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Regulation of the Rat Pituitary Gonadotropin-Releasing Hormone Receptor

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SUMMARY

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It is apparent that pituitary gonadotropin release changes quantitatively during various endocrine states, such as those found during ovarian cyclicity, lactation, aging, and ovariectomy. In the present work, we have used a radioligand prepared from a nondegradable, superagonist of gonadotropin-releasing hormone (GnRH) to determine the number and binding affinity of GnRH receptors during these endocrine states. While receptor affinity was unaltered (range 1.6–2.7 × 10^{10} M^{-1}), marked differences were observed in the receptor number throughout the estrous cycle [maximal in late diestrus II and proestrus immediately preceding the luteinizing hormone (LH) spike]. Lactating animals and old animals also had diminished concentrations (number per milligram of protein) of GnRH receptors as compared with young precycling females. Ovariectomy increased the number of receptors, and injections of estradiol benzoate to ovariectomized animals reversed this increase within 3 hr. Throughout the study, elevated receptor numbers were generally associated with elevated LH levels, although this alone did not appear to be sufficient for increased LH secretion. The present results suggest that regulation of the GnRH receptor may be one mechanism through which gonadotrope sensitivity is regulated.

INTRODUCTION

Pituitary gonadotropin release to the peripheral circulation varies markedly during the lifetime of the rat and reflects the reproductive state of the animal. Gonadotropin release from the pituitary is stimulated by GnRH,[4] which binds to specific plasma membrane receptors (1) and initiates the intracellular events leading to increased LH and FSH secretion (2). The magnitude of LH release can be influenced both by the concentration of GnRH in the hypothalamic-hypophysial portal system and the gonadotrope responsiveness to stimulation. Responsiveness is related to the number and binding affinity of functional GnRH receptors, the efficiency of stimulus-secretion coupling, and the intracellular level of gonadotropin available for release. Significant fluctuations in gonadotropin release are associated with onset of puberty, normal estrus or menstrual cycling, pregnancy and lactation, female postcycling periods in old age, and gonadectomy. Because receptor number and affinity appear to be major regulatory sites in many systems, we sought to examine the GnRH receptor during endocrine states which are characterized by marked changes in gonadotropin release.

METHODS

General

Long-Evans female rats (hooded) were purchased from Charles River Breeding Laboratories, Wilmington, Mass., or obtained from our continuous colony. Animals were housed in a 22° room with a 14:10 hr (light:dark) cycle. Food and water were provided ad libitum. Animals were killed between 2 p.m. and 4 p.m. unless otherwise indicated. Upon autopsy, any animal found to have microscopic anatomical anomalies of the kidneys, liver, gonads, pituitary, or heart or evidence of disease (wheezing, unkept coat, or diarrhea) was excluded from the study.

Animals from a variety of endocrine states were used in the present studies.

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[4] The abbreviations used are: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; BSA, bovine serum albumin; TRH, thyrotropin-releasing hormone; PP, pseudopregnant; CE, constant-estrus.

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4-Day Cycling Rats. Two separate groups of young adult females (60–90 days old) which exhibited at least two consecutive 4-day cycles (assessed by vaginal smears) were killed between 2 a.m. and 4 a.m. and between 2 p.m. and 4 p.m. on the indicated day of the cycle. Pituitaries were collected over a 2-week period with several weeks between the two groups. Anterior pituitaries were removed and stored frozen at −40° prior to the binding assay. Blood was collected and serum was frozen for radioimmunoassay of luteinizing hormone (using antiserum LHS-5, LH for iodination, LH I–5, and standard, RP1, provided by the National Institute of Arthritis, Metabolism and Digestive Diseases Hormone Distribution Office and the National Pituitary Agency) or 17β-estradiol [antiserum previously characterized (3), standard and tracer obtained from Sigma Chemical Company, St. Louis, Mo., and New England Nuclear Corporation, Boston, Mass., respectively]. For LH, a 20- to 50-μl sample of serum was assayed. This assay was sensitive to 1 ng/tube with intra-assay variance of 7% and interassay variance of 12%. For estradiol, serum was first extracted into ether as described previously (3). This assay was sensitive to 10 pg/tube, and intra-assay and interassay variance were 10% and 13%, respectively.

Noncycling rats (old and lactating). Pituitaries and sera were collected from animals of different ages observed to be in constant estrus or pseudopregnant. In our colony, the state of constant estrus develops in the majority of rats at approximately 1 year of age. This condition persists until about 20 months of age, at which time the rats become pseudopregnant. In young adult female rats these conditions are observed in approximately 5% of animals. Thus, young adult, middle-aged, and old constant-estrus and old pseudopregnant animals were studied to evaluate the effects of these states on the GnRH receptor concentration and binding affinity. Pituitaries and sera were collected, stored, and assayed as described above for cycling female rats.

Mother rats were killed at the time of pup weaning and their pituitaries and sera were treated as described above.

Intact and ovariectomized/estrogen-treated rats. Female pups (14 days old) were ovariectomized or sham-operated (controls) and returned to their mothers until weaning, at 21 days of age. Ovariectomized and sham-operated weanlings received injections 10 days after surgery of 10 μg of estradiol benzoate in 0.2 ml of sesame oil or of sesame oil alone. Groups were killed at 3 or 12 hr following the initial injection (at 6 a.m.) or after seven daily doses (as indicated). Pituitaries and sera were collected, stored, and assayed as described above.

Radioligand Preparation

A superactive, degradation-resistant analogue of GnRH, D-Ser(t-Bu)^6-des-Gly^{10} EA (Buserelin, obtained from Dr. V. Wagner, Hoescht-Roussel Pharmaceuticals, Inc., Somerville, N. J.) was iodinated using chloramine T and purified by CM-cellulose column chromatography, as described previously (4). Maximal bindability of the radioligand was assessed by binding with excess plasma membrane receptor. The membrane fraction used was prepared from weanling rats, and maximal bindability ranged from 30% to 50%. Specific activity (assessed by self-displacement assay) was 850–1250 μCi/μg for different preparations.

Membrane Preparation

Thawed anterior pituitaries (n = 4–15) were homogenized in ice-cold 0.25 M sucrose in 10 mM Tris-HCl, pH 7.4 (100 μl/pituitary), then centrifuged at 700 × g, 4°, for 5 min. The supernatant was centrifuged at 11,000 × g for 30 min and the resulting membrane fraction was resuspended with 10 mM Tris-HCl, pH 7.4. Following centrifugation at 11,000 × g for 20 min, the pellet (membrane fraction) was resuspended to 1 mg of protein per milliliter of 10 mM Tris-HCl, pH 7.4. Protein was determined by the method of Bradford (5), using BSA as a standard. Membranes prepared from frozen pituitaries retained 90–95% of the binding capacity as compared with freshly prepared pituitaries of the same age. The binding affinity was unchanged. Membrane fractions were assayed immediately after preparation. Membrane fractions from other tissues were prepared as described for pituitaries, for assessment of tissue specificity of radioligand binding.

Binding Assay

The binding assay was conducted in polypropylene microfuge tubes which were precoated overnight with 1% BSA. The assay contained approximately 20,000 cpm of ^{125}I-labeled Buserelin and 30 μg of membrane protein, in the presence or absence of 10^{-5} M GnRH. In some competition experiments, the amount of added GnRH was altered as indicated. To determine the specificity of binding, des-pGlu^1-GnRH (Bachem, Torrance, Calif.), D-Ala^6-GnRH (Peninsula Laboratories, Inc., San Carlos, Calif.), D-Phe^2-D-Ala^6-GnRH (Wyeth Laboratories, Philadelphia, Pa.) TRH (Peninsula Laboratories), arg^8-vasopressin (Calbiochem, San Diego, Calif.), or somatostatin (Beckman Instruments, Fullerton, Calif.) were added at the indicated concentrations. The final assay volume was 500 μl adjusted with 10 mM Tris-0.1% BSA. Following a 2-hr incubation on ice, bound and free hormone were separated by centrifugation (Eppendorf microfuge, 12,800 × g, 5 min) through 100 μl of 10% (w/v) sucrose. The tips of the tubes were cut off and radioactivity was determined by γ-spectroscopy. Most (80–85%) of the membrane-bound radioactivity was displaceable by excess competing ligand. Less than 1.5% of the total added radioactivity, or about 10% of the nondisplaceable counts, were found to bind to the tube tip itself (assayed in the absence of any membrane). For some kinetic studies, bound hormone and free hormone were separated by the method of rapid filtration as previously described (4). Incubations were performed in triplicate. Specific binding was determined by subtracting nonspecific binding (in the presence of excess unlabeled GnRH) from total binding (no GnRH). Scatchard analysis of the binding data, using linear regression analysis to establish best-line fit, was used to determine the number and binding affinity of GnRH receptors. The t-test was used to determine significance of differences. Membrane fractions prepared from groups of animals killed at different times were assayed in two or more binding studies with different batches of radiolabel, to distinguish results due to assay variability. Since results were similar, regardless of the

batch of radiolabel used and between the groups of animals, data in such cases were pooled and are expressed as means ± standard error of the mean.

Electrophoresis

Samples were applied to strips (1.5 cm wide) of Whatman 3 MM chromatography paper held horizontally between two reservoirs of 50 mM sodium phosphate buffer, pH 8.5. A 400-V potential was maintained for 60 min. The strips were then dried and cut into 1-cm lengths; radioactivity was determined by γ-spectroscopy.

RESULTS

The time course for ^{125}I-labeled Buserelin binding to the rat pituitary plasma membrane fraction (4°) is shown in Fig. 1. Equilibration was reached within 2 hr and appeared to be stable for at least 5 hr. To assess the stability of the labeled compound during the incubation period, aliquots of the incubation medium from a 2-hr incubation and authentic ^{125}I-labeled Buserelin were compared for mobility on paper electrophoresis as described under Methods. This system can resolve free iodide and ^{125}I-labeled Buserelin, and provides a good indication of the appearance of degradation products. The profiles of the two samples were indistinguishable (Fig. 2) and suggested that no measurable (<1%) degradation products were released during the incubation period.

The competitive binding characteristics of the radioligand receptor assay are shown in Fig. 3. Buserelin and another superagonist, D-Ala^6-GnRH, were approximately 40 times more potent than GnRH itself in inhibiting binding of ^{125}I-labeled Buserelin to the receptor. A GnRH antagonist, D-Phe^2-D-Ala^6-GnRH, and a biologically inactive analogue, des-pGlu^1-GnRH, were less potent competitors than GnRH in the binding assay. TRH showed no inhibition at a concentration of 10^{-5} M, while arg^8-vasopressin and somatostatin inhibited binding by 34% and 39%, respectively, at this concentration.

Scatchard analysis of ^{125}I-labeled Buserelin binding to pituitary membranes was consistent with only a single class of binding sites. Similarly, ovarian membrane preparations revealed a single class of binding sites (Fig. 4).

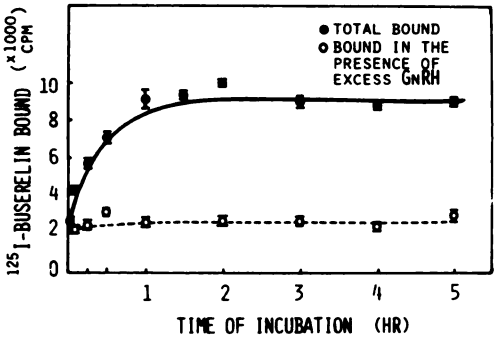

FIG. 1. *Time course for equilibration of binding of ^{125}I-labeled Buserelin with rat pituitary membranes*

Triplicate aliquots, containing 50 μg of membrane protein and 40,000 cpm of radiolabel were removed at times indicated and rapidly filtered as described under Methods. Nonspecific binding to membrane and filters was measured in the presence of 10^{-5} M GnRH.

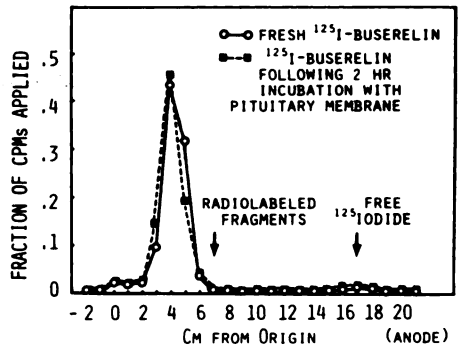

FIG. 2. *Paper electrophoretic profile of iodinated ligand and ligand after incubation with pituitary membrane*

Arrows indicate migration distances of other radioactive, but non-bindable, fractions from CM-cellulose column elution of iodination mixture. The labeled fraction, which demonstrates specific, high-affinity binding to pituitary membranes, migrates 3–5 cm from the origin, as shown, under the conditions of this system.

For both pituitary and ovarian membranes, the binding affinities were similar, although ovarian membranes had approximately one-tenth as many sites per milligram of protein. No specific binding was found in membrane preparations from heart, lung, kidney, cerebral cortex, liver, spleen, or adrenal.

Scatchard analyses were performed on data for ^{125}I-labeled Buserelin binding to membranes prepared from young rats killed at the indicated stage of the estrous cycle (Fig. 5). Although the affinity of the receptor for the radioligand remained unchanged throughout the estrous cycle (K_a = 1.6–2.7 × 10^{10} M^{-1}), the number of receptors per milligram of protein changed markedly. Figure 6 shows the concentration of GnRH pituitary receptors throughout the estrous cycle. Receptor concentration was lowest on the morning of estrus and gradually increased to a plateau on the afternoon of diestrus II, through proestrus. The most rapid change in receptor number during any 12-hr period (a 2.3-fold decrease), occurred between the afternoon of proestrus and the morning of estrus.

The contribution of estrogen to the regulation of GnRH receptor was evaluated in rats which were ovariectomized prior to the onset of ovarian cyclicity (Table

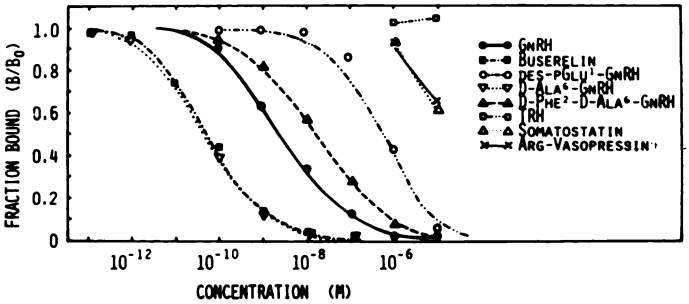

FIG. 3. *Hormone and GnRH analogue specificity of the radioligand-receptor assay in rat pituitary membranes*

Increasing concentrations of the competing peptides were added to 25 μg of rat membrane protein and ^{125}I-labeled Buserelin (40,000 cpm). Following a 2-hr equilibration on ice, samples were centrifuged and pellets were counted as described under Methods. Data are expressed as ratios of specifically bound counts to total displaceable counts. Each curve is determined by data pooled from several experiments.

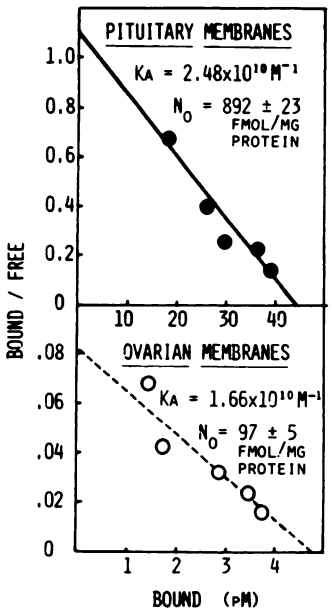

FIG. 4. *Scatchard analyses of ^{125}I-labeled Buserelin binding to ovarian and pituitary membrane fractions from young precycling female rats.*

The affinity constant (K_a) and number of binding sites per milligram of protein (N_0) were established by linear regression analysis.

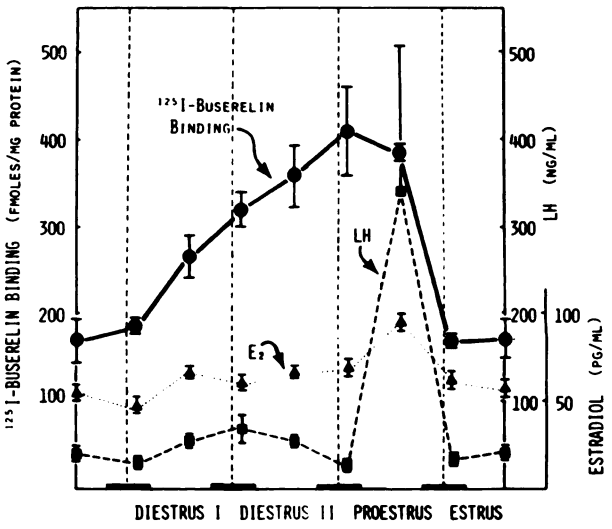

FIG. 6. *GnRH pituitary receptor binding and serum levels of estradiol and LH throughout the rat 4-day estrous cycle*

Dark bars across *bottom* indicate periods of darkness; *vertical dashed lines* separate days. Binding data were collected in three binding assays from two sets of cycling rats and were analyzed by Scatchard plot and linear regression, as in Fig. 5.

1). Twenty-four-day-old rats which had been ovariectomized at fourteen days of age had twice the number of pituitary GnRH receptors as compared with sham-operated rats of the same age. Receptor affinity did not vary significantly (K_a = 1.1–1.9 × 10^{10} M^{-1}). Within 3 hr of administration of 10 μg of estradiol benzoate (in oil, s.c.) receptor number returned to sham levels, and LH serum concentration dropped 86% from that of nontreated ovariectomized rats. Twelve hours after the initial estradiol injection, both receptor number and LH serum concentration were again above sham levels, although not as high as levels of nontreated ovariectomized rats. Receptor concentrations were lower in all of the 31-day-old animals as compared with 24-day-old rats, although the patterns of increased levels with ovariectomy and of partial reversal with estradiol administration were similar. In contrast, basal serum LH was higher in 31-day-old rats than 24-day-old animals, although the response to ovariectomy and 7 days of estradiol replacement was similar to that of the younger animals.

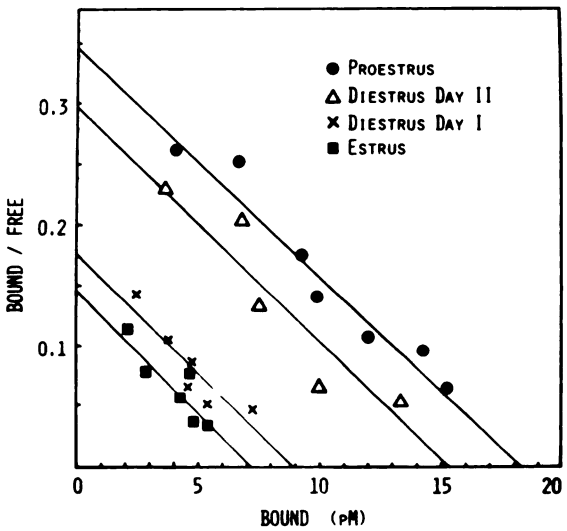

FIG. 5. *Scatchard plot of GnRH receptor binding in pituitaries collected between 2 a.m. and 4 a.m. from young estrous cycling rats*

Pituitary membrane (20–40 μg of protein) was incubated with 40–600 pM ^{125}I-labeled Buserelin as described under Methods. Data from pituitaries collected at 2 p.m. to 4 p.m. are not included for graphic clarity. All points lie betwen the estrous and proestrous values shown, and the slopes described by linear regression were not different from the ones shown. Data values were determined from triplicate samples of three binding studies from two separately collected groups of animals.

Pituitary receptor concentration and LH serum levels in mother rats killed at the time pups were weaned is shown in Table 1. Both the number of receptors and LH serum concentration were very low as compared with those of young, nonlactating rats.

Receptor number in pituitaries from PP and CE rats (20 to 24 months old) was compared with that of 30-day-old pups (Fig. 7). Since these old animals differ from weanlings both in age and in endocrine status, 6- and 12-month-old spontaneously CE rats were also examined. For all ages of CE rats, ^{125}I-labeled Buserelin binding was dramatically decreased compared with that of weanling females (Table 2). A similar decrease was observed for old PP rats. Receptor affinity was not different between the groups, while receptor concentration in the CE and old animals was 18–24% that in the young.

DISCUSSION

The radioiodinated GnRH analogue (Buserelin) used in the present study binds to a single class of high-affinity (1–2 × 10^{10} M^{-1}) sites in the anterior pituitary plasma membrane fraction. Clayton *et al.* (6) suggested that receptor assays using iodinated GnRH as a radioligand were complicated by rapid hormone proteolysis and li-

TABLE 1

Affinity and number of the GnRH receptors following ovariectomy (OVX) [and estradiol (E_2) replacement] and lactation

Data shown are means ± standard error of the mean determined from representative Scatchard plots from a group of pooled pituitaries (n = 4–15). Incubations were performed in triplicate, and K_a and number of sites were determined by linear regression analysis. The K_a values of the treated pups were not significantly different from 24-day sham-operated (Sham) animals, assessed by a two-tailed t-test. Individual LH serum values were determined.

Pups		K_a	No. of binding sites	Serum LH
Age	Treatment			
		$\times 10^{10}$ M^{-1}	*fmoles/mg protein*	*ng/ml*
24 days	Sham, day 14	1.1 ± 0.2	438 ± 15	100 ± 16
24 days	OVX, day 14	1.9 ± 0.3	888 ± 32[a]	964 ± 159[a]
24 days	OVX, day 14; killed 3 hr after E_2 injection	1.5 ± 0.3	430 ± 27[b]	139 ± 35[b]
24 days	OVX, day 14; killed 12 hr after E_2 injection	1.5 ± 0.1	696 ± 16[c]	551 ± 95[d]
31 days	Sham, day 14; oil s.c. days 24–31	1.7 ± 0.2	282 ± 12[a]	204 ± 30[e]
31 days	OVX, day 14; oil s.c. days 24–31	1.8 ± 0.2	442 ± 11[f]	1452 ± 213[f]
31 days	OVX, day 14; E_2 s.c. days 24–31	1.3 ± 0.2	408 ± 21[g]	938 ± 135[g]
Mothers at weaning		0.9 ± 0.2	159 ± 12[a,f]	120 ± 19[h]

[a] $p < 0.001$ versus 24-day sham-operated.
[b] Not significant versus 24-day sham-operated, $p < 0.001$ versus 24-day OVX.
[c] $p < 0.001$ versus 24-day OVX and 24-day OVX, 3-hr E_2.
[d] $p < 0.01$ versus 24-day OVX; $p < 0.05$ versus 24-day OVX, 3-hr E_2.
[e] $p < 0.01$ versus 24-day sham-operated.
[f] $p < 0.001$ versus 31-day sham-operated.
[g] Not significant versus 31-day OVX, $p < 0.001$ versus 31-day sham-operated.
[h] Not significant versus 24-day sham-operated, $p < 0.05$ versus 31-day sham-operated.

gand interaction with a low-affinity binding site. These authors suggested that degradation-resistant analogues of GnRH were superior choices as radioligands used for quantitation of the GnRH receptor.

We have used a sensitive electrophoretic system to confirm that ^{125}I-labeled Buserelin is not degraded under the assay conditions described for the present work. The radioiodinated derivative was displaced by GnRH agonists, and antagonists in general correlated with their reported potencies (7). TRH did not displace binding, while high concentrations of vasopressin and somatostatin partially inhibited ^{125}I-labeled Buserelin binding. Inhibition of radioligand binding to pituitary receptor by somatostatin has been reported previously (8), and a somatostatin analogue has been shown to have some biological properties similar to GnRH (9).

Of the nine tissues examined, only pituitary and ovary showed specific binding of ^{125}I-labeled Buserelin. While it is unlikely that hypothalamic GnRH could reach a

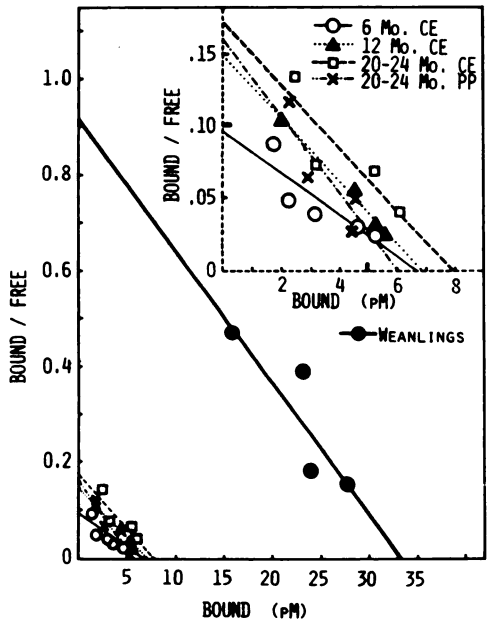

FIG. 7. *Scatchard analysis of GnRH receptor binding in young precycling and older CE and PP rats*

Inset is detail of *lower left*. Values were determined from two separate groups of animals, assayed in triplicate.

TABLE 2

Affinity and number of the GnRH receptor during spontaneously occurring endocrine states

Receptor data for each age group were obtained from Scatchard analysis of binding with membranes prepared from one group of pooled pituitaries (n = 6–12). K_a values were not significantly different from each other ($p < 0.05$ in two-tailed t-test). Numbers of binding sites are expressed as means ± standard error of the mean of the x-intercept. Receptor number is significantly lower ($p < 0.001$) for all groups versus weanlings. LH values are the means ± standard error of the mean of 6–12 samples. Serum LH titers that were 10-fold higher than the mean values, possibly due to stress-induced release, were not included in the calculated values.

Treatment	K_a	No. of binding sites	Serum LH
	$\times 10^{10}$ M^{-1}	*fmoles/mg protein*	*ng/ml*
Weanlings	2.8 ± 0.9	669 ± 28	66 ± 13
CE			
6 mo old	1.4 ± 0.3	134 ± 9	163 ± 42
12 mo old	2.3 ± 0.2	136 ± 2	151 ± 18
20–24 mo old	2.2 ± 0.8	158 ± 9	112 ± 24
Pseudopregnant, 20–24 months old	2.8 ± 1.0	118 ± 7	100 ± 27

significantly high level to stimulate the ovarian receptor, the possibility remains that another biologically cross-reactive molecule could utilize this receptor. Occupancy of the ovarian receptor has been correlated with suppression of stimulated granulosa cell steroidogenesis (10).

The concentration of pituitary GnRH receptors varied markedly between groups of animals of different ages and endocrine states, although no significant changes in receptor affinity were observed. While the concentration of serum LH correlated well with receptor levels in some cases (both increased following ovariectomy, both decreased during lactation), increases in measurable receptors were not uniformly associated with elevated LH levels. For example, receptor concentration was highest in weanling pups, when secretion of gonadotropins was relatively low. In mature cycling rats, receptor concentration fluctuated with the estrous cycle, although the concentration was increased by late on diestrus II, 1 day prior to the LH surge and prior to the period of increased pituitary responsiveness to exogenous GnRH administration (11–13). In contrast, receptor number fell in parallel with serum concentration of LH, between the afternoon of proestrus and the morning of estrus. The possibility that the decreased radioligand binding on estrus was due to endogenous GnRH present at the time of sacrifice is unlikely, since dissociation of GnRH from its receptor is very rapid (data in ref. 14) and appreciable amounts would not remain bound to the receptor during the period of membrane isolation and assay incubation. Similar patterns of receptor changes throughout the estrous cycle have been reported recently by other investigators (14, 15). One explanation for an apparent discrepancy in phase between increased receptor concentration and serum levels of LH is that the binding sites identified by the assay during diestrus and in prepubertal rats are secretion mechanisms. Thus, elevated GnRH receptor levels appear to be necessary, but not sufficient, for elevated LH release. Other factors, such as GnRH concentration in the portal circulation and postreceptor modulation by other hormones, also may regulate the secretory function of the gonadotropes.

Possible modulators for the levels of GnRH receptors are the gonadal steroids, since they appear to have direct actions on the pituitary (16, 17) and since LH release is well-correlated with elevated estradiol. However, estrogen effects *in vivo* are apparently bimodal (18), and the regulation may be markedly more complicated. Increased pituitary sensitivity to GnRH at proestrus has been shown (19). Park *et al.* (20) reported that ovariectomy of 5-day cycling rats in diestrus I blocked the normal increase in receptor number on proestrus and this inhibition could be overcome by estradiol. Precycling weanling rats were used in the present investigation of GnRH receptor regulation by estradiol, since these animals have not yet been exposed to high levels of estradiol nor to the influence of other cyclic hormonal changes characteristic of the mature female rat. The surges of GnRH and gonadotropins and the fluctuations of the steroids may contribute to the changes in GnRH receptor number during the estrous cycle. Although the effects of cyclic hormonal changes are avoided by the use of prepubertal rats, certain developmental changes can be seen. Receptor numbers are higher at age 24 days than at age 14 days (while pups are still suckling) and higher than at any older stage we have examined (data not shown). Our findings are consistent with values reported by Chan *et al.* (21) of a 5-fold higher number of GnRH receptors in 20-day-old females than in estrus-stage adults. The developmental decrease in receptor concentration, superimposed upon experimental manipulation, may explain the lower numbers in the 31-day-old rats as compared with 24-day-old animals (Table 1). Ovariectomy in the young rats resulted in a doubling of pituitary GnRH receptor number and was associated with a 9- to 10-fold increase in LH secretion. These changes were rapidly reversed upon administration of estradiol. These data indicate that the concentration of available GnRH receptors may be altered rapidly and that some of the actions of estradiol in this tissue may be mediated by regulation of GnRH receptor levels.

Receptor concentration and affinity were also determined in postpartum lactating animals whose gonadotropin output was particularly low. Lactation results in suppression of gonadotropin secretion, both basal and postcastration (22), and in decreased pituitary responsiveness to GnRH, in both single and sequential doses, as compared with values obtained from cycling rats (23). We found that both ^{125}I-labeled Buserelin binding to pituitary membranes and LH secretion in lactating rats was dramatically reduced as compared with those in nonlactating females. This suggests that the lack of gonadotrope responsiveness to GnRH, in lactating rats, is due to low levels of GnRH receptors.

Senescence of the female reproductive system is characterized by the loss of regular ovarian function and the onset of patterns of constant vaginal estrus, repetitive pseudopregnancies, or an anestrous state. Old CE and PP rats are less responsive to gonadal steroid sensitization of GnRH stimulation than are younger adults (24). Pituitary GnRH receptor concentration was much reduced in 20- to 24-month-old animals as compared with precycling weanlings. However, a similar decrease in binding was seen in pituitaries from young (6-month-old) and middle-aged (12-month-old) rats that demonstrated a spontaneous CE vaginal smear. These data suggest that changes in pituitary function seen in senescence may be determined, at least in part, by the state of ovarian function and are not solely a function of the age of the animal.

In the present study we determined the binding affinity and number of GnRH receptors during naturally occurring endocrine states. These studies demonstrate alterations in GnRH receptor number, but not in binding affinity, during normal physiological events, including vaginal cyclicity, lactation, and old age. In addition, ovariectomy and replacement with estradiol benzoate were shown to result in modulation of receptor levels. Because functional receptor levels can effectively alter target cell sensitivity (25) and because relatively elevated receptor levels appear necessary for elevated serum LH, the possibility remains that gonadotrope sensitivity is regulated in part by altered receptor levels.

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Note added in proof. Since acceptance of the manuscript, another report has been published (26) which indicates that castration of adult rats results in a 2-fold increase in GnRH binding capacity of the pituitary within 7 days. As shown in the present study, this effect is reversed by steroid administration. These authors have indicated that administration of rabbit antiserum, prepared against GnRH, concomitant with castration, inhibits the rise in both GnRH receptor number and LH increase. They have shown that changes in pituitary GnRH receptors parallel previously demonstrated changes in hypothalamic secretion of GnRH. The authors have suggested that GnRH probably regulates its own receptor *in vivo* and that gonadal steroids may influence pituitary GnRH receptors by changing hypothalamic GnRH secretion.

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