding GnRH receptor requires continued GnRH stimulation. This dependence probably reflects, at least in part, a GnRH-induced increase in GnRH receptor gene transcription. Indeed, transcription of a reporter gene linked to the 5'-flanking region of the mouse GnRH receptor gene is increased markedly during stimulation by a GnRH agonist (Albarracin *et al.*, 1994).

Withdrawal of endogenous GnRH support by passive immunization significantly decreased tissue concentrations of mRNA encoding GnRH receptor, without effecting comparable change in tissue concentration of the receptor itself. Similar divergence between tissue concentrations of the receptor and receptor mRNA has been noted by Turzillo *et al.* (1995a) and Adams *et al.* (1996) and is likely to reflect differences in the relative stability of mRNA and protein rather than variation in GnRH dependence. Indeed, we have shown that more extended exposure to anti-GnRH-containing sera significantly reduces tissue concentrations of both GnRH receptor and receptor mRNA in wethers (Sakurai *et al.*, 1997).

The results presented here demonstrate that physiological concentrations of oestradiol increase tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor in orchidectomized sheep. Oestradiol also increases concentrations of GnRH receptor and mRNA encoding GnRH receptor in ovariectomized sheep (Turzillo *et al.*, 1994). This oestrogenic effect is likely to reflect a direct action of oestradiol at adenohypophyseal loci. Indeed, an oestradiol-induced increase in the tissue concentration of GnRH receptor and mRNA encoding GnRH receptor is evident in orchidectomized sheep made deficient in GnRH by hypothalamo-pituitary disconnection (Gregg and Nett, 1989; Turzillo *et al.*, 1995a). Similarly, oestradiol-induced augmentation of tissue concentrations of mRNA encoding GnRH receptor has been reported in sheep, even after prolonged administration of a GnRH agonist (Brooks and McNeilly, 1994). This oestrogenic response is also evident *in vitro* (Wu *et al.*, 1994). Collectively, these data indicate that oestradiol increases tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor via a GnRH-independent mechanism.

Oestradiol-induced change in the steady-state concentration of mRNA encoding GnRH receptor may reflect a change in rate of gene transcription or a change in mRNA stability, or

both. The relative significance of these potential oestrogen-sensitive processes is not known. However, increased gene transcription is likely to account, at least in part, for the oestradiol-induced increase in steady-state concentrations of mRNA encoding GnRH receptor reported in the present study since the oestradiol-induced increase in GnRH receptor is suppressed by actinomycin D (Gregg *et al.*, 1990). Kimura *et al.* (1994) have suggested that the oestradiol-induced increase in mRNA encoding thyrotrophin-releasing hormone receptor is due to oestrogen-specific enhancement of both thyrotrophin-releasing hormone receptor gene transcription and mRNA stability.

Taken together, these data demonstrate that maintenance of steady-state concentrations of mRNA encoding GnRH receptor in pituitary tissue requires continued GnRH stimulation. In contrast, oestradiol-induced increase in tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor is independent of GnRH and is manifest even in sheep made deficient in GnRH by passive immunization.

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