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# THE ROLE OF STEROID HORMONES IN THE REGULATION OF GONADOTROPIN SECRETION

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SUMMARY

An acute rise in serum gonadotropins occurs in the immature rat on castration indicating the role of gonadal steroids in gonadotropin regulation. In the castrated male rat testosterone brings about restoration of gonadotropins to intact levels at doses that are "physiological" as judged by seminal vesicle and ventral prostate weights. The suppression of LH takes place at lower doses than FSH. Estradiol prevents the post-castration rise of gonadotropins in the male rat at a dose level of 0·3% of the testosterone dose. Using a variety of techniques, it has been demonstrated that receptors for estradiol are found in the hypothalamus and pituitary of both male and female rats in similar concentrations. No such high affinity receptors are found for androgens in either sex although androgens appear to interfere with estrogen binding. The regulation of gonadotropin secretion in the male thus may be mediated by conversion of androgens to estrogens and the interference manifested by them in estrogen binding. The conversion to estrogens appears to be of importance in the control of FSH because androgens not convertible to estrogens such as 5α-dihydrotestosterone and 5α-androstane-3α, 17β-diol are even less effective than testosterone in suppressing FSH. However, for the suppression of LH, such a conversion does not appear necessary. Conclusive evidence for a positive feedback effect of estradiol on the pituitary leading to greater sensitivity to LHRH in the release of LH has been provided by studies in the cyclic rat and a pituitary stalk-sectioned rat model. Progesterone in the absence of estrogens does not have any effect on gonadotropins. In the presence of estrogens the effect may be stimulatory or inhibitory depending on dose.

Although the relationship between the gonads and pituitary secretion of gonadotropins has been studied by several investigators, the precise interactions involved and the role played by individual gonadal steroids in the regulation of gonadotropin secretion are still poorly understood. This paper further explores the intricate relationship between gonadal steroids and gonadotropin secretion in the immature and mature rat. The immature rat turned out to be an excellent animal preparation for some of the initial studies because of the sensitive pituitary-gonadal axis in both the male and female rat. Furthermore, the absence of daily cyclic changes in the female was an additional advantage. A possible drawback to the use of the immature animals is the change of sensitivity of the hypothalamic-pituitary axis to the feedback effect of steroids at puberty suggested by Byrnes and Meyer in 1951 [1], and confirmed by McCann and Ramirez in 1964 [2], using bioassay techniques for LH. This concept was challenged by Swerdloff *et al.* in 1972 [3]. Recent work in our laboratory [4] has confirmed a dramatic change in the amount of estradiol required for preventing the post-castration rise of FSH and LH in mature and immature rats. The difference, however, was quantitative, the pattern of suppression of FSH and LH being very similar. Therefore, the immature rat model does provide meaningful information on the regulation of gonadotropin secretion.

*Steroid-gonadotropin relationship in the immature state*

The dramatic rise in the secretion of FSH and LH after castration in the adult rat is well documented by several classical experiments and more recently by Gay and Midgley[5], and Yamamoto *et al.*[6], using radioimmunoassay techniques for the measurement of serum gonadotropins. A sensitive feedback system in the neonatal rat was apparent from the work of Goldman *et al.*[7] who found significantly higher levels of serum LH in such rats one day after castration. In order to establish whether the rise in gonadotropins after castration was an acute phenomenon based on sensitive feedback by gonadal steroids, serum FSH and LH were measured in 26 day old male and female rats of the Sprague–Dawley strain obtained from the Holtzman Company at various intervals of time after castration [8]. The results obtained are shown in Table 1. In male rats, serum FSH was significantly elevated within 8 hours of castration ($P < 0.01$) and continued to rise till 24 h after castration. Serum LH was elevated significantly by 12 h ($P < 0.05$) with further rise at 24 and

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Table 1. Serum gonadotropins before and after castration in immature rats (26 days old)

| | Hours after castration | Male | Female |
|---|---|---|---|
| FSH | 0 | 638·3 ± 64·3 | 374·2 ± 30·2 |
| NG/ML | 4 | 733·3 ± 186·6 | 490·0 ± 236·5 |
| RP-1 | 8 | 1328·6 ± 290·2† | 723·3 ± 103·7 |
| | 12 | 1463·0 ± 167·1† | 607·5 ± 99·9 |
| | 24 | 2478·6 ± 201·7† | 1630·0 ± 320·1† |
| | 48 | 2770·0 ± 194·0† | 2664·3 ± 265·6† |
| LH | 0 | 16·6 ± 3·9 | 22·3 ± 8·3 |
| NG/ML | 4 | 13·0 ± 3·0 | 19·0 ± 6·2 |
| RP-1 | 8 | 70·7 ± 27·0 | 20·2 ± 7·2 |
| | 12 | 87·0 ± 19·6* | 17·4 ± 3·8 |
| | 24 | 111·3 ± 13·2† | 58·3 ± 58·3 |
| | 48 | 190·0 ± 15·8† | 180·4 ± 36·7† |

* $P < 0.05$ vs. 0 h.
† $P < 0.01$ vs. 0 h.

48 h. In female rats, the elevation of gonadotropins also occurred acutely, nevertheless at a slower pace as compared to male rats. The first significant rise in FSH was not seen until 24 h postcastration ($P < 0.01$). Even though serum LH appeared higher than the controls at 24 h post-castration, statistical significance was only reached in the 48 h post-castration sample. These experiments therefore indicate that even in the immature state, small quantities of gonadal steroids are secreted which are responsible

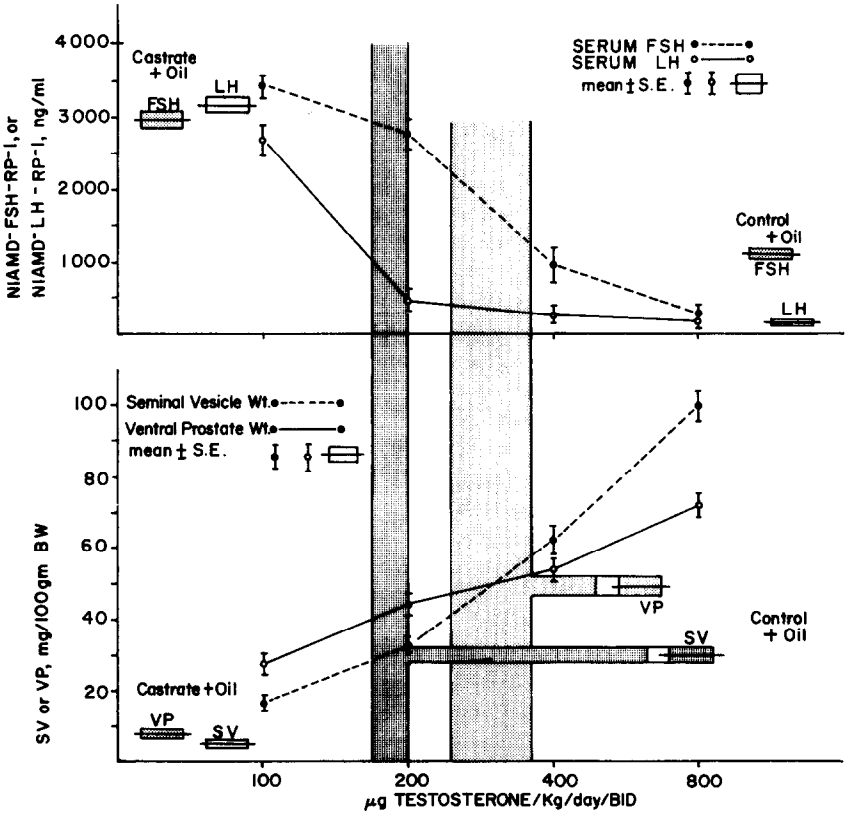


Fig. 1. Serum FSH and LH, and seminal vesicle (SV) and ventral prostate (VP) weights in male rats orchidectomized at 26 days of age and treated with testosterone for 7 days. On the abscissa scale is testosterone dosage, in μg/kg/day; the scale is logarithmic. On the ordinate is ng/ml of serum FSH or LH (top) and SV and VP, mg/100 g body weight (bottom). Each point represents the mean value from 7 animals ± one S.E.M. Values and limits from 7 castrated and 7 intact controls are also indicated. The weights of SV and VP from intact controls have been extended over to the plot of treated animals, and then extrapolated up and down to indicate a physiologic dosage range (PDR), or an approximate dosage of administered hormone which would result in organ weights of the same magnitude as the intact controls.

for maintaining the secretion of gonadotropins at low levels consistent with the state of immaturity.

*Steroids responsible for gonadotropin control in the immature state*

If the conclusions drawn by the preceding experiments were correct, then it would be possible to control the post-castration rise of gonadotropins by the administration of physiological doses of various steroids. To test the validity of this hypothesis, male rats orchidectomized at 26 days of age were treated with testosterone for seven days [9]. The doses administered ranged from 100–800 μg/kg/day and were administered in 0·1 ml corn oil. The doses were divided in two equal amounts and were injected at 12 h intervals. Castrate control rats and intact rats were also studied over the same period of time, the only treatment being the injection of the corn oil. Ventral prostate and seminal vesicle weights and serum FSH and LH were measured in all groups of animals at the end of the treatment period. The "physiologic dose range" of testosterone was projected as the dose of the steroid which produced ventral prostate and seminal vesicle weights comparable to those found in the intact corn oil-treated controls. The results are shown in Fig. 1. In the castrate animals, serum FSH and LH levels are very high and seminal vesicle and ventral prostate weights are low as compared to the controls. Based on seminal vesicle weights, a dose range of 180–200 μg/kg/day of testosterone appeared to be the "physiological dose range" because at this dose level the seminal vesicle weights of the castrate rats treated with testosterone were comparable to those found in intact controls. At this dose level, serum LH was comparable to that found in intact animals, whereas serum FSH was no different from castrate levels. A higher dose of testosterone (250–350 μg/kg/day which was still within the "physiologic dose range" as judged by ventral prostate weights) was able to suppress both serum FSH and LH in castrate animals to intact control levels.

The experiments described above clearly demonstrate that the testicular production of testosterone can account for the control of FSH and LH secretion by the pituitary in the immature male rat. The suppression of LH in the immature castrated male rat

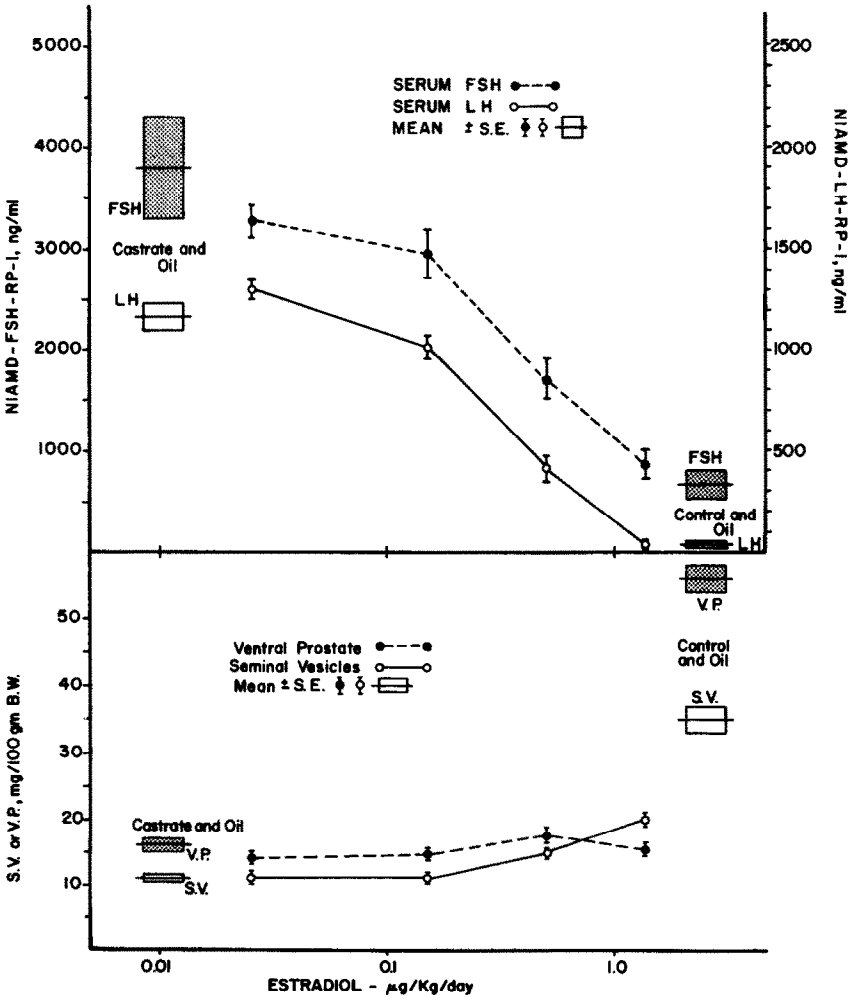


Fig. 2. Serum FSH and LH, and seminal vesicle (SV) and ventral prostate (VP) weights in male rats orchidectomized at 26 days of age and treated with estradiol-17β for 7 days. The dose of estradiol necessary to restore serum FSH and LH to control levels is 0·3% of the testosterone dose.

with testosterone propionate was reported earlier by McCann and Ramirez[2]. However, these investigators reported that higher than physiologic levels were required for such suppression. Differences in the length of the treatment period and a less sensitive method for LH measurement may be responsible for some of the differences in results. The observations by our group [9, 10] that higher levels of testosterone were necessary to suppress FSH as compared to LH have been confirmed by several investigators [11–13].

Estradiol is well known to bring about inhibition of gonadotropins in both male and female rats. In order to determine the dose of estradiol required to prevent the post-castration rise of gonadotropins, the experiment was repeated using estradiol instead of testosterone. The results of estradiol administration to castrate male rats is shown in Fig. 2. Suppression of both FSH and LH to intact control levels occurred at the 1·33 μg/kg/day dosage and there appeared to be no difference in the pattern of suppression between FSH and LH in sharp contrast to the observation with testosterone. Therefore, it is conceivable that estradiol may play a role in the control of FSH secretion in the male.

The conversion of androgens such as testosterone and 4-androstene-dione to estrogens has been demonstrated in the human *in vivo* [14, 15], and in the hypothalamic tissue of rats *in vitro* [16, 17]. Our results indicate that 400 μg/kg/day of testosterone is equivalent to 1·33 μg/kg/day of estradiol in its gonadotropin suppressing activity. This 0·3% conversion is well within the conversion ratio found *in vivo* [14, 15].

*Steroid receptor interaction in the hypothalamus and pituitary*

An alternative approach to the question of steroidal specificity in feedback control of gonadotropin secretion was the measurement of steroid receptor binding capacity in the anterior pituitary and hypothalamus. It was felt that initial differential uptake and retention of estrogens or androgens might shed further light upon the specific roles of these classes of steroid hormones within these glands. Receptor assays were performed by protamine precipitation technique [18] under conditions of saturating steroid concentration, wherein the quantity, moles steroid bound/mg cytosol protein, was a constant as demonstrated for the anterior pituitary in Fig. 3. Such analyses afforded readily reproducible data as exemplified graphically in Fig. 4 for estradiol in the pituitary and hypothalamus. In summary of some of our results [18, 19, 20], Table 2 is a compilation of equilibrium data from Scatchard analyses of estradiol binding in anterior pituitary and hypothalamic cytosol from male and female rats. Clearly equivalent concentrations of binding moieties of equal affinity were found in males and females. Moreover, immaturity or even the absence of gonads was without influence upon these parameters. Anterior pituitary cytosol contained ten times as much receptor as hypothalamic cytosol.

These findings suggested that estrogenic mediation of gonadotropin secretion might be effected by endogenous factors other than concentration or affinity of receptor interactions, such as changing blood levels of steroid hormones at various phases of the cycle. In this regard, the influence of androgens within the anterior pituitary and hypothalamus was considered as a consequence of one of four possible phenomena: (a) specific high-affinity androgen receptors were present which were independent of estradiol–receptor interactions, (b) high-affinity binding of androgens to

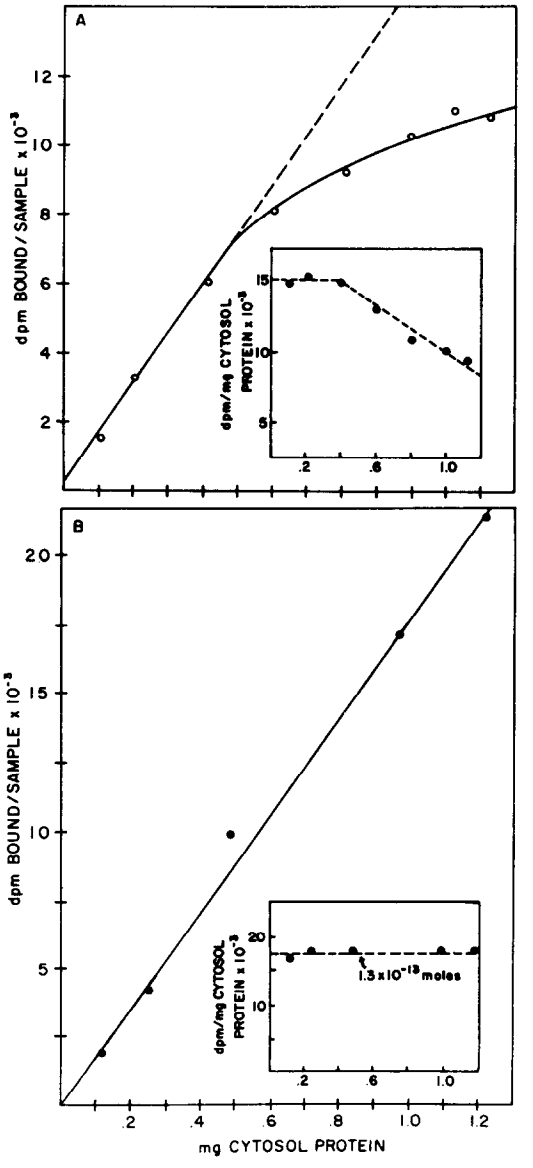


Fig. 3. Relationship between anterior pituitary cytosol receptor binding of estradiol and cytoplasmic protein concentration in the presence of limiting (A) and non-limiting (B) concentrations of steroid. Aliquots of cytosol were incubated with a given concentration of $^3$H-estradiol for 18 hours at 4°C, following which the specific binding was determined by protamine precipitation of the complex. Steroid concentrations utilized were: (A) 0·08 nM and (B) 0·20 nM. Units on the abscissa are identical for the insets and the graphs. Linear correlation in (B) was highly significant ($r > 0{\cdot}99$, $P < 0{\cdot}001$).

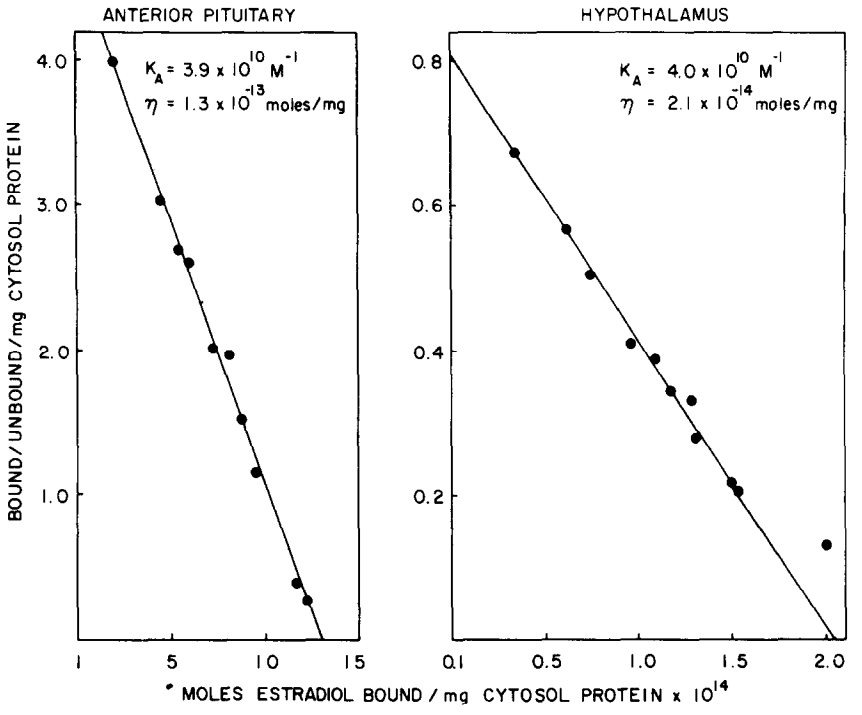


Fig. 4. Representative Scatchard plot analyses of the estradiol–receptor interactions in the anterior pituitary and hypothalamus. The contribution of non-specific binding, as determined by the difference between samples incubated in the presence and absence of a 100-fold molar excess of unlabeled estradiol, has been eliminated from each determination. The concentration of binding sites ($n$), in moles bound per mg cytosol protein, was determined as the extrapolated $x$-intercept; $K_A$ was calculated from the slope. Linear regression analysis in each case afforded correlation coefficients, $r > 0.99$ ($P < 0.001$).

the estradiol receptor occurred, reducing the effective concentration of estrogen receptor, (c) low-affinity interactions of androgens with the estrogen receptor, under conditions of changing androgen/estrogen ratios, altered the initial rate of formation of the estrogen-receptor complex, or (d) low affinity interactions of androgens resulted in physiological responses independent of those initiated by estradiol. Distinction among these possibilities was attempted by measurement of cytoplasmic binding of androgens under both kinetic and equilibrium conditions.

Numerous experimental techniques were employed for detection of androgen receptors. Exhaustive dialysis was effective in removing $^3$H-testosterone from preincubated anterior pituitary cytosol (Fig. 5b), while identical samples preincubated with $^3$H-estradiol retained appreciable levels of bound steroid (Fig. 5a). Removal of testosterone from cytosol by this procedure followed a pattern similar to disruption of the testosterone–BSA complex, a known low-affinity interaction. The results shown in Table 3 further demonstrate lack of specific testosterone binding in the anterior pituitary; in addition, similar data are presented for dihydrotestosterone binding in the hypothalamus. Successive subjection of steroid-incubated samples to procedures which remove free or loosely-associated steroid results in loss of androgens to an unmeasurable level, but appreciable quantities of estradiol remain protein-bound. Sucrose gradient sedimentation analysis of androgen-incubated anterior pituitary cytosol samples occasionally showed very small amounts of radioactivity in the 8S region;

Table 2. Estradiol receptor binding site concentration and equilibrium association constants for the corresponding reactions in anterior pituitary and hypothalamic cytosol

| Group | Anterior pituitary | | Hypothalamus | |
|---|---|---|---|---|
| | $n(10^{-13}$ moles bound per mg cytosol protein) | $K_A$ ($10^{10}$ $M^{-1}$) | $n(10^{-14}$ moles bound per mg cytosol protein) | $K_A$ ($10^{10}$ $M^{-1}$) |
| Immature female | 1·1 ± 0·4 | 3·5 ± 0·8 | 2·0 ± 0·1 | 5·9 ± 1·2 |
| Intact female | 1·2 ± 0·1 | 3·5 ± 0·4 | 1·6 ± 0·2 | 4·4 ± 0·2 |
| Castrate female | 1·5 ± 0·3 | 3·5 ± 0·1 | 1·8 ± 0·3 | 4·3 ± 0·3 |
| Immature male | 1·1 ± 0·2 | 3·8 ± 0·3 | 1·4 ± 0·5 | 5·9 ± 0·2 |
| Intact male | 1·6 ± 0·3 | 3·9 ± 0·4 | 1·5 ± 0·4 | 4·4 ± 0·6 |
| Castrate male | 1·6 ± 0·2 | 3·2 ± 0·1 | 1·9 ± 0·3 | 5·5 ± 0·9 |

Binding parameters were determined from Scatchard plot analysis of equilibrium binding data, as exemplified in Fig. 4. Each value represents the mean ± S.E. of at least three separate determinations.

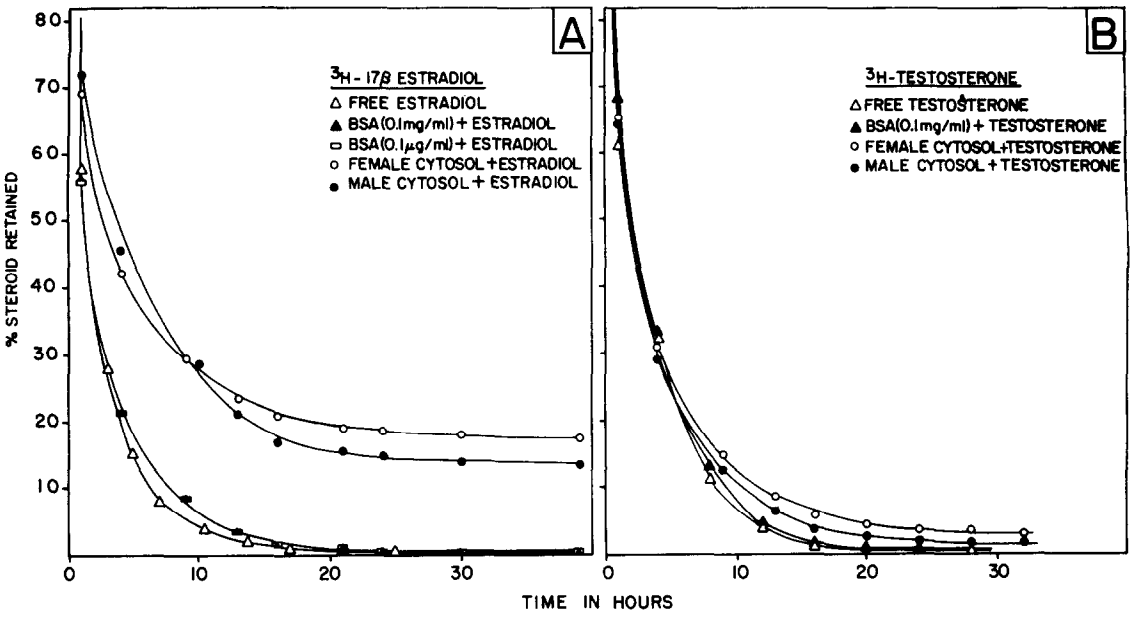


Fig. 5. Analysis of high-affinity steroid-protein interactions by exhaustive sequential redialysis. Anterior pituitary cytosol samples were incubated with the designated steroids for 2 h at 4°C. They were then pipetted into prepared dialysis bags and dialyzed successively against outside buffer solutions of twice the inside volume. Fresh outside solution was introduced at each interval measured, and the amount of steroid retained at that interval was determined as the difference between the initial content of the bag and the amount removed by dialysis to that point. (A) $^3$H-Estradiol concentration was $3{\cdot}0 \times 10^{-9}$ M in all samples except female cytosol, which was $2{\cdot}25 \times 10^{-9}$ M in steroid. Protein concentration of male cytosol was 1·8 mg/ml; of female cytosol, 1·7 mg/ml. (B) $^3$H-testosterone concentration was $2{\cdot}3 \times 10^{-9}$ M in all samples. Cytosol protein was 1·4 mg/ml for male samples, 1·5 mg/ml for female.

these results were not reproducible and occurred only when large amounts of cytosol protein were applied to the gradient. While our findings do not preclude the possibility that androgen receptors do exist in these tissues as several other investigators have reported [21, 22], it would appear that the concentration of such receptors must be very low and the binding sites would probably be saturated at physiological androgen concentrations. Under these conditions, it is difficult to imagine a dynamic role for androgen receptors in the regulation of changes in gonadotropin levels. Data from $^3$H-androgen-incubated cytosol samples were no more indicative of high-affinity binding than were those obtained by measurement of $^3$H-estradiol binding to unlabeled-androgen-preincubated samples; thus, strong binding of androgens to the estradiol receptor binding sites, resulting in subsequent modification of estrogen action, was not a tenable explanation. Analyses of binding of estrogens and androgens at various time intervals following incubation with cytosol were performed in order to determine whether labile high-affinity complexes were formed which would be undetectable at equilibrium; no indication of this phenomenon was observed[18].

The data in Table 3 show that significant binding of androgens can be detected following equilibrium dialysis and that this binding is of moderate affinity in comparison to that of the estradiol–receptor interaction. Although low-affinity binding of androgens may be of prime physiological significance in the

Table 3. Analysis of relative strengths and amounts of steroid-protein interactions following incubation of cytosol with estradiol, testosterone or dihydrotestosterone

| Cytosol source | Sequential treatment | % Steroid bound | |
|---|---|---|---|
| Anterior pituitary | | Estradiol | Testosterone |
| | Equilibrium dialysis | 42·8 | 5·3 |
| | Redialysis | 9·7 | 1·6 |
| | Gel filtration | 9·4 | 0 |
| Hypothalamus | | Estradiol | Dihydrotestosterone |
| | Equilibrium dialysis | 15·5 | 8·0 |
| | Redialysis | 3·8 | < 0·5 |

Cytosol samples were initially subjected to equilibrium dialysis for 72 h at 4°C. The dialyzed sample was then redialyzed against 4 VOL. of buffer for 24 h. Finally, the redialyzed sample (anterior pituitary only, since radioactivity retained in hypothalamic sample was too low to permit accurate analysis by gel filtration) was eluted from a column of Sephadex G-200 and radioactivity in the fractions was measured.

regulation of estradiol binding, it is not possible to assess this interrelationship in an *in vitro* system at equilibrium, since estradiol can effectively compete for its sites by virtue of its high affinity for the receptor. It occurred to us, however, that it might be possible to detect androgen modification of estrogen binding by measuring the effects of androgens upon the initial rate of formation of the estradiol–receptor complex. It was necessary to characterize the kinetics of the reaction initially in the absence of androgen. As shown in Fig. 6, the association rate reaction follows second-order kinetics for at least the first 10 min of reaction. The specificity of this reaction is evidenced by the fact that it can be completely eliminated by either preincubation or co-incubation with excess unlabeled estradiol, but is unaffected by excess cortisol. The ease and reproducibility of this kinetic analysis[20] allowed us to examine the influence of preincubation with androgens upon the association rate constant of the estradiol-receptor reaction. As shown in Table 4, preincubation with progressively increasing amounts of dihydrotestosterone resulted in successive decreases in the association rate constant for the subsequent interaction of estradiol with its receptor. Testosterone was ineffective in this regard at levels where dihydrotestosterone was a highly potent inhibitor. When the system was allowed to come to equilibrium, testosterone and dihydrotestosterone were weak and approximately

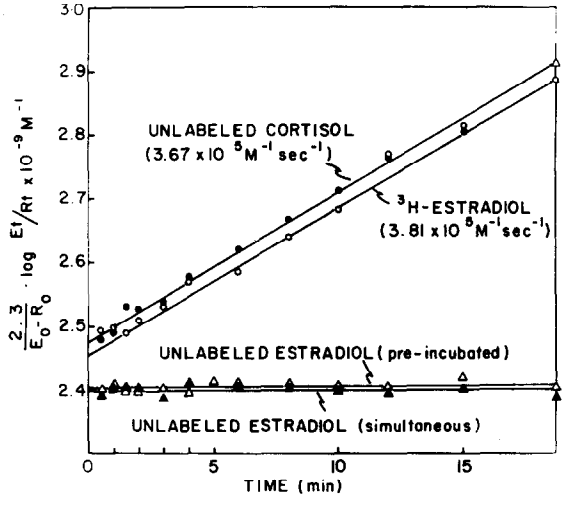


Fig. 6. Second-order association rate plot of the interaction between estradiol and the pituitary cytosol receptor. The $[R]_0$ value for the cytosol sample used was 0·365 nM, calculated as the product of the protein concentration of the reaction mixture (3·02 mg/ml) and the binding site concentration (n = 0·12 pmol/mg) of cytosol protein, determined by Scatchard analysis of saturation data). Initial [$^3$H]-estradiol concentration ($[E]_0$) was 0·50 nM. One sample (○) demonstrated the association reaction when [$^3$H]-estradiol was added at time zero. Two other samples were preincubated for 2 h with 50 nM of either unlabeled cortisol (●) or unlabeled estradiol (△), and the reaction was then performed as described in [20]. In another sample, the labeled and unlabeled estradiol were added together at time zero (▲). Linearity of the plots was established by regression analysis, yielding $r > 0{\cdot}99$ ($P < 0{\cdot}001$).

Table 4. Influence of preincubation with various steroids upon the initial formation of anterior pituitary cytosol estradiol receptor complex

| Steroid | Fold Molar Excess | % Inhibition |
|---|---|---|
| Estradiol | 50 | 100 |
| | 100 | 100 |
| Cortisol | 100 | 0 |
| Testosterone | 20 | 0 |
| | 50 | 0 |
| Dihydrotestosterone | 10 | 17 |
| | 50 | 56 |
| | 100 | 66 |
| | 500 | 79 |
| | 1000 | 87 |

Samples of cytosol were incubated with the designated level of each steroid (unlabeled) for 2 h, following which time $^3$H-estradiol was added and the association reaction was monitored as described [20]. The association rate constants were measured as the slope of the linear second order kinetic plot and inhibition is designated as the per cent decrease in rate constant.

equivalent inhibitors of the estradiol–receptor interaction, since a 1000-fold molar excess of either compound was required to effect 50% inhibition of estradiol binding. These findings indicate that dihydrotestosterone, but not testosterone, engages in low-affinity interactions which decrease the rate of formation of the estradiol–receptor complex. It is not difficult to visualize a significant role for this modifying effect in the control of gonadotropin secretion when one considers how crucial the effective levels of estradiol are to the manifestation of the feedback control mechanism. The possibility that low-affinity interactions occur between androgens and intracellular proteins completely independent of the estrogen receptor, and that such complex formation may be of physiological significance, cannot be disregarded on the basis of these studies.

*Action of androgens that cannot be aromatized to estrogens on gonadotropins in the castrated rat*

The work of several investigators has shown that testosterone may be converted to 5α-dihydrotestosterone prior to manifestation of biological activity within the cell and the subject has been reviewed by Wilson and Gloyna[23]. That 5α-reduced androgens cannot be aromatized to estrogens has been shown also by the work of several investigators and the subject has been reviewed by Engel[24]. The conversion of testosterone to 5α-dihydrotestosterone was shown to occur both in the pituitary and the hypothalamus by Jaffe[25], and subsequently confirmed by several studies. The effect of 5α-dihydrotestosterone on serum FSH and LH was studied by us in the 26-day-old castrated rat [9, 10] and the results are shown in Fig. 7. Based on the ventral prostate weights, the "physiological dose range" was estimated to be 250–300 μg/kg/day. Serum LH was suppressed to intact control levels at the 200 μg/kg/

day dose level. Serum FSH, although somewhat lowered was still significantly higher than intact controls ($P < 0.01$) even at the 400 μg/kg/day dose level. These findings emphasize the control of LH secretion at physiological levels by androgens that are not aromatized to estrogens with poor suppression of FSH. Recently, attention has focused on a further reduction of 5α-dihydrotestosterone to 5α-androstane-3α,17β-diol [26, 27]. This compound is also not converted to estrogens and was potent in suppressing LH much more readily than FSH (Fig. 8), a finding that was similar to the results with 5α-dihydrotestosterone. The 3β reduction product of 5α-dihydrotestosterone (5α-androstane-3β,17β-diol) however, was ineffective in modifying both FSH and LH in doses up to 2000 μg/kg/day.

*Differences in response between estradiol and a synthetic estrogen, mestranol (3-methoxy-17α-ethynyl estra-1,3,5(10)-triene-17β-ol)*

The effectiveness of estradiol in suppressing the post-castration rise of gonadotropins has also been studied by us in the female rat [28]. Just as in the male rat, estradiol brought about a parallel pattern of lowering of both FSH and LH, and the "physiological replacement dose level", as judged by uterine weight, was between 0·15 to 0·2 μg/kg/day (Fig. 9). Similar observations were made with estrogens readily convertible to estradiol *in vivo* such as estrone and estradiol benzoate [28]. However, the synthetic estrogen, mestranol, showed preference for the suppression of LH as compared to FSH at lower dose levels (Fig. 10). An explanation of this difference is not readily apparent and may lie in the differences in receptor binding as compared to estadiol in the hypothalamus and the pituitary.

*The role of estrogen receptors in the pituitary*

The high affinity binding of estrogen by pituitary cytosol has been regarded by several investigators as estradiol–receptor interaction based on indirect evidence on the role of estradiol in modulating pituitary function. To prove such a role, an animal preparation in which the influence of the hypothalamus

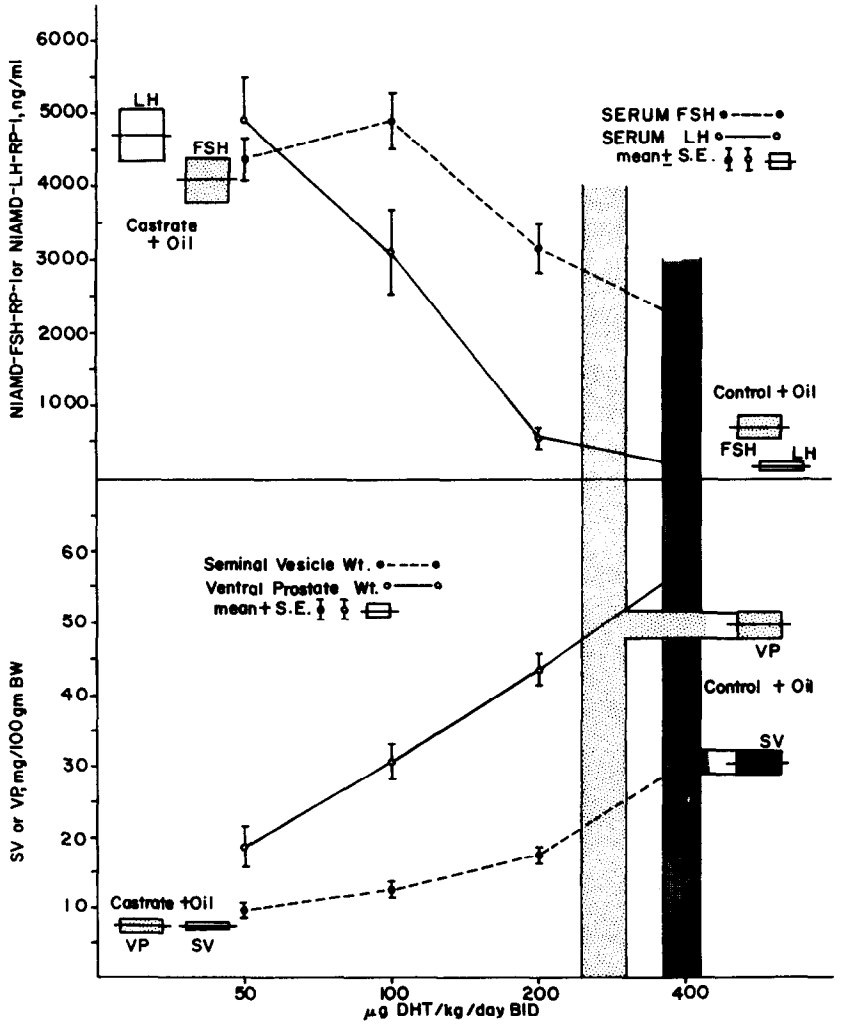


Fig. 7. Serum FSH and LH, and seminal vesicle (SV) and ventral prostate (VP) weights in male rats orchidectomized at 26 days of age and treated with 5α-dihydrotestosterone (DHT) for 7 days. See Fig. 1 for more details.

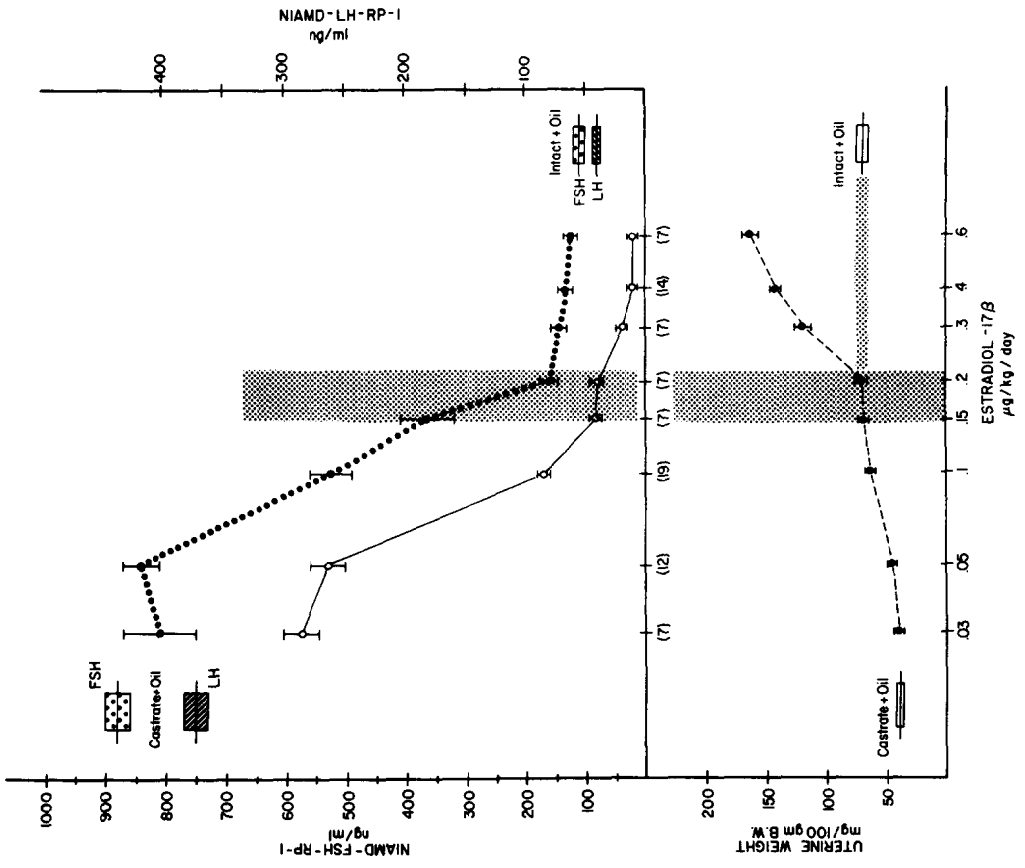


Fig. 9. Serum FSH and LH, and uterine weights of female rats ovariectomized at 26 days of age and treated with 17β-estradiol for 5 days post-operatively. The physiologic dosage range is projected based on uterine weight. See Fig. 1 for more details.

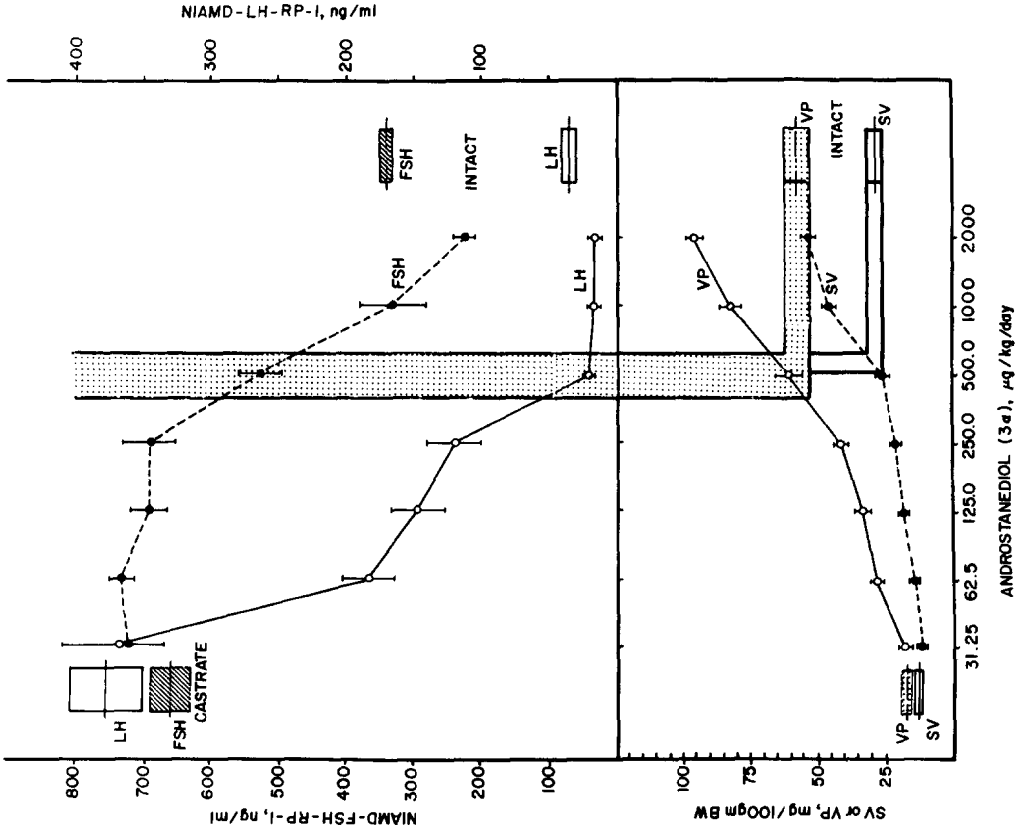


Fig. 8. Serum FSH and LH, and seminal vesicle and ventral prostate weights in male rats orchidectomized at 26 days of age and treated with various doses of 5α-androstane-3α, 17β-diol for 7 days. See Fig. 1 for more details.

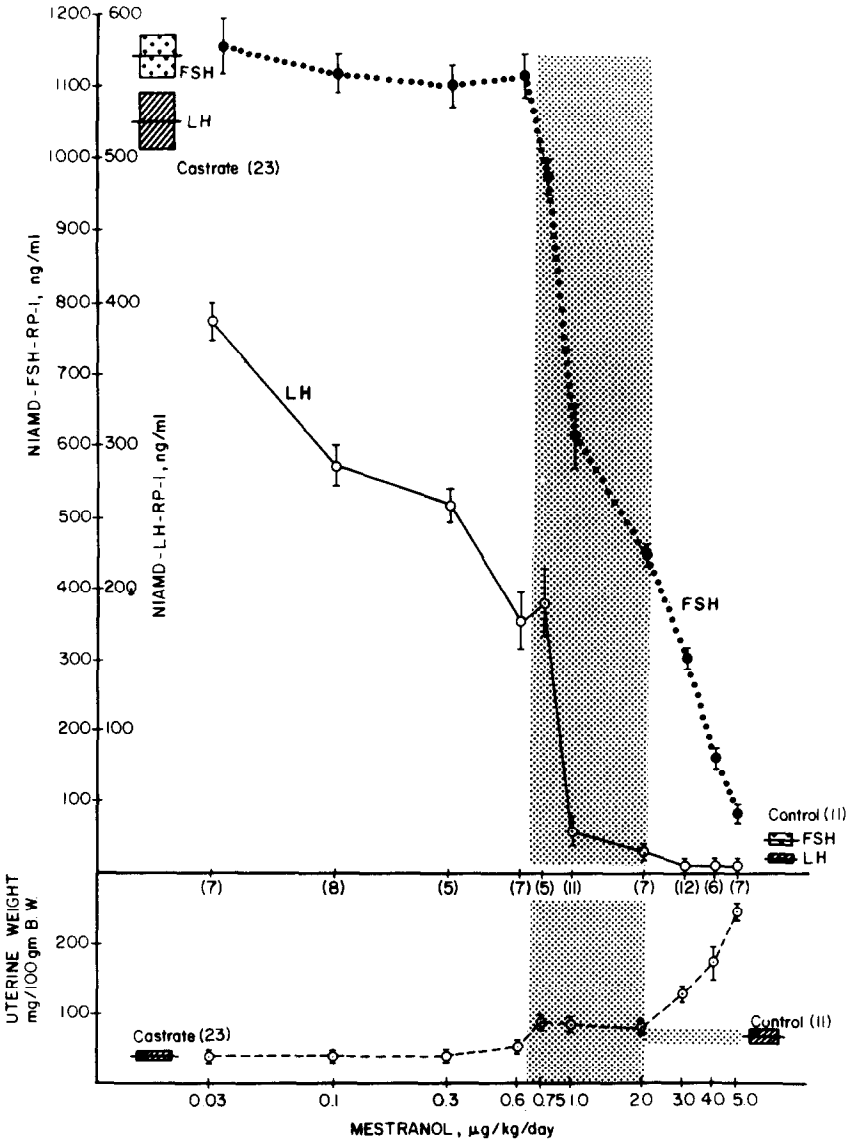


Fig. 10. Serum FSH, LH and uterine weights of female rats ovariectomized at 26 days of age and treated with varying doses of mestranol for 5 days post-operatively. See Fig. 9 for details.

has been either minimized or eliminated is highly desirable. Such an animal model was perfected in our laboratory by surgically cutting the vascular connection between the hypothalamus and pituitary and inserting a metallic barrier to prevent regeneration [29]. Table 5 shows the validity of this preparation in the ovariectomized rat as judged by serum FSH, LH, and prolactin. A dose response with LH was obtained when various quantities of LRF were injected [30]. Studies were then carried out to evaluate if estrogen treatment would potentiate the effect of LRF in LH release [29]. Table 6 shows a dramatic potentiation of the effect of LRF by estradiol, thus demonstrating conclusive evidence of a direct effect of estradiol on the pituitary.

To further establish the receptor role of the specific estrogen-pituitary cytoplasmic interaction, the sensitivity of the pituitary in releasing LH to a standard dose of exogenous LRF was studied at various times of the rat estrus cycle [31]. An excellent correlation

Table 5. Serum gonadotropins in the ovariectomized and the ovariectomized stalk-sectioned rat

| | Gonadotropins in RP-1/ml | | |
|---|---|---|---|
| | FSH | LH | Prolactin |
| Ovariectomized | 763 ± 48* | 706 ± 40 | 7 ± 2 |
| Ovariectomized stalk-sectioned | 29 ± 3 | 3 ± 1 | 35 ± 4 |

* S.E.M.

Table 6. Effect of LH-RH and estradiol benzoate (EB) on LH release in the ovariectomized stalk sectioned rat

| Pretreatment regimen for six days | Basal | Serum LH (RP-1/ml) After 50 ng LH-RH Intracarotid +10 | +60 | +120 |
|---|---|---|---|---|
| Untreated | 4 ± 1* | — | — | — |
| Estradiol benzoate 1 μg/kg/day | 2 ± 0·4 | — | — | — |
| LH-RH 2 ng, 3×/d.sc | 4 ± 1 | 32 ± 12 | 9 ± 3 | 8 ± 2 |
| LH-RH (2 ng) + EB | 4 ± 1 | 379 ± 156 | 89 ± 26 | 48 ± 26 |

* S.E.M.

between the decrease in cytosol estrogen-receptor binding (due to nuclear translocation) and responsiveness to LRF is evident in Fig. 11. The phenomenon of depletion of the cytosol estrogen-receptor binding after a single dose of estradiol and subsequent replenishment several hours later by a cycloheximide sensitive step in the rat pituitary, hypothalamus and uterus has been recently demonstrated in our laboratory [32].

*The role of progesterone in gonadotropin control*

The role of progesterone in the regulation of gonadotropin secretion has been studied by several investigators and its effects may be suppressive or stimulatory depending on the state of estrogenic stimulus. The early experiments of McCann[33] and Naller *et al.*[34] suggested that in the castrated rat, progesterone does not have any effect on LH levels. These findings have now been confirmed by our group [35] using radioimmunoassays and have been extended to FSH as well. It is significant in this regard to note that anterior pituitary cytosol, when preincubated with progesterone, showed no decrease in the initial velocity of formation of estradiol–receptor complex, even when the amount of progesterone used was 1000-fold in excess of estradiol [36]. However, in the castrate rat treated with a constant low dose of estradiol, progesterone can further suppress or elevate the level of circulating FSH and LH and these actions are dependent on the dose of progesterone used (Fig. 12) [37, 38]. These results emphasize that doses of estrogen and progesterone that are unable to suppress gonadotropins individually, are very effective when used in combination with each other. Furthermore, in obtaining the desired effect, the ratio of the steroids may be critical.

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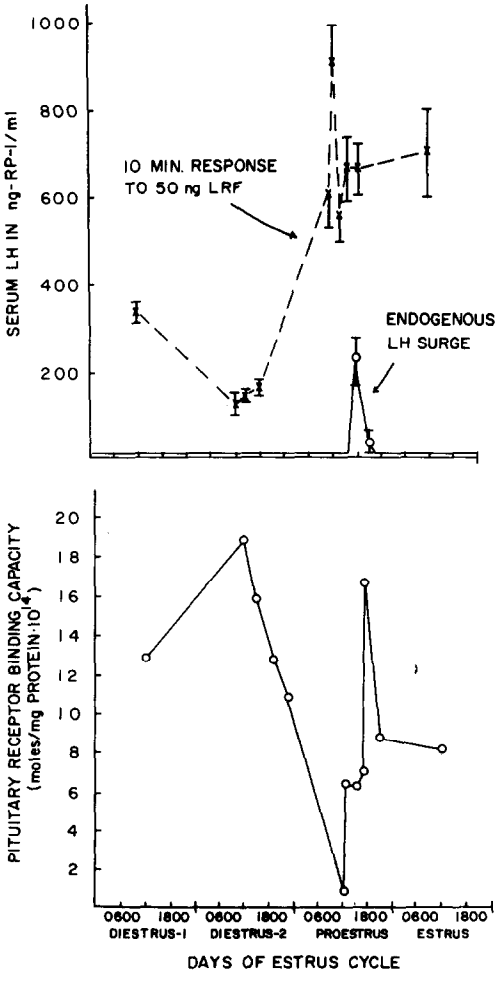


Fig. 11. Pituitary cytosol estradiol receptor binding and release of LH in response to LRF at various times in the 4 day cyclic rat. Maximum sensitivity to LRF and lowest receptor binding occurred around noon on proestrus.

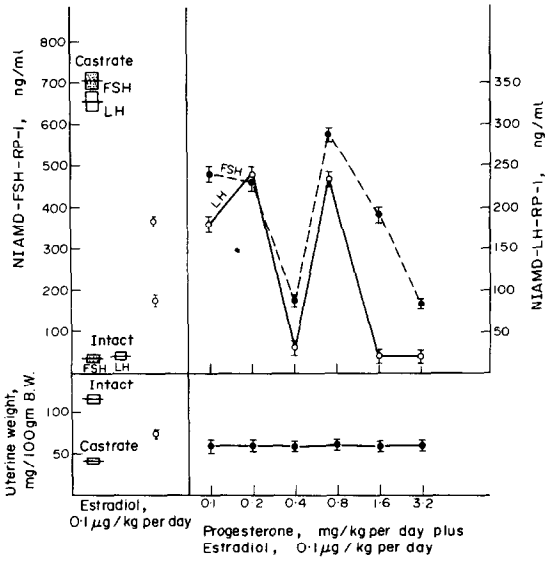


Fig. 12. The dose-dependent effect of various doses of progesterone in the castrate female rat treated with a low dose of estradiol.

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