BIOLOGY OF REPRODUCTION 24, 889–901 (1981)

Relative Importance of the Adenohypophyseal and Gonadal Sites of Inhibitory Action of LHRH Agonists

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ABSTRACT

Assessment of the role of endogenous LH release and direct gonadal action of LHRH agonists in the loss of gonadal LH receptors induced by treatment with gonadotropin-releasing peptides was first made by comparing the effect of single administration of 1 μg of [D-Ala^6, des-Gly-NH$_2$^{10}]-LHRH ethylamide, [D-Ala^6]LHRH-EA, 12 h before or immediately after hypophysectomy (HYPOX) on LH receptor levels measured 48 h after surgery in male and female adult rats. While administration of the LHRH agonist before HYPOX leads to a 50% loss of testicular LH receptors, only a 20% decrease is seen in HYPOX animals, thus suggesting a predominant role of endogenous LH release in the desensitization process in male animals. Treatment with increasing doses (1 to 100 ng/day) of another LHRH agonist, [D-Ser(TBU)^6]LHRH-EA, causes a 90% loss of testicular LH receptors in intact rats at the highest dose used (100 ng daily for 9 days) while the same treatment decreases LH receptors by only 35% in HYPOX animals. As measured by the steroidogenic response to 10 μg oLH in vivo, while treatment of intact animals with [D-Ser(TBU)^6]LHRH-EA leads to a marked blockage of the testicular steroidogenic pathway at the level of 17-hydroxylase and 17,20-desmolase activities with a corresponding increase of 5α-reductase activity, minimal or no signs of changes of enzymatic activity are seen after identical treatment in HYPOX animals. Chronic treatment (1 month) with the LHRH agonist in intact rats leads to degenerative changes of the seminiferous tubules, while no effect is observed in HYPOX animals, thus suggesting the essential role of the pituitary gland. Moreover, adrenalectomy does not influence the inhibitory effect of treatment with LHRH agonists on the loss of testicular LH receptors in HYPOX animals, thus eliminating the role of the adrenals in the action of LHRH agonists. Contrary to the results obtained in male animals, single administration of the LHRH agonist in female rats has similar inhibitory effects on ovarian gonadotropin receptors when administered either before or after hypophysectomy. Moreover, treatment with increasing doses of [D-Ser(TBU)^6]LHRH-EA leads to a similar inhibition of ovarian LH receptor levels in intact and HYPOX female animals. The present data show that LHRH agonist-induced endogenous LH release plays a predominant role in the desensitizing effect of treatment with LHRH agonists on testicular LH receptors and steroidogenesis as well as on inhibition of spermatogenesis; in the female, the direct gonadal effect, at least on LH and FSH receptors, appears to play a more important role than in male animals.

INTRODUCTION

Since the antifertility effects of LHRH agonists are parallel to their LH-releasing activity (Cusan et al., 1979), it has been suggested that endogenous LH release leading to a secondary loss of gonadal receptors and blockage of steroidogenesis is responsible for the antifertility effects observed after such treatment (Auclair et al., 1977a,b; Labrie et al., 1978; Kledzik, 1978a,b). However, the observation by Rippel and Johnson (1976) that administration of a potent LHRH agonist to hypophysectomized (HYPOX) immature rats prevents the hCG-induced increase in ovarian weight already suggested a direct ovarian site of action of the peptide. A direct in vivo action of LHRH agonists has also been observed by other groups at both the ovarian (Mayer et al., 1979; MacDonald and Beattie, 1979; Hsueh and Erickson, 1979a) and testicular (Arimura et al., 1979; Hsueh and Erickson, 1979b) levels. Moreover, LHRH agonists have been shown to

Accepted January 6, 1981.
Received May 12, 1980.
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inhibit steroidogenesis in granulosa and luteal cells in culture (Clayton et al., 1979; Hsueh and Erickson, 1979a; Massicotte et al., 1980, 1981), thus providing strong support for the direct inhibitory action of these peptides on ovarian function in the rat.

Valid assessment of the relative importance of the pituitary and gonadal sites of the antifertility action of LHRH agonists can be achieved only under in vivo conditions. Although LHRH and some of its agonistic analogues have been shown to have direct gonadal effects in HYPOX animals, pharmacological doses of the LH-releasing peptides have been used and no dose-response study has been performed. The present study compares the effects of increasing doses of the LHRH agonist [D-Ser(TBU)^6, des-Gly-NH_2 ^{10}] LHRH ethylamide on testicular and ovarian gonadotropin receptors and steroidogenesis in intact and HYPOX male and female rats.

MATERIALS AND METHODS

Animals

Intact or HYPOX male or female Sprague-Dawley rats were obtained from Canadian Breeding Farms, St. Constant, Québec, housed two per cage at 20–22°C on a 14L:10D schedule (lights on at 0500 h), and given food and water ad libitum. HYPOX animals were given drinking water containing 5% glucose and 0.9% NaCl. For studies using females in diestrus I, only animals showing three consecutive and regular 4-day cycles were used. All animals were killed by decapitation within 20 sec of removal from their cage.

Treatments

Ten animals were used in each group. In the first series of experiments, adult male and 4-day cycling rats in diestrus I were injected once with 1 μg of [D-Ala^6, des-Gly-NH_2 ^{10}] LHRH ethylamide, [D-Ala^6] LHRH-EA (provided by Drs. M. Götz and R. Deghenghi, Ayerst Research Labs., Montréal) or the vehicle alone (0.2 ml 1% gelatin–0.9% NaCl) 12 h before or immediately after parapharyngeal hypophysectomy and sacrificed by decapitation 2 days after surgery. In the second experiment, HYPOX adult male rats were injected every 2nd day for 1 month with 100 ng of [D-Ser(TBU)^6] LHRH-EA or the vehicle alone for histological examination of the testes. The potency of the two LHRH agonists used has been described (Labrie et al., 1978; Cusan et al., 1979; Sandow et al., 1978b). To assess a possible mediatory role of the adrenals in the action of LHRH agonists, groups of adult HYPOX animals were adrenalectomized (ADRX) under ether anesthesia by the lumbar approach. HYPOX and HYPOX-ADRX animals were then injected daily with 100 ng of [D-Ser(TBU)^6] LHRH-EA or the vehicle alone before sacrifice for measurement of testicular LH and PRL receptors.

In the last series of experiments, intact or HYPOX (3 days earlier) male (200–225 g) or female (120–130 g) rats were used. The female animals were injected s.c. with 25 IU of pregnant mare serum gonadotropin, PMSG, (Equinex, Ayerst Research Labs., Montréal). This first injection was followed 72 h later by the administration of 5 IU of hCG (provided by Dr. J. P. Raynaud, Centre de Recherches Roussel-UCLAF, Romainville). Four days after hCG administration, female animals were injected daily with increasing doses (1, 3, 10, 30, or 100 ng) of [D-Ser-(TBU)^6]-LHRH-EA (provided by J. Sandow, Hoechst AG, Frankfurt) or with the vehicle alone (1% gelatin–0.9% NaCl) for 9 days. Identical treatments with the LHRH agonist were performed in intact and HYPOX male rats. The animals were sacrificed by decapitation 24 h after the last injection. In studies performed with intact or HYPOX male rats, animals were sacrificed under basal conditions or 2 h after s.c. injection of 10 μg of oLH (S1397A) (provided by Dr. M. R. Sairam, Institut de Recherches Cliniques, Montréal), while all female rats were killed under basal conditions. Organs and blood were then collected for RIA of LH, FSH, PRL, and steroids, radioreceptor assays, and organ weight measurements. The completeness of hypophysectomy was assessed by examination of the sella turcicae and by measurement of plasma LH levels.

LH/hCG, FSH and PRL Receptor Assays

Immediately after decapitation of the rats, the testes and ovaries were weighed and kept at −20°C until assayed for gonadotropin receptor levels as described (Auclair et al., 1977a,b; Kledzik et al., 1978a,b), except that [^{125}I]-hGH was used instead of [^{125}I]-oPRL as tracer for measurements of testicular prolactin receptors. Specificity of [^{125}I]-hGH binding to the testicular homogenate is shown in Fig. 1. For measurement of the specificity of [^{125}I]-hGH, hCG (CR119, 11600 IU/mg) was supplied by the Center for Population Research of the NICHHD, while ovine LH (NIH-L-5-S19, 1.01 X NIH-LH, SD), oFSH (NIH-FSH-S12, 1.25 X NIH-FSH-S1), hFSH (LER-1801-3, 4019 IU/mg), oPRL, (35 IU/mg), hGH (NIH-hGH-I-4), bGH (NIH-GH-B-18), hGH (NIH-GH-H2160E), and oTSH (NIH-TSH-S8) were gifts of the National Pituitary Agency, NIH.

Steroid Assays

Testicular and plasma steroids were extracted with ether and separated on LH-20 columns. Chromatography and radioimmunoassays were performed as described (Bélanger et al., 1980b).

Pituitary and Plasma LH, FSH, and PRL Assays

After decapitation of the rats, anterior pituitaries were immediately homogenized in a glass-Teflon homogenizer in 5 ml of PBS (50 mM sodium phosphate, 0.1 M NaCl, pH 7.4) buffer before centrifugation at 2300 X g for 10 min. The supernatant was kept at −20°C for RIA determination. LH, FSH, and PRL were measured in adenohypophyseal homogenate and plasma by double-antibody radioimmunoassays using rat hormones and rabbit antisera supplied by Dr. A. F. Parlow for the NIAMDD Rat Pituitary Hormone Program as described (Drouin and Labrie, 1976).

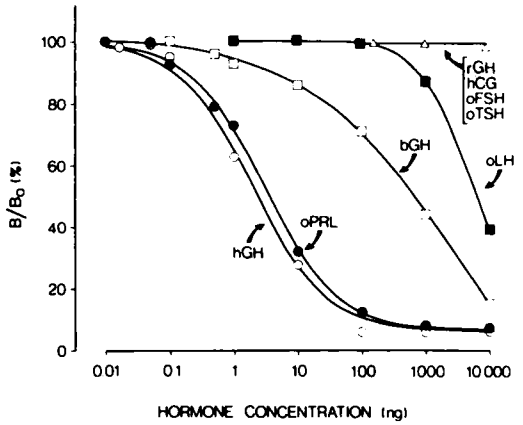

FIG. 1. Specificity of [^{125}I]-hGH binding to the testicular homogenate. Homogenate (20 mg) was incubated in the presence of a saturating amount of [^{125}I]-hGH (i.e., 200,000 dpm; sp act 55 μCi/μg) and the indicated unlabeled hormone for 12–16 h at 23°C in a final incubation volume of 0.6 ml.

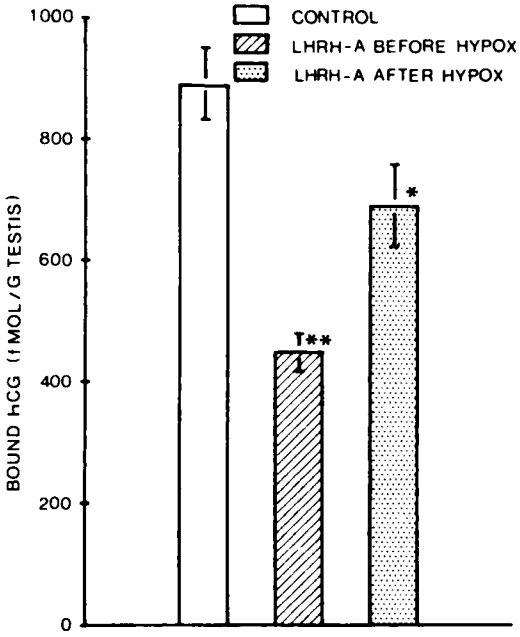

FIG. 2. Comparative effect of single injection of 1 μg of [D-Ala^6] LHRH-EA 12 h before or immediately after HYPOX on testicular LH receptor levels in adult rats. The animals were sacrificed 2 days after surgery. Control animals are HYPOX.

Morphological Studies

Testes from three or four rats from control and [D-Ser(TBU)^6] LHRH-EA-treated groups were fixed by perfusion with Bouin's fluid. Testes were then postfixed by immersion in the same fixative for 2 days before embedding in paraffin. Cross sections were cut at 5 μm and stained with PAS-hematoxylin. For each testis, 10 sections obtained at different levels were examined as described (Pelletier et al., 1978).

Calculations

Radioimmunoassay data were analyzed using a program based on model II of Rodbard and Lewald (1970). Statistical significance was assessed according to the multiple range test of Duncan-Kramer (Kramer, 1956). All radioimmunoassay and radioreceptor assay data are presented as means ± SEM of duplicate determinations.

RESULTS

Studies in Male Rats

As illustrated in Fig. 2, the administration of 1 μg of [D-Ala^6] LHRH-EA, 12 h before HYPOX, causes a 50% ($P<0.01$) decrease of testicular LH receptor levels measured 48 h after surgery. When administered immediately after HYPOX, the analogue produces only a 20% ($P<0.05$) loss of LH binding sites.

Since the previous data show that a relatively high dose of the LHRH agonist, [D-Ala^6]-LHRH-EA, leads to a much greater loss of testicular LH receptors in intact than HYPOX animals, we next investigated in more detail the effect of increasing doses of an LHRH agonist of comparable potency, [D-Ser(TBU)^6]-LHRH-EA, on various parameters of gonadal function in intact and HYPOX rats.

In agreement with our previous data obtained with [D-Leu^6] LHRH-EA (Auclair et al., 1977b) and [D-Ala^6] LHRH-EA (Labrie et al., 1978), a maximal inhibition of testis ($P<0.05$) and seminal vesicle ($P<0.01$) weight was obtained in intact animals at the daily dose of 30 ng of [D-Ser(TBU)^6] LHRH-EA. Treatment with the LHRH agonist had no effect on the already low testis and seminal vesicle weights observed after HYPOX (data not shown).

As illustrated in Fig. 3A, treatment of intact rats with the daily doses of 1 or 3 ng of [D-Ser-(TBU)^6] LHRH-EA leads to a 35–50% stimulation of testicular LH receptor levels, while the 10, 30, and 100 ng doses decrease LH receptors by 20%, 65%, and 90%, respectively. By contrast, in HYPOX animals (Fig. 3B), the 1 and 3 ng doses have no effect while the 10, 30, and 100 ng doses reduce LH receptor levels by only 20%, 40% and 35%, respectively. Since treatment with the LHRH agonist leads to decreased testis weight in intact animals, it is important to note that similar effects are obtained when LH receptors are expressed in fmoles per testis

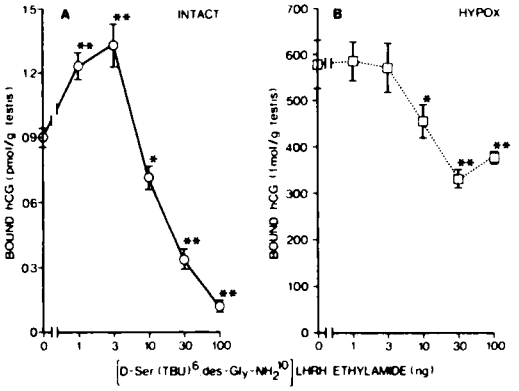

FIG. 3. Effect of daily treatment for 9 days of (A) intact or (B) HYPOX (3 days earlier) rats with increasing doses of [D-Ser(TBU)^6] LHRH-EA on testicular LH/hCG receptor levels. The animals were sacrificed 24 h after the last injection of the LHRH agonist or vehicle alone (1% gelatin–0.9% NaCl).

(Table 1) or per gram testis (Fig. 3). It can also be seen in Table 1 that treatment with the LHRH agonist has no effect on testicular FSH receptors while only the highest dose (100 ng) has an inhibitory effect on prolactin receptors in intact rats, and no effect is seen in the absence of the adenohypophysis.

Since chronic treatment with LHRH agonists exerts inhibitory effects at the pituitary level (Sandow et al., 1978a; Labrie et al., 1978; Cusan et al., 1979), plasma and pituitary LH, FSH, and PRL levels were measured in the same groups (Table 2). While treatment with the two highest doses of [D-Ser-(TBU)^6] LHRH-EA leads to increased basal plasma FSH levels by about 50% ($P<0.01$) in intact male animals, a similar elevation of basal plasma LH levels is seen at the 100 ng dose of the LHRH agonist. Although long-term treatment with LHRH agonists is known to lead to changes in pituitary gonadotropin levels (Cusan et al., 1981), no significant change is observed after 10 days in male animals (Table 2). There is much evidence for a role of prolactin in testicular steroidogenesis in the rat (Hafiez et al., 1972; Bélanger et al., 1979). It is thus interesting to see that treatment with doses of [D-Ser-(TBU)^6] LHRH-EA from 3 to 100 ng leads to a progressive and marked decrease of basal plasma prolactin levels in the male rat (Table 2).

Since treatment of adult male rats with LHRH agonists leads to an important blockage of the testicular steroidogenic pathway at the level of 17-hydroxylase and 17,20-desmolase activities (Auclair et al., 1977a,b; Bélanger et al., 1979, 1980a), we next investigated a possible effect of similar treatment on the testicular steroidogenic pathway in the absence of the pituitary gland.

As illustrated in Fig. 4, treatment of intact rats with increasing doses of [D-Ser(TBU)^6]-LHRH-EA leads to a biphasic effect on basal testicular levels of pregnenolone, progesterone, 17-OH-pregnenolone, and 17-OH-progesterone: an inhibitory effect at the 10 and 30 ng doses is followed by a tendency to normal or even high (17-OH-pregnenolone) values at the 100 ng dose. Basal levels of androst-5-ene-3β,17β-diol, testosterone, and androstane-3β,17β-diol are markedly reduced after 9 days of treatment with doses of the LHRH agonist higher than 10 ng while the concentration of androstane-3α, 17β-diol is increased.

TABLE 1. Effect of daily treatment for 9 days of intact or HYPOX adult male rats with increasing doses of [D-Ser(TBU)^6] LHRH-EA on testicular LH/hCG, FSH, and PRL receptor levels. Data are expressed as fmoles/testis (means ± SEM).

Dose of [D-Ser(TBU)^6] LHRH-EA (ng)	Receptor levels (fmoles/testis)					
	LH	FSH	PRL	LH	FSH	PRL
	Intact			HYPOX		
Control	1348 ± 65	211 ± 11	232 ± 20	406 ± 34	165 ± 9	40 ± 3
1	1904 ± 104**	180 ± 10	206 ± 14	404 ± 20	152 ± 5	45 ± 6
3	2025 ± 143**	195 ± 4	222 ± 12	408 ± 28	140 ± 7	39 ± 3
10	1104 ± 110	222 ± 13	232 ± 15	302 ± 26**	134 ± 8	32 ± 2
30	423 ± 44**	207 ± 13	201 ± 12	217 ± 10**	158 ± 6	38 ± 4
100	147 ± 8**	205 ± 15	162 ± 21**	249 ± 10**	163 ± 7	40 ± 4

**$P<0.01$; experimental vs control.

TABLE 2. Effect of daily treatment for 9 days of intact adult male and immature PMSG-hCG-treated female rats with increasing doses of [D-Ser(TBU)^6] LHRH-EA on plasma and pituitary LH, FSH, and PRL levels.

Dose of [D-Ser(TBU)^6] LHRH-EA (ng)	Plasma hormone levels (ng/ml)						Pituitary hormone levels (μg/pituitary)					
	LH	FSH	PRL	LH		PRL	LH	FSH	PRL	LH	FSH	PRL
	Male			Female			Male			Female		
0	29 ± 3.6	608 ± 44	18.8 ± 2.9	25 ± 4.8		14 ± 3	308 ± 19	173 ± 15	6.25 ± 0.29	171 ± 20	113 ± 9	304 ± 27
1	29 ± 2.9	621 ± 35	16.4 ± 1.8	21 ± 2.6		11 ± 2.1	338 ± 13	207 ± 9	6.92 ± 0.49	161 ± 18	109 ± 9	206 ± 8.3**
3	23 ± 1.8	626 ± 17	10.2 ± 2**	23 ± 2.4		18 ± 4.6	328 ± 15	209 ± 15	6.38 ± 0.54	180 ± 11	101 ± 7	241 ± 14*
10	25 ± 1.6	650 ± 44	8.0 ± 1.5**	26 ± 4.5		12 ± 2.2	273 ± 12	215 ± 13	7.01 ± 0.52	211 ± 15	110 ± 8	259 ± 14*
30	34 ± 4.0	919 ± 90**	5.3 ± 0.2**	47 ± 8.9**		22 ± 3.8	280 ± 20	222 ± 13	6.22 ± 0.37	164 ± 12	106 ± 13	240 ± 17*
100	46 ± 8.1*	991 ± 99**	4.8 ± 0.7**	54 ± 6.2**		20 ± 3.3	243 ± 16	186 ± 13	6.20 ± 0.42	103 ± 10**	68 ± 2*	183 ± 12**

*P<0.05; experimental vs control.

**P<0.01; experimental vs control.

When the steroidogenic response is examined 2 h after s.c. administration of 10 μg of oLH, there is a marked decrease of the testicular levels of 17-OH-progesterone, androst-5-ene-3β,17β-diol, and testosterone after treatment with doses of the LHRH agonist higher than 10 ng (Fig. 5). While treatment with the 30 and 100 ng doses of the LHRH agonist causes a marked decline in the testicular 17-OH-progesterone levels, the concentration of 17-OH-pregnenolone is increased. As indicated by the levels of pregnenolone and progesterone, no signs of blockage at early steps in the steroidogenic pathway are observed after treatment with the LHRH agonist. It is of interest that while low doses (3 and 10 ng) of [D-Ser(TBU)^6] LHRH-EA stimulate the response of testosterone and androst-5-ene-3β, 17β-diol, higher doses have a marked inhibitory effect. While basal levels of 5α-dihydrotestosterone are unchanged after treatment with the LHRH agonist, it can be seen that the response to oLH is slightly increased at the 10 and 30 ng doses. Both basal and oLH-induced androstane-3α,17β-diol levels are increased after treatment with 10 to 100 ng of [D-Ser(TBU)^6] LHRH-EA, thus suggesting increased 5α-reductase activity.

A very different pattern of basal as well as oLH-stimulated testicular steroid levels is found in HYPOX animals. While testicular pregnenolone and 17-OH-pregnenolone levels are not detectable in these animals, treatment with any dose (1 to 100 ng) of the LHRH agonist leads to a marked inhibition of basal testicular levels of all steroids (Fig. 6). Moreover, in contrast with the marked changes of the steroidogenic response to oLH observed in intact animals treated with the LHRH agonist, little or no effect of the same treatment can be seen in HYPOX animals (Fig. 7). In fact, only a slight (∼25%, $P<0.05$) inhibition of the testosterone response is seen after treatment with the two highest doses of the LHRH agonist, while the 17-OH-progesterone response is increased ($P<0.01$) and no significant change is seen on the response of the seven other steroids measured.

We have observed that chronic treatment with LHRH agonists can lead to a marked inhibition of spermatogenesis in the rat (Pelletier et al., 1978), significant degenerative changes of the seminiferous tubules being observed after 2 weeks of treatment. To determine if the LHRH agonist can produce these effects by a direct action at the testicular level,

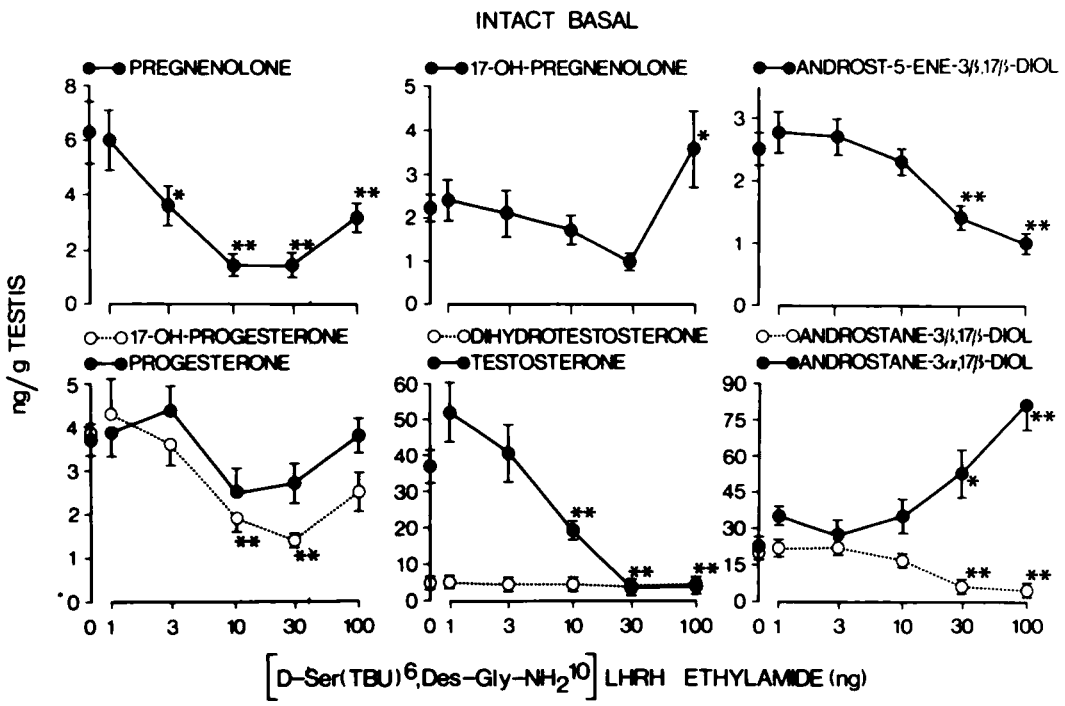

FIG. 4. Effect of daily treatment for 9 days of intact adult rats with increasing doses of [D-Ser(TBU)^6] LHRH-EA on basal testicular levels of pregnenolone, 17-OH-pregnenolone, androst-5-ene-3β, 17β-diol, progesterone, 17-OH-progesterone, testosterone, 5α-dihydrotestosterone, androstane-3α, 17β-diol, and androstane-3β, 17β-diol. The animals were sacrificed 24 h after the last injection of the LHRH agonist.

HYPOX rats were injected every 2nd day with 100 ng of [D-Ser(TBU)^6] LHRH-EA for 1 month before histological examination. As illustrated in Fig. 8, treatment of HYPOX rats with the LHRH agonist has no effect on the histology of the testis.

Finally, since the presence of adrenal LHRH receptors has been reported (Bernardo et al., 1978), we have investigated the possibility that some of the effects of LHRH agonists could be mediated by the adrenals. To investigate this possibility, the LHRH agonist was administered for 9 days to HYPOX rats and HYPOX animals ADRX 3 days after HYPOX. As illustrated in Fig. 9, administration of 100 ng of [D-Ser-(TBU)^6] LHRH-EA for 9 days leads to the same inhibitory effects on LH and PRL receptors in both groups of animals.

Studies in Female Rats

The comparative effect of a single injection of 1 μg of [D-Ala^6] LHRH-EA 12 h before or immediately after HYPOX on the level of ovarian LH and FSH receptors is illustrated in Fig. 10. When injected before HYPOX, [D-Ala^6] LHRH-EA inhibits LH receptor levels by 65% while a 40% decrease is seen when the peptide is administered immediately after HYPOX. In the same groups, FSH receptors are decreased by 45% and 35%, respectively.

To follow up the most useful information obtained by comparing the effect of increasing doses of [D-Ser(TBU)^6] LHRH-EA in intact and HYPOX male rats (Fig. 3), a similar experiment was performed in female animals. As illustrated in Fig. 11, the injection of 1, 3, or 10 ng of [D-Ser(TBU)^6] LHRH-EA in intact rats has no significant effect on ovarian LH receptor levels while treatment with the 30 and 100 ng doses decreases LH receptor levels by 35% and 95% ($P<0.01$), respectively. In HYPOX animals, a significant loss of ovarian LH receptors (30%, $P<0.05$) is seen in animals treated with as little as 3 ng of the LHRH agonist. The daily 10, 30, 100 ng doses of the peptide decrease ovarian LH binding sites by 40%, 82%, and 94%, respectively.

When the effect on ovarian FSH receptors is examined, a 25% decrease ($P<0.05$) is observed

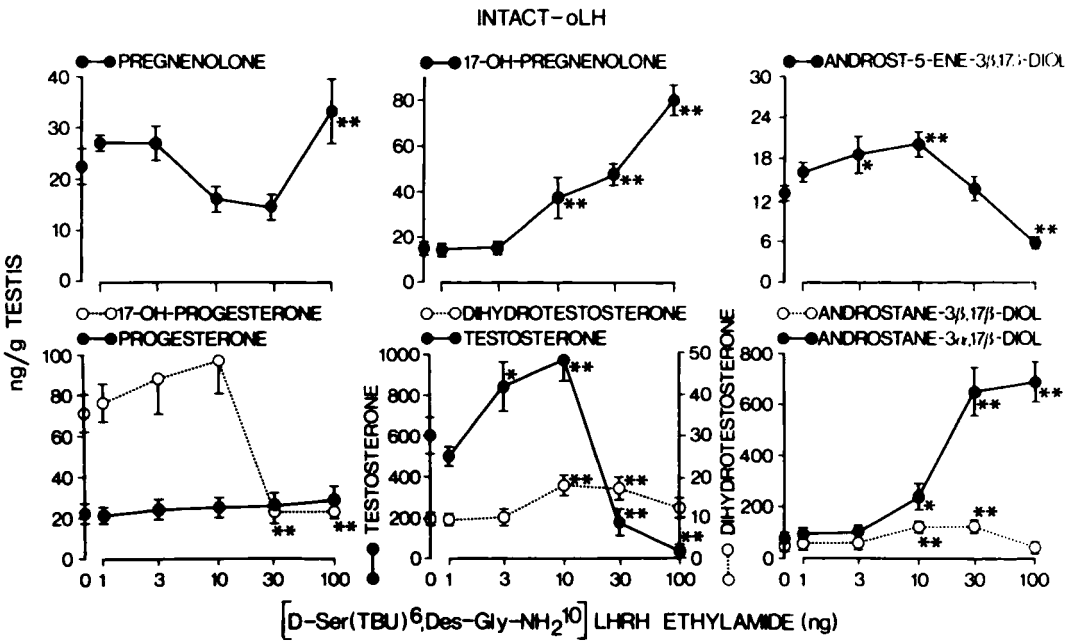

FIG. 5. Effect of daily treatment for 9 days of intact adult rats with increasing doses of [D-Ser(TBU)^6]-LHRH-EA ethylamide on oLH-stimulated testicular levels of pregnenolone, 17-OH-pregnenolone, androst-5-ene-3β,17β-diol, progesterone, 17-OH-progesterone, testosterone, 5α-dihydrotestosterone, androstane-3α,17β-diol, and androstane-3β,17β-diol. The animals were sacrificed 24 h after the last injection of the LHRH agonist and 2 h after administration of 10 μg of oLH.

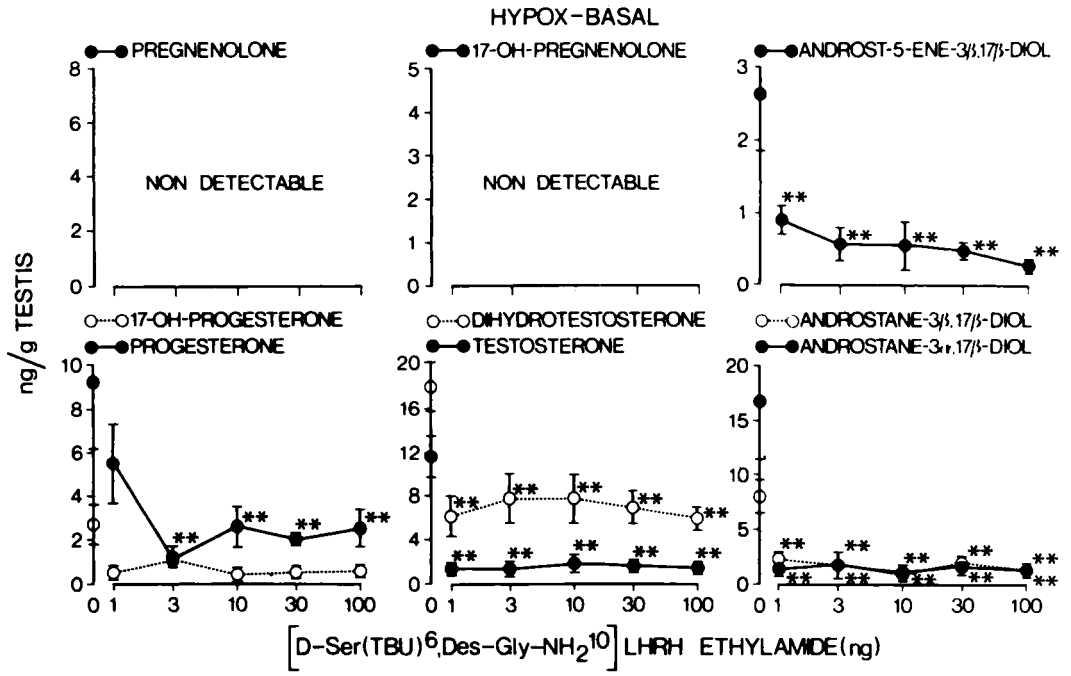

FIG. 6. Effect of daily treatment for 9 days of HYPOX (3 days earlier) adult rats with increasing doses of [D-Ser-(TBU)^6] LHRH-EA on basal testicular levels of pregnenolone, 17-OH-pregnenolone, androst-5-ene-3β, 17β-diol, progesterone, 17-OH-progesterone, testosterone, 5α-dihydrotestosterone, androstane-3α,17β-diol, and androstane-3β,17β-diol. The animals were sacrificed 24 h after the last injection of the LHRH agonist.

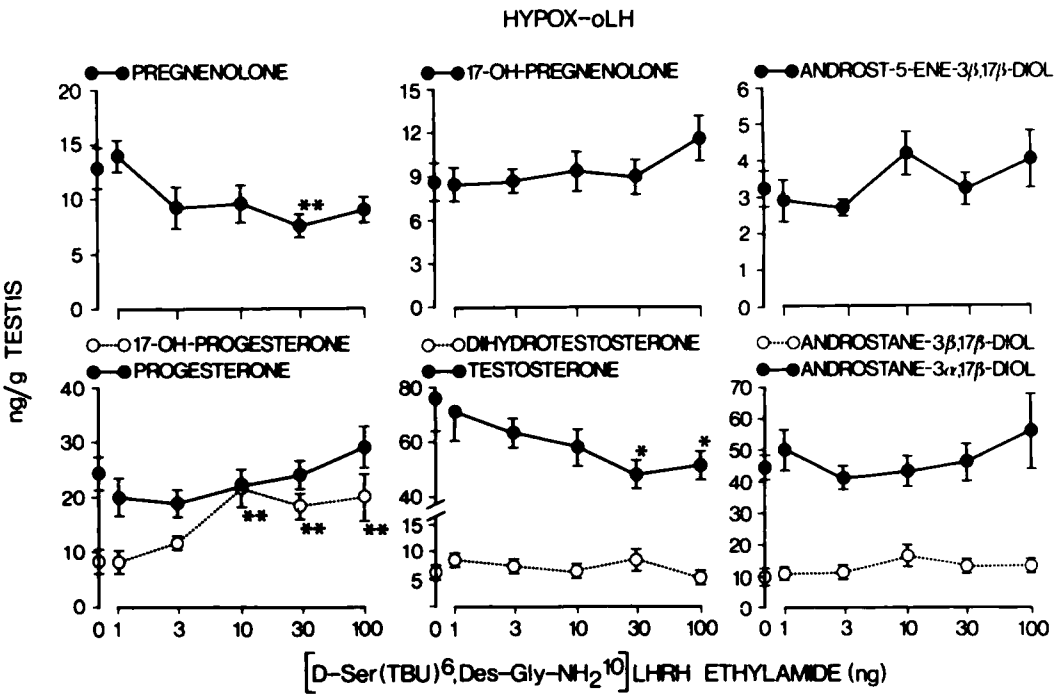

FIG. 7. Effect of daily treatment for 9 days of HYPOX (3 days earlier) adult rats with increasing doses of [D-Ser-(TBU)^6] LHRH-EA on oLH-stimulated testicular levels of pregnenolone, 17-OH-pregnenolone, androst-5-ene-3β,17β-diol, progesterone, 17-OH-progesterone, testosterone, 5α-dihydrotestosterone, androstane-3α,17β-diol and androstane-3β,17β-diol. The animals were sacrificed 24 h after the last injection of the LHRH agonist and 2 h after the administration of 10 μg of oLH.

in intact rats after daily treatment with doses of 3 to 30 ng while the 100 ng dose of the LHRH agonist inhibits by 64% ($P<0.01$). In HYPOX animals, the 1 to 10 ng doses have no effect while a 45–50% inhibition is seen with the 30 and 100 ng doses ($P<0.01$) (data not shown). Since treatment with the LHRH agonist had no significant effect on ovarian weight, similar effects on LH and FSH receptors are seen when the data are expressed per ovary (Table 3) instead of per 100 mg ovary. It can also be seen in Table 3 that ovarian prolactin receptor levels were not affected by treatment with the LHRH agonist. Treatment with doses of the LHRH agonist ranging from 3 to 30 ng leads to 25–30% decrease ($P<0.05$) of uterine weight in intact animals while the 100 ng dose inhibits uterine weight by 40% ($P<0.01$). In HYPOX animals, 33% ($P<0.05$), 37%, and 62% ($P<0.01$) of inhibition of uterine weight is observed with the 10, 30, and 100 ng doses, respectively (data not shown).

As observed with male animals, treatment of female rats with [D-Ser(TBU)^6] LHRH-EA for 9 days leads to an ~50% increase of basal plasma LH levels at the 30 and 100 ng daily doses (Table 2). This increase of basal LH levels is accompanied, at the 100 ng dose, by reduced pituitary LH and FSH content. Although the wide fluctuations of basal plasma prolactin levels in female rats can mask inhibitory effects, treatment with all doses of the LHRH agonist inhibits pituitary PRL content.

DISCUSSION

The present data show that single or repeated administration of low doses of LHRH agonists can lead to a loss of testicular and ovarian LH receptors in the absence of the pituitary gland. However, after single administration in adult animals, the LH receptor loss observed in HYPOX rats is reduced compared with the effect seen in intact animals. The decreased potency of LHRH agonists on testicular LH receptor levels in the absence of the pituitary gland is well illustrated by the finding that higher doses of the LHRH agonist

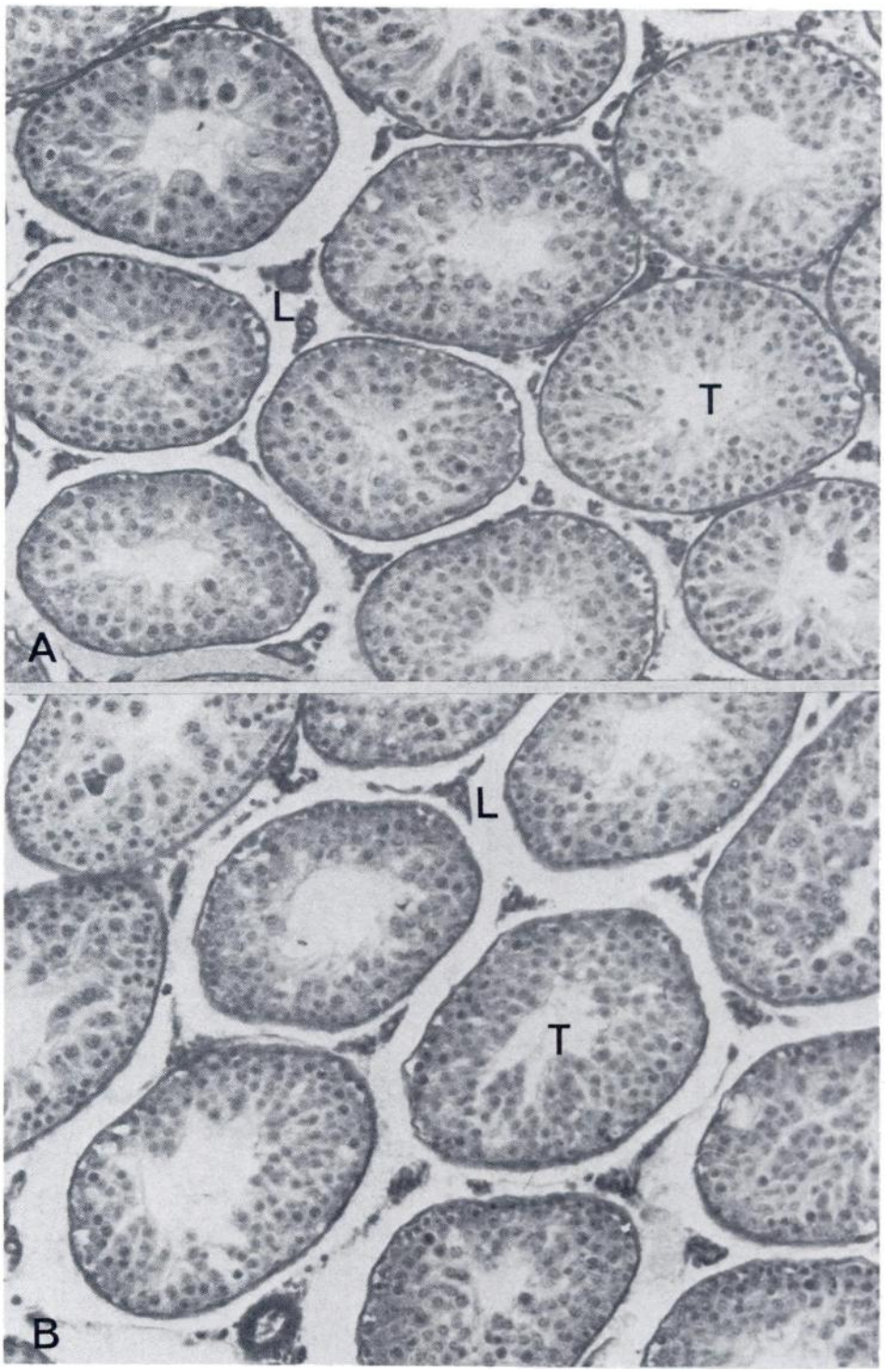

FIG. 8. Section through (A) a control HYPOX rat testis and (B) the testis of a HYPOX rat treated with [D-Ser(TBU)^6] LHRH-EA (100 ng/every 2nd day) for 4 weeks. No difference can be observed between the two groups. T, seminiferous tubules; L, Leydig cells. X160.

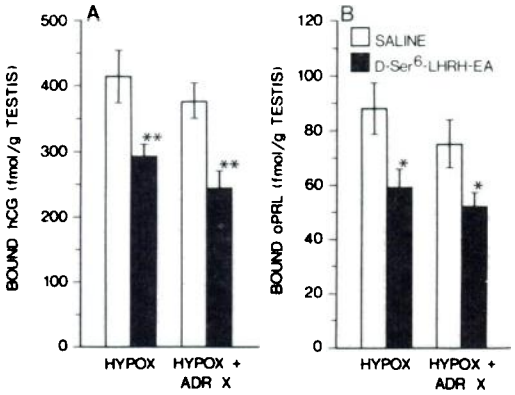

FIG. 9. Effect of daily treatment for 9 days of HYPOX or HYPOX (3 days earlier) and ADRX adult rats with 100 ng of [D-Ser(TBU)^6] LHRH-EA on (A) testicular LH/hCG or (B) PRL receptor levels. The animals were sacrificed 24 h after the last injection of the peptide or vehicle alone (1% gelatin–0.9% NaCl).

are required after HYPOX and that the maximal loss of LH receptors is markedly reduced in the absence of the pituitary gland. However, in PMSG-hCG-treated immature rats, the potency of increasing doses of the LHRH agonist is similar in intact and HYPOX animals, thus suggesting a greater impact of the direct inhibitory action of LHRH agonists on luteal than on Leydig cells. The present data extend previous observations obtained with high doses of LHRH agonists in HYPOX animals (Hsueh and Erickson, 1979a,b; Arimura et al., 1979) and provide detailed information on the accompanying changes of steroidogenesis in male rats.

The present findings of an inhibitory effect of treatment with daily doses of 10 to 100 ng of [D-Ser(TBU)^6] LHRH-EA on uterine weight in HYPOX rats is at variance with the observations of Corbin and Beattie (1975) and Jones (1979) who reported a stimulatory and no effect, respectively. The difference may be due to different ages of the animals or different time intervals between HYPOX and start of treatment. In the present study, the first injection was given 3 days after surgery while in the the two other studies, there were delays of 5–6 weeks (Jones, 1979) and 4 months (Corbin and Beattie, 1975). The inhibition of uterine weight by treatment with the LHRH agonist is likely due to the direct inhibitory effect of the peptide on ovarian steroidogenesis (Hsueh and Erickson, 1979a; Clayton et al., 1979; Massicotte et al., 1980, 1981).

Since there is increasing evidence for a role of prolactin in the control of testicular steroidogenesis (Hafiez et al., 1972; Bélanger et al., 1979), the marked decrease of basal plasma prolactin levels in intact male rats observed after treatment with daily doses of 3 to 100 ng of [D-Ser(TBU)^6] LHRH-EA may well contribute to the decreased androgen formation observed during such treatment. Moreover, since testosterone is known to stimulate prolactin secretion (Kalra et al., 1973), the decreased plasma testosterone levels observed after treatment with LHRH agonists are likely to be responsible for the inhibition of prolactin

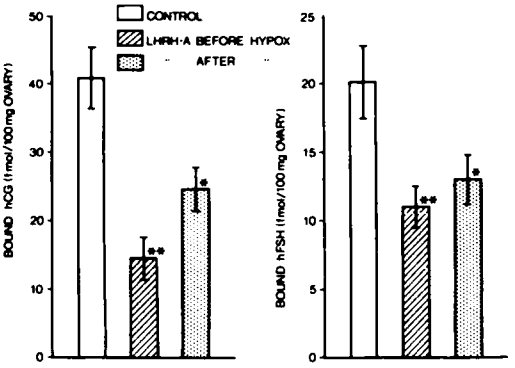

FIG. 10. Comparative effect of single injection of 1 μg of [D-Ala^6] LHRH-EA 12 h before or immediately after HYPOX (performed on diestrus I) on ovarian LH and FSH receptors in adult rats. The animals were sacrificed 2 days after surgery. Control are HYPOX.

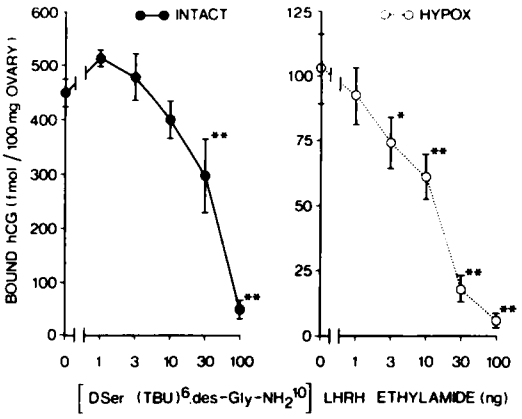

FIG. 11. Effect of daily treatment for 9 days of intact or HYPOX (3 days earlier) rats with increasing doses of [D-Ser(TBU)^6] LHRH-EA on ovarian LH/hCG receptors. The animals were sacrificed 24 h after the last injection of the peptide.

TABLE 3. Effect of daily treatment for 9 days of intact or HYPOX adult female rats with increasing doses of [D-Ser(TBU)^6] LHRH-EA on ovarian LH, FSH, and PRL receptor levels.

Dose of [D-Ser(TBU)^6] LHRH-EA (ng)	Receptor levels (fmoles/ovary)					
	LH	FSH	PRL	LH	FSH	PRL
	Intact			HYPOX		
Control	389 ± 41	25 ± 3	203 ± 30	49 ± 81	3.3 ± 0.7	105 ± 15
1	527 ± 59	24 ± 2.8	278 ± 45	44 ± 31	4.3 ± 0.7	107 ± 17
3	455 ± 62	19 ± 1.9	220 ± 46	37 ± 6.7	2.7 ± 0.3	85 ± 10
10	345 ± 28	19 ± 1.9	198 ± 16	28 ± 3.7**	2.8 ± 0.3	81 ± 9
30	202 ± 63**	17 ± 1.7*	225 ± 34	10 ± 3**	1.6 ± 0.2*	105 ± 3
100	45 ± 11**	13 ± 3*	151 ± 30	3 ± 1**	2.0 ± 0.4*	105 ± 8

*P<0.05; experimental vs control.

**P<0.01; experimental vs control.

secretion. On the other hand, partial removal of the inhibitory feedback action of androgens (Ferland et al., 1976) and possibly also inhibin (Lagacé et al., 1979) could explain the stimulatory effect of treatment with the LHRH agonist [D-Ser(TBU)^6] LHRH-EA on basal plasma LH and FSH levels.

While the main testicular androgen secreted in response to oLH in intact animals is testosterone, it can be seen in Fig. 5 that after treatment with [D-Ser(TBU)^6] LHRH-EA the marked inhibition of the testosterone response is compensated by a marked increase of androstane-3α, 17β-diol levels, thus suggesting an increase of testicular 5α-reductase and 3α-dehydrogenase activities following treatment with the LHRH agonist. The increased 5α-reductase activity could also explain the observation of normal or only slightly reduced levels of testicular and plasma 5α-dihydrotestosterone concentration in the presence of an almost complete inhibition of testosterone levels (Bélanger et al., 1980a) (Figs. 4, 5).

It is of interest that treatment with low daily doses (3 or 10 ng) of the LHRH agonist [D-Ser(TBU)^6] LHRH-EA can lead to an increased testosterone response to oLH. This increased testosterone response to oLH is observed in the presence of a 40% increase and a 30% decrease of testicular LH receptor levels, respectively (Fig. 5). These data show a clear dissociation between the level of LH receptors and the testosterone response to oLH. Moreover, they raise the possibility that treatment with low doses of LHRH agonists can stimulate Leydig cell function while high doses have potent inhibitory effects.

While treatment of adult male rats with LHRH agonists leads to a loss of LH receptor levels accompanied by a marked blockage of the steroidogenic pathway at the level of 17-hydroxylase and 17,20-desmolase and a stimulation of 5α-reductase activities (Bélanger et al., 1979, 1980a; Labrie et al., 1980), the same treatment of HYPOX animals leads to a smaller loss of testicular LH receptors and a minimal or no effect on the steroidogenic pathway as measured by testicular steroid levels 2 h after the administration of 10 μg of oLH, although the basal levels of all steroids are reduced.

The present data suggest that endogenous LH release induced by the LHRH agonists is probably the main factor responsible for the loss of gonadotropin receptors, blockage of the steroidogenic pathway, as well as inhibition of spermatogenesis observed after treatment of intact male animals with LHRH agonists. However, in the PMSG-hCG-treated immature female rats, the similar potency of the LHRH agonist to induce loss of ovarian LH receptors both in intact and HYPOX animals suggests that the direct gonadal site of action may be more important. In adult female animals, however, comparison of the inhibitory effect of a single dose of LHRH agonist before or immediately after HYPOX (Fig. 10) suggests that endogenous LH release also plays an important role in the LHRH agonist-induced loss of LH receptors.

High affinity LHRH binding sites having a specificity similar to the anterior pituitary

receptors for a large series of LHRH agonists have recently been described in ovarian (Reeves et al., 1980) and testicular (Lefebvre et al., 1980; Sharpe et al., 1980) tissue. Although the mechanisms involved remain to be assessed, it is quite likely that these gonadal receptors play a role in the direct inhibitory effects of LHRH agonists at the gonadal level. The finding of an inhibitory effect of LHRH agonists on FSH- and hCG-induced cyclic AMP accumulation in porcine and rat granulosa and luteal cells (Massicotte et al., 1980, 1981; Behrman et al., 1980) suggests that ovarian and probably also testicular LHRH receptors are negatively coupled to adenylate cyclase in gonadal tissue.

ACKNOWLEDGMENTS

F. L. is an associate of the Medical Research Council of Canada. This study was supported by a grant from the Medical Research Council of Canada.

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