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Receptor Depletion and Replenishment Processes: In vivo Regulation of Gonadotropin Receptors by Luteinizing Hormone, Follicle Stimulating Hormone and Ethanol in Rat Testis

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ABSTRACT

The effect of *in vivo* injections of human luteinizing hormone (hLH), human follicle stimulating hormone (hFSH) and ethanol on gonadotropin receptor sites in rat testes was studied. We found that hLH (32 IU) and hFSH (30 IU) reduced the number of their respective binding sites shortly after administration. The hLH was more rapid and effective in its action than hFSH. Hormone specificity was also evident in the depletion process. The hLH could not effectively deplete testicular hFSH receptors during the first 3 days and *vice versa*.

Prolonged administration of ethanol (5 ml of 20% v/v ethanol in saline/injection; 2 injections/day for 7 days) also led to depletion of gonadotropin receptor sites in rat testes but without the specificity displayed by hLH and hFSH. The receptor replenishment process commenced when ethanol was withdrawn and was essentially complete by Day 20 (or 13 days after ethanol withdrawal). The ability of ethanol to reduce gonadotropin binding through receptor depletion may be a possible cause of testicular atrophy, reduced steroidogenesis and impotency, problems frequently encountered in chronic alcoholics.

INTRODUCTION

It has been shown that *in vivo* administration of LH/hCG to male rats results in temporary desensitization of testicular functions: receptor loss, diminished adenylate cyclase activity and reduced steroidogenesis in testis (Hsueh et al., 1976, 1977; Purvis et al., 1977; Saez et al., 1978; Sharpe, 1976; Tsuruhara et al., 1977). Although loss of gonadotropin receptors in rat testis is believed to be a phenomenon specifically regulated by the gonadotropins themselves (Haour and Saez, 1977; Hsueh et al., 1976, 1977; Sharpe, 1976), it has recently been reported that experimental induction of diabetes in rats led to reduction in the concentration of gonadotropin receptors in testis. When insulin was administered, the binding capacity of the testis was fully restored (Charreau et al., 1978). It would, therefore, seem that loss of gonadotropin receptors may not be solely regulated by gonadotropins; other mechanisms need to be explored.

Chronic alcoholics frequently encounter such problems as testicular atrophy, sterility, and impotency (Klatskin, 1960; Van Thiel et al., 1974). The Leydig cells in testes of these people appeared normal histologically (Rather, 1947) but plasma testosterone concentrations were greatly reduced. These findings have been recently confirmed by studying the effects of ethanol ingestion on the levels of steroidogenic output in rats (Van Thiel et al., 1975) and in mice (Badr and Bartke, 1974). Serum testosterone levels in those rodents were indeed drastically reduced and it was suggested that reduced food intake associated with chronic ethanol ingestion was not responsible for the depressed testicular function (Van Thiel et al., 1975). In those studies, however, gonadotropin receptor contents were not measured and causes of reduced steroidogenic output by the testis remained speculative.

Previous studies from this laboratory have suggested that gonadotropin receptors in testicular homogenates were lost following *in vitro* ethanol treatment and that high-affinity gonadotropin binding sites could be recovered

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in the soluble form (Bhalla and Reichert, 1974a,b; Bhalla et al., 1976; Bhalla, 1977, 1978). The *in vivo* effect of ethanol on LH/FSH receptor concentrations in rat testis has not been studied.

This study describes the process of LH/hCG and FSH receptor depletion following ethanol administration *in vivo*. When ethanol was withdrawn, receptor replenishment commenced and the gonadotropin receptor contents in rat testis were fully restored in 20 days. To rule out artifacts due to methodology in estimation of B_{max} values by Scatchard analysis of the binding data under our experimental conditions, parallel studies were also carried out with *in vivo* administration of either hLH or hFSH. The results support our previous observations that ethanol soluble factors (Bhalla and Reichert, 1974; Bhalla et al., 1976; Bhalla, 1977, 1978) may be involved in the modulation of testicular gonadotropin response.

MATERIALS AND METHODS

Mature male rats of the Sprague-Dawley strain (∿50–60 days old and weighing ∿250–300 g) were chosen for these studies. They were kept under standard conditions of temperature (23–24°C) and light. Chloramine T was purchased from Eastman Kodak, magnesium chloride and sodium metabisulphite from Mallinckrodt, sucrose from Fisher Scientific Co., egg albumin (twice crystallized) from Schwartz/Mann, carrier-free Na[^{125}I] from Amersham-Searle Corp.

Hormones

Highly purified hLH (LER 960 and 11/19) and hFSH (LER 1991) prepared by Dr. L. E. Reichert, Jr., of Emory University in Atlanta, Georgia, were supplied by the NPA and NIAMDD, NIH, Bethesda, MD. The biologic activity of the hLH preparation was 4760 IU/mg and of the hFSH preparation was 3600 IU/mg. LER-907 used as a standard in radioimmunoassay of LH was obtained from NPA and NIAMDD. It had a biologic potency of 20 IU of FSH and 60 IU of LH/mg.

Preparation of [^{125}I]-labeled hLH and [^{125}I]-labeled hFSH

Gonadotropins (hLH and hFSH) were iodinated by the chloramine T method, modified to allow the retention of biologic activity as described previously (Bhalla and Reichert, 1974a; Bhalla et al., 1976). Separation of radiolabeled hLH or hFSH from other reagents was accomplished by gel filtration through Sephadex G-100. The specific activities and the recoveries of the labeled hormones were calculated by the method of Hunter and Greenwood (1962). The specific activities of [^{125}I]-labeled hLH or hFSH were 18–20 μCi/μg. The labeled hormones were diluted to a final concentration of 7.5×10^{-14} moles (2.5 ng)/50 μl using 0.01 M phosphate buffer (pH 7.5) containing 5 mM $MgCl_2$, 0.1 M sucrose and 0.1% (w/v) egg albumin (egg albumin-phosphate buffer) and stored frozen in several vials.

In Vivo Administration of hLH

Adult male rats of the Sprague-Dawley strain (60 days old, 250–300 g body weight) were divided into 14 groups of 8 rats each. Rats in the first 6 groups received 1 i.p. injection of hLH (LER 11/19, 32 IU/injection) at 1000 h and were sacrificed by groups 6 h later at 1600 h and on Days 1, 2, 3, 7 and 11 after the injection. Rats in the second 6 groups received 2 injections of hLH (LER 11/19, 32 IU/injection, 1 injection/day for 2 consecutive days) and were also sacrificed by groups 6 h later at 1600 h and on Days 1, 2, 5, 9 or 14 following the last injection of hLH. The remaining 2 groups of rats (controls) received injections of saline and were sacrificed at 6 h and on Day 12 following the injection. The purpose of the control was to detect and quantitate any age-dependent alteration in LH receptor concentration. The rats were sacrificed by ether asphyxiation. Testes were removed for binding studies and blood collected for the determination of serum hLH and hFSH levels (see below).

In Vivo Administration of hFSH

Rats were divided into 7 groups (5 rats/group), 5 of which received a single injection of hFSH (LER 1991; 30 IU/rat) and were sacrificed 6 h later and on Days 2, 3, 7 and 11. The remaining 2 groups of rats served as controls as described previously. Blood samples were also collected as described above and the sera obtained were stored frozen for FSH radioimmunoassay.

In Vivo Administration of Ethanol

Ethanol in saline (20%, v/v) was administered i.p. to 9 groups of male rats weighing 250–300 g. Each group of rats (9–13 rats/group) received 2 injections (5 ml each) of ethanol saline every day at 0900 h and at 1300 h for 7 days after which the ethanol was withdrawn. The animals were sacrificed at the indicated time intervals (Days 1, 3, 5, 7, 9, 11, 13, 15 or 20). In this regimen that group of rats sacrificed on Day 1 received only 2 injections of ethanol and the group sacrificed on Day 7 received 14 injections of ethanol. After Day 7, ethanol was withdrawn during the receptor replenishment phase for those rats sacrificed on Days 9, 11, 13, 15 or 20. A group of control rats received similar injections of 0.9% saline without ethanol and it was found that saline injections did not affect the receptor site concentrations. Serum samples were also collected and stored as described above.

Preparation of 360 × g Pellets

The testicular homogenates (360 × g pellets) used in gonadotropin binding studies were prepared as follows: testes of rats from each group were pooled, decapsulated, suspended in egg ablumin phosphate buffer in a ratio of 1 ml buffer/g of tissue and homogenized in a teflon pestle tissue grinder. The homogenate was filtered through a single layer of cheesecloth, then centrifuged at 360 × g for 10 min at 4°C. The supernatant was removed and the pellet was recentrifuged at 20,000 × g for 10 min at 4°C to expel

excess buffer. The compact pellets were then weighed on a Mettler analytical balance and stored frozen in separate vials under liquid N_2.

Binding Studies

The binding assays were carried out using 360 × g pellets which were stored frozen in vials under liquid N_2 in semisolid form (unsuspended in buffer, see above). Tissue preparation stored this way has been found to yield reproducible results and the loss of receptors which takes place when tissue preparation is suspended in buffer during thawing is appreciably reduced (unpublished observations). The testicular homogenate prepared from pooled testes was divided among several vials, each containing ∿3–4 g of the homogenate. The content of each vial was resuspended in buffer so that every 50 μl of the suspension was equivalent to 20 mg (wet tissue weight) of the homogenate. Binding assays were performed in a total reaction volume of 250 μl using 2.5 ng of [^{125}I]-labeled hLH or [^{125}I]-labeled hFSH in the presence of a wide range of the mass of homologous unlabeled hormone (0, 1, 1.5, 2, 2.5, 3, 3.5, 5, 10, 15, 20, 25, 50, 75, 100 and 1000 ng) at a homogenate concentration of 20 mg (wet tissue weight) which was equivalent to ∿1.28% of the wet weight of a whole decapsulated testis. All the reactants were predissolved in egg albumin phosphate buffer [0.01 M phosphate buffer, pH 7.5, containing 5 mM $MgCl_2$, 0.1 M sucrose and 0.1% (w/v) egg ablumin]. The assay mixture was incubated at 37°C in a metabolic shaker for 30 min and the tubes were centrifuged at 1500 × g for 20 min. The supernatants were discarded and pellets counted for radioactivity in a Beckman gamma 310 counter.

For each set of experiments, there were tubes which contained only [^{125}I]-labeled hormone in the quantity used in that particular study (total count tubes). In addition, there were tubes which contained all reactants except homogenate (blank tubes). The blank tubes were treated in the same manner as assay tubes (Bhalla, 1978) and the radioactivity associated with them was subtracted from that of the assay tubes to yield radioactivity due to the binding of homogenate alone. The binding of the radioligand to glass tubes was comparable to that found in the presence of excess unlabeled hormone concentrations (Bhalla, 1978).

Data Analysis

The testicular homogenate (360 × g pellet) used in binding was prepared from testes pooled from the 5–13 rats sacrificed at each indicated time interval after hormone or ethanol administration. This procedure was adopted to acquire sufficient experimental material for the necessary assays to be carried out. The statistical information given in each figure legend pertains to the linear regression analysis performed on the data from each assay undertaken in duplicate and averaged. The averages of the duplicate data points were converted into hormone saturation curves and then to Scatchard plots according to the procedures described earlier (Charreau et al., 1978; Tsuruhara et al., 1977; Bhalla, 1978). The method of unweighted least squares and a Hewlett-Packard 9830 A computer were used to calculate the parameters for various lines of regression. The relative quality of the data (probability values) was assessed by applying Student's t test and by considering the significance of parameter values derived from each regression. The Standard Errors (SE) for serum LH, FSH and ethanol concentrations were calculated using a Hewlett-Packard 9830 A computer.

Measurements of Serum Alcohol and Serum hLH and hFSH

Serum alcohol levels were measured by Dr. Robert E. Sobel of the Department of Pathology of the Medical College of Georgia, Augusta, GA according to the method described by Bergmeyer (1965). Serum hLH and hFSH were assayed by radioimmunoassay technique using double antibody with LER 907 as an internal standard (Midgley, 1966).

RESULTS

Effects of a Single Injection of hLH on the Concentration of Testicular hLH Receptors

After a single injection of hLH (32 IU/rat), the concentration of gonadotropin binding sites in rat testis was slightly increased at 6 hours[2] and declined thereafter (Fig. 1, upper panel). By then, most of the radioligand binding to testicular receptors was not associated with the high affinity binding class, thereby resulting in a horizontal slope (Fig. 1, upper panel, see below). After a dramatic decrease in receptor site concentrations at Day 3, the process of receptor replenishment began and the testicular gonadotropin contents were fully restored to normal values by Day 11 (Fig. 1, lower panel) to Day 14 (data not shown).

At each of the homogenate concentrations tested, the binding isotherms were found to be linear when total hormone present did not exceed 20 ng (a combination of 2.5 ng labeled and 15 ng unlabeled hormone). The binding detected within this range of hormone concentrations is due to the high affinity class of hormone binding sites. However, deviation from linearity occurred when hormone mass

[2] The apparent B_{max} values (apparent number of hormone binding sites) obtained from control animals at 6 h were similar to those obtained from untreated controls (0 h). The apparent number of hLH binding sites at Day 1 after a single injection of hLH was negligible and that for Day 7 was slightly lower than the value obtained for Day 11 (2.2 ng/20 mg tissue). The data points from those days are not shown for the sake of clarity. There was considerable overlapping between those data points and those for Day 2 and Day 11, respectively.

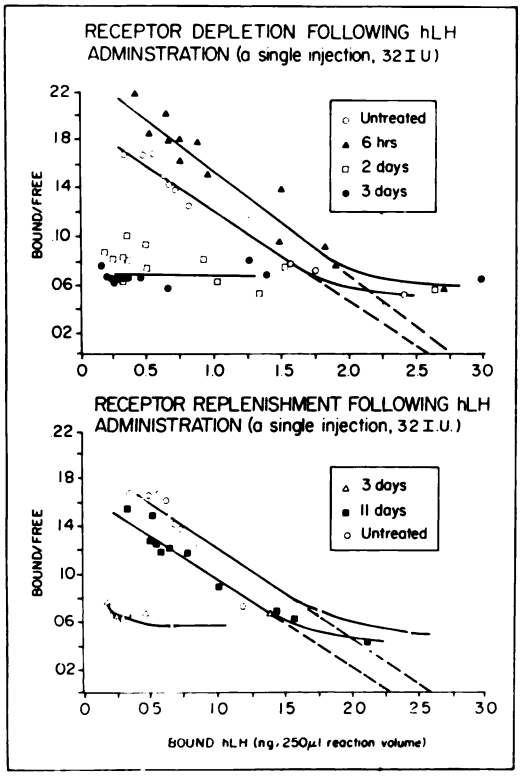

FIG. 1. Scatchard plots of the binding data obtained using testicular homogenate prepared from rats sacrificed at different time intervals following a single injection of hLH (32 IU).

The upper panel shows the Scatchard plots of the binding data obtained using rat testicular homogenates prepared from rats sacrificed at 6 h, 1 or 3 days following a single injection of hLH. The testicular homogenates were prepared as described under Materials and Methods and the binding studies were carried out in duplicate as described previously (Bhalla, 1978). The competitive inhibition plots were converted to hormone saturation curves and then to Scatchard plots as described under Materials and Methods. The equation for the line corresponding to "Untreated" (control) is $y = -0.076x + 0.20$ ($r = 0.97$; $P<0.001$). The equation for the line corresponding to 6 h is $y = -0.087x + 0.24$ ($4 = 0.98$; $P<0.001$). The x-axis intercept values (B_{max}) for "Untreated" and 6 h are 2.64 and 2.81 ng/250 µl, respectively.

Lower panel shows the Scatchard plots of the binding data obtained using testicular homogenate prepared from rats sacrificed at Day 11. The equations for lines corresponding to "Untreated" and 11 days are $y = -0.076x + 0.20$ and $y = -0.074x + 0.171$, respectively. The regression coefficients (r) are 0.97 for both lines and the P values are <0.01. The B_{max} values for "Untreated" and 11 days are 2.64 and 2.33 ng/250 µl, respectively.

was greater than 20 to 25 ng (data not shown). This behavior resulted in data points which fall on a horizontal line as discussed previously. Because this binding was displaceable by high unlabeled hormone concentrations in the range of 25–300 ng, we feel that this residual binding of the radioligand is associated with a second class of binding sites (Fig. 1). Heterogeneity in hFSH binding sites has been established (Bhalla and Reichert, 1974c; Abou-Issa and Reichert, 1976; Maghuin-Rogister et al., 1978); this phenomenon has now been detected for gonadotropin (Bhalla, 1978; Rao, 1978) and other hormones such as glucagon (Sonne et al., 1978).

To determine whether the reduction in binding sites was the result of increased receptor occupancy at the time of animal sacrifice rather than of a reduction in their number, serum hLH levels were measured using a double-antibody radioimmunoassay (Table 1). When serum gonadotropin levels were high, receptor number was not reduced but increased slightly (upper panel of Fig. 1; Table 1) and when serum gonadotropin values returned to normal levels at Day 2, testicular receptor contents continued to decline (Fig. 1; Table 1). A similar relationship existed between the serum FSH levels and FSH binding sites in rat testis (Table 2). In both cases, the responses were somewhat inconsistent with the concept of receptor occupancy; in the former case, a rapid association of hormone with receptor sites was expected and, in the latter instance, the rate of dissociation by the assumption of receptor occupancy was somewhat slower. It took ~8–10 days before the number of binding sites was fully restored (Fig. 2, lower panel). To clarify further the relationship between hormone and receptor during the binding process, an experiment was performed in which 6 groups of rats were treated with 2 i.p. injections of hLH/day for 2 consecutive days.

Effect of 2 Injections of hLH

After the second injection, the animals were sacrificed according to the schedule given in Materials and Methods. The Scatchard plots of the binding data obtained are shown in Fig. 2. At 6 h after the second injection, when circulating levels of gonadotropins were persistently high (Table 1; upper panel of Fig. 2), the gonadotropin receptor concentrations were still detectable but declined to only negligible values by Day 1 (48 h after the first injection or 24 h after the second). This time period correlated well with the loss of receptors observed at 48 h after a single injection of hLH (Fig. 1). The

TABLE 1. Serum hLH levels in animals after a single and after 2 injections of hLH. Mean ± SEM.

Particulars	Serum hLH values (ng/ml)[a]	
	Single injection	2 injections
Untreated	46.6 ± 8.2	46.6 ± 8.2
6 h	1138.4 ± 116.9	1392.8 ± 178.4
Day 1	126.8 ± 218.8	93.5 ± 4.2
Day 2	52.6 ± 6.4	63.0 ± 0.5
Day 3	43.7 ± 6.6	...[b]
Day 5	...[b]	46.5 ± 5.8
Day 7	54.0 ± 9.6	...[b]
Day 9	...[b]	42.0 ± 6.4
Day 11	43.4 ± 8.9	...[b]
Day 14	44.2 ± 8.2	45.5 ± 8.9

[a]The Standard reference used was LER 907.

[b]Animal sacrifice was not scheduled at that time.

[3]The level of ethanol in blood considered lethal for human beings is in the range of 400–500 mg/100 ml (Haggard et al., 1940; Haag et al., 1951). For rats, the value is considerably higher, 890–1000 mg/100 ml (Haggard et al., 1940; Haag et al., 1951). The route of administration does not affect the absorption of ethanol; the LD_{50} values for ethanol administered orally, s.c. and i.p. are the same (Mailing, 1970). The clearance rate for alcohol is 628 mg/kg BW/h for 30-day-old rats; it is 330 mg/kg/h for 68-day-old rats (Wallgren, 1970). For rats used in our study (60-day old, 250–300 g average BW) this means a clearance of 83–100 mg of ethanol/hour. According to Table 3, the serum ethanol content in rats receiving ethanol was 320–431 mg/100 ml at the time of sacrifice (1600 h) and was negligible by 2400 h. This would still leave enough time for these animals to feed themselves. The body and testicular weights of the experimental animals were indeed found to be normal and comparable to those of the controls during the entire course of the study. Due to the relatively short period of ethanol administration (1 week as compared to 4 or more weeks for some studies), pair-fed controls and liquid diet were not used. This system, however, is being employed to study the effect of prolonged administration of ethanol on the depletion and replenishment processes of testicular gonadotropin receptors.

[4]The B_{max} value for untreated controls in Fig. 3 is 3.56 ng/20 mg tissue as compared to untreated controls in Figs. 1 and 2 (2.63 ng/20 mg tissue). The reason for this discrepancy is unclear, but we now have the evidence which indicates that the density of gonadotropin receptor/unit mass of testicular homogenates changes with age of the experimental animals (unpublished observations). Concerning the variations in these two studies, it is believed that the age of animals, as provided by the suppliers, was slightly overestimated yielding high B_{max} values reported in Fig. 3 and elevated FSH levels reported in Table 3. See Dohler and Wuttke (1975) for details on changes in serum gonadotropins as a function of animal age.

response, as discussed previously, was difficult to explain on the basis of receptor occupancy. The process of receptor replenishment was also too slow (10–12 days following depletion) to be explained adequately by hormone dissociation of occupied binding sites (see below). We feel that both of these results can be explained equally well by receptor dissociation and new receptor synthesis (Bhalla, 1978; Rojas and Bhalla, 1979).

In Vivo Injection of hFSH

The number of hFSH receptor sites also decreased following a single injection of hFSH, although the difference was more apparent on Day 3 than on Day 1 or 2 (Table 2). The relationship between the estimated number of FSH binding sites in testis and serum FSH concentrations was very similar to that seen with hLH (see above).

Effects of In Vivo Injections of Ethanol

In vivo administration of ethanol[3] (5 ml of 20% ethanol in saline; 2 i.p. injections/day/rat for 7 days) resulted in a progressive diminution of LH and FSH receptor contents in rat testis (upper panels of Figs. 3, 4). The number of LH and FSH binding sites/20 mg tissue (Fig. 3 for hLH; Fig. 4 for hFSH) increased slightly by Day 1 and thereafter decreased ∿10–12% by Day 5 (after 10 injections) and ∿30–50% by Day 7 (after 14 injections). These differences were statistically highly significant[4] and varied depending upon the age of the experi-

TABLE 2. B_{max} values for hFSH in testicular homogenates and serum hFSH levels in rats sacrificed at different time intervals following a single injection of hFSH (30 IU). Mean ± SEM.

Particulars	B_{max} values[a] (ng/250 μl/20 mg homogenate)		Serum hFSH levels[b] (ng/ml)
Untreated	0.36	r>0.96; P<0.001	172.1 ± 13.3
6 h	0.35	r>0.96; P<0.001	9015.2 ± 119.7
Day 2	0.33	r>0.96; P<0.001	418.3 ± 13.0
Day 3	0.24	r>0.89; P<0.05	240.6 ± 21.4
Day 7	0.35	r>0.79; P<0.05	151.9 ± 12.7
Day 11	0.35	r>0.94; P<0.01	138.3 ± 20.1

[a] B_{max} values were obtained from Scatchard analysis. The Scatchard data were analyzed as described in Materials and Methods.

[b] Serum hFSH levels expressed here were based upon LER 907 as standard.

mental animals. In certain preliminary studies, we have found that under identical experimental conditions aqueous ethanol administered *in vivo* depleted LH/hCG binding sites from testes of 100-day-old rats much more effectively than from testes of younger animals (unpublished observation). This observation could be the result of binding sites being synthesized at a faster rate in younger animals than in older ones. This postulate is under current investigation. Withdrawal of ethanol after Day 7 resulted in replenishment of gonadotropin receptors; normal values were attained after Days 18–20 (lower panels of Figs. 3, 4). In spite of chronic administration of aqueous ethanol, the mortality of rats was less than 1% and the body weights were comparable to normal control rats. Serum alchohol levels during the receptor depletion and replenishment phases are shown in Table 3. During the entire study, gonadotropin levels remained normal (Table 3).

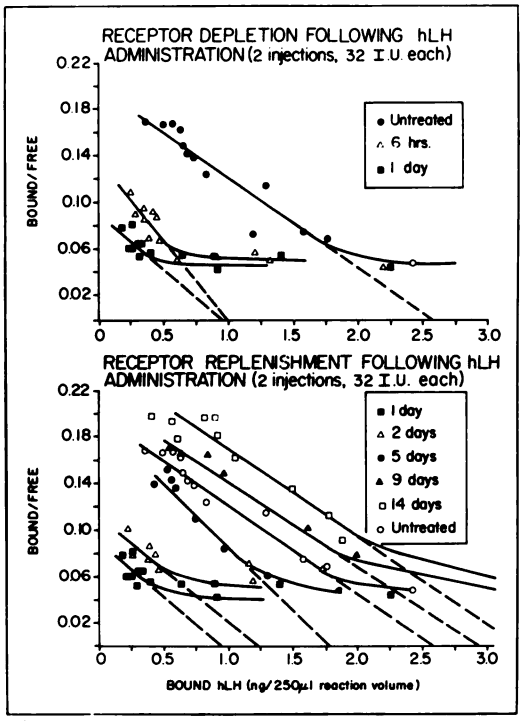

FIG. 2. Scatchard analysis of the binding data obtained using testicular homogenate prepared from rats at different time intervals following two injections of hLH (32 IU/rat/day for 2 consecutive days). The binding experiments were carried out as described under the legend of Fig. 1 except that testicular homogenates were prepared according to the experimental protocol described under Materials and Methods.

Upper panel shows the Scatchard analysis of the binding data obtained from testicular homogenates prepared from rats sacrificed at 6 h or 1 day following 2 injections of hLH. The equations for lines corresponding to "Untreated" and 6 h are $y = -0.076x + 0.20$ ($r = 0.97$; $P<0.001$), $y = -0.14x + 0.14$ ($r = 0.86$; $P<0.01$), respectively. The B_{max} values for those lines given in the same order and including the one on Day 1 are 2.643, 1.004 and 0.974 ng/250 μl, respectively.

Lower panel shows the Scatchard plots of the binding data obtained using testicular homogenates prepared from "Untreated" rats and from rats sacrificed at 1, 2, 5, 9 or 14 days following the last injection of hLH. The equations for lines corresponding to 2, 5, 9, 14 days and "Untreated" are, respectively: $y = -0.094x + 0.091$; $y = -0.089x + 0.11$ ($r = 0.75$, $P<0.1$); $y = -0.108x + 0.20$ ($r = 0.97$, $P<0.001$); $y = -0.069x + 0.22$ ($r = 0.99$, $P<0.001$); $y = -0.071x + 0.24$ ($r = 0.94$, $P<0.001$); and $y = -0.076x + 0.20$ ($r = 0.97$, $P<0.001$). The B_{max} values for the lines given in the same order are 1.27, 1.82, 3.11, 3.35 and 2.64 ng/250 μl, respectively. The regression analysis for Day 1 is not given.

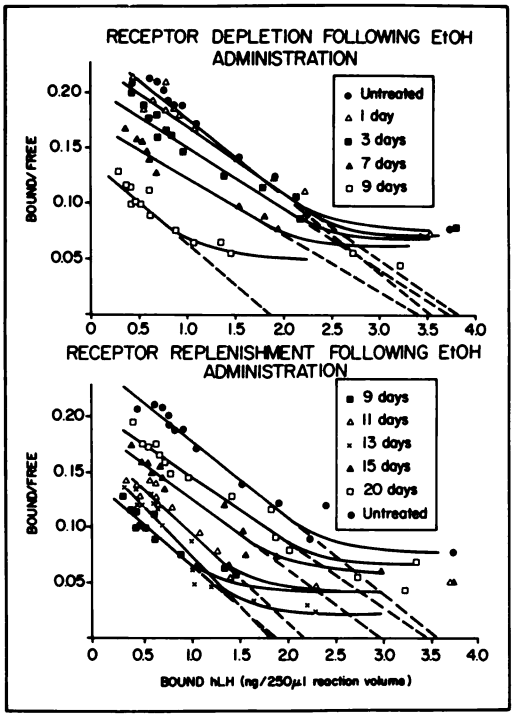

FIG. 3. Levels of LH receptors in rat testis during ethanol administration and ethanol withdrawal. The procedures for *in vivo* ethanol injections are given in Materials and Methods. The rats were sacrificed at different time intervals and testicular homogenates were prepared. The binding experiments were performed as described in Materials and Methods.

Upper panel shows the Scatchard analysis of the binding data obtained using testicular homogenates prepared from rats sacrificed on Day 1, 3 or 7 (during the period of ethanol administration). Ethanol was withdrawn on Day 7; 1 group of rats were sacrificed on Day 9 and the data shown along with those exposed to ethanol. The equations for lines corresponding to "Untreated," Days 1, 3, 7 and 9 are, respectively: $y = -0.070x + 0.25$ ($r = 0.99$, $P<0.001$); $y = -0.061x + 0.24$ ($r = 0.97$, $P<0.001$); $y = -0.055x + 0.21$ ($r = 0.97$, $P<0.001$); $y = -0.097x + 0.20$ ($r = 0.94$, $P<0.001$); and $y = -0.074x + 0.14$ ($r = 0.91$, $P<0.001$). The B_{max} values for the various lines in the same order are 3.56, 3.85, 3.78, 2.04 and 1.90 ng/250 µl, respectively.

Lower panel shows the Scatchard plots of the binding data obtained using testicular homogenates prepared from rats sacrificed at 9, 11, 13, 15 or 20 days from the beginning of EtOH administration (or 2, 4, 6, 8 or 13 days after withdrawal of EtOH following a 7-day EtOH administration schedule). The equations for the lines corresponding to "Untreated," Days 9, 11, 13, 15 and 20 are, respectively: $y = -0.070x + 0.25$ ($r = 0.99$, $P<0.001$); $y = -0.074x + 0.14$ ($r = 0.91$, $P<0.001$); $y = -0.08x + 0.18$ ($r = 0.97$, $P<0.001$); $y = -0.091x + 0.17$ ($r = 0.94$, $P<0.001$); $y = -0.065x + 0.19$ ($r = 0.96$, $P<0.001$); and $y = -0.059x + 0.21$ ($r = 0.97$, $P<0.001$). The x-intercept values for the lines in the given order are 3.56, 1.90, 2.19, 1.82, 2.98 and 3.47 ng/250 µl, respectively.

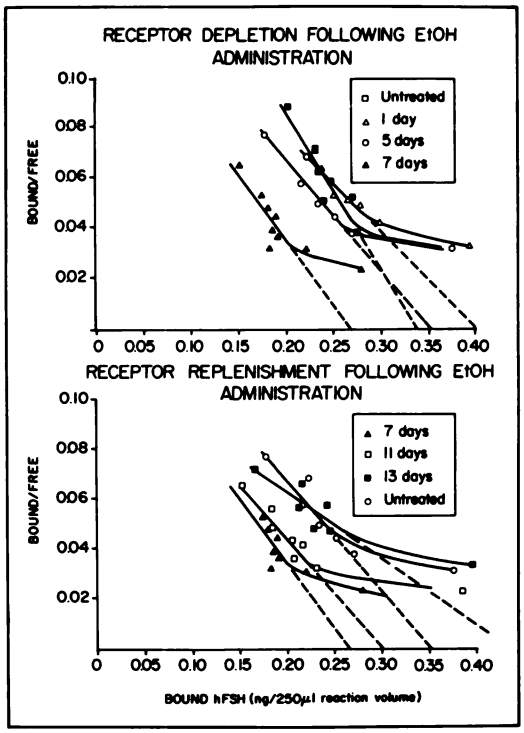

FIG. 4. Levels of FSH receptors in rat testis during ethanol administration and ethanol withdrawal. The experiment was carried out as described under Fig. 3 except that FSH receptor levels were measured rather than LH receptors.

Upper panel shows the Scatchard plots of the binding data at Days 1, 5 and 7. The equation for lines corresponding to "Untreated," 1, 5 and 7 Days are, respectively: $y = -0.44x + 0.16$ ($r = 0.93$, $P<0.001$); $y = -0.38x + 0.15$ ($r = 0.94$, $P<0.01$); $y = -0.61x + 0.21$ ($r = 0.91$, $P<0.01$); and $y = -0.51x + 0.14$ ($r = 0.84$, $P<0.01$). The x-intercept values for the various lines in the same order are 0.34, 0.40, 0.36 and 0.27 ng/250 µl, respectively.

Lower panel shows the Scatchard plot of the binding data obtained using testicular homogenates prepared from "Untreated" rats and rats sacrificed at 7, 11 and 13 days from the beginning of ethanol administration (or 0, 4 and 6 days after withdrawal of EtOH following an EtOH administration schedule of 7 days duration). The equations for lines corresponding to "Untreated," 7, 11 and 13 days are, respectively: $y = -0.44x + 0.16$ ($r = 0.93$, $P<0.01$); $y = -0.51x + 0.14$ ($r = 0.84$, $P<0.01$); $y = -0.43x + 0.13$ ($r = 0.95$, $P<0.001$); and $y = -0.28x + 0.12$ ($r = 0.08$, $P<0.05$). The x-intercept values for the lines in the given order are 0.36, 0.27, 0.30 and 0.43 ng/250 µl, respectively.

DISCUSSION

Alterations in the number of LH-specific receptor sites detectable in ethanol treated rats appear to be the result of receptor loss rather

TABLE 3. Serum alcohol and gonadotropin levels during the ethanol-induced depletion and replenishment phases of LH/hCG and FSH receptors in rat testes. Mean ± SEM.

Particulars[a]	Serum alcohol levels (mg/100 ml)	Serum gonadotropin levels (ng/ml)[c]	
		LH	FSH
Untreated	0	42.9 ± 8.9	201.1 ± 17.7
Day 1	431.0 ± 4.3	45.0 ± 8.8	228.3 ± 10.9
Day 3	431.0 ± 0.3	43.9 ± 8.8	230.1 ± 11.9
Day 5	396.0 ± 0.8	41.4 ± 7.3	188.1 ± 16.9
Day 7[b]	320.0 ± 5.7	38.2 ± 7.9	139.4 ± 9.3
Day 9	0	40.8 ± 8.8	165.2 ± 22.3
Day 11	0	43.5 ± 8.4	175.5 ± 21.6
Day 13	0	42.0 ± 7.4	157.4 ± 20.4
Day 15	0	38.9 ± 5.8	174.6 ± 16.4
Day 20	0	42.9 ± 6.9	159.4 ± 12.7
Untreated	0	65.0 ± 22.0	150.1 ± 14.8

[a]Serum alcohol and gonadotropin levels were estimated at indicated time intervals during the ethanol administration schedule. Details of alcohol and hormone assays are given in Materials and Methods.

[b]Denotes the time when ethanol administration was discontinued.

[c]Values are based on LER 907 standards.

than of occupancy. This has been directly demonstrated by the measurement of serum LH levels during the entire course of ethanol administration. Serum LH levels remain normal and the negative regulations of ethanol on LH receptor contents remain superficially similar to those brought about by the hormone itself (Hsueh et al., 1976, 1977; Haour and Saez, 1977; Purvis et al., 1977; Sharpe, 1976). The effects of ethanol, however, are not tissue-specific. Although LH selectively depletes its own receptors and the levels of LH receptors remain unaffected by FSH under the experimental conditions used, the present study has clearly shown that both LH and FSH receptors can be affected by ethanol; as a result, Leydig and Sertoli cell functions are probably also affected. These findings may explain why problems such as impotency, reduced steroidogenesis and testicular atrophy are frequently encountered in chronic alcoholics.

The decrease in LH/FSH receptors after ethanol administration parallels the inhibition of hormone-specific adenylate cyclases (unpublished observations). Therefore, the number of high affinity receptor sites in the testis is a critical factor in determining the maximal attainable cAMP production (Rojas and Bhalla, 1979).

Although the precise mechanism by which ethanol and gonadotropins deplete LH/FSH receptors is unknown, we believe that these effects are brought about through membrane perturbation processes. Similar effects were seen when testicular tissues were treated with ethanol *in vitro* (Bhalla and Reichert, 1974a,b), and soluble factors which bind LH and FSH with high affinity and specificity were recovered in tissue-free extracts following *in vitro* ethanol treatment (Bhalla et al., 1976; Bhalla, 1977, 1978). Although we favor receptor loss via shedding into the surrounding fluid as one of the possible mechanisms of receptor down-regulation (Bhalla, 1978), there is other evidence which seems to support the concept of hormone and/or hormone-receptor complex internalization (Ascoli and Puett, 1978; Goldstein and Brown, 1977). The relationship between either of these two hypotheses and physiologic response remains to be determined. Other facets of the problem which need to be investigated are the fate of the binding sites after gonadotropin binding and the processes involved in the biosynthesis of these binding sites. Studies to correlate the concentration of ethanol-soluble factors with the levels of adenylate cyclase activity are in progress to resolve these issues.

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