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Modulation of testicular lutropin receptors in the developing male rat

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Abstract. In the developing male rat around 40 days of age, the testis appears to contain the maximum amount of lutropin receptors per unit weight. During this period, circulating levels of testosterone markedly increase without the concomitant major surges in lutropin levels. The increased sensitivity and responsiveness of tests to basal levels of circulating lutropin during this period is accompanied by enhanced serum prolactin levels suggesting that this hormone may be involved in this process. The finding that prolactin treatment of pubertal rats for 3 days induced the formation of more testicular lutropin receptors supports the above premise. However, short-term immunoneutralisation of endogenous prolactin did not significantly alter the specific binding of [^{125} I]-labelled lutropin to testicular membranes. Interestingly, during development, a close correction exists between receptor occupancy and capacity of the tissue to bind labelled lutropin. The apparent dissociation between serum lutropin levels, on the one hand and tissue occupancy and free receptor contents on the other, suggests that factors other than lutropin (presumably prolactin) are involved in the modulation of the sensitivity and the responsiveness of the testis to lutropin during early development.

Keywords. Radioreceptor assay; radioimmunoassay; tissue-bound hormone; prolactin; puberty; lutropin receptor; ontogeny.

Introduction

Recent investigations in the modulation of the testicular lutropin (LH) receptors in the rat have demonstrated that these receptors undergo marked fluctuations under several experimental conditions (Dufau *et al.,* 1978). Thus, hypophysectomy, chronic administration of large doses of LH, human chronic gonadotropin (hCG) or dexamethasone leads to 'down regulation of LH receptor' phenomenon accompanied by desensitisation of the testis (Thanki and Steinberger, 1976; Frowein and Engel, 1975; Sharpe, 1976; Hsueh *et al.,* 1976; Chen and Payne, 1977; Purvis *et al.,* 1977; Saez *et al.,* 1977; Raff, 1976). On the other hand, treatment with follicle stimulating hormone (FSH) has been shown to enhance significantly the LH receptors in the rat testis (Chen *et al.,* 1976; Ketelslegers *et al.,* 1978). Furthermore, there is now abundant evidence to show that prolactin (PRL) could augment testicular responsiveness to LH in terms of the steroid hormone, testosterone production implying a permissive role for PRL at the testis (Odell and Swerdloff, 1976; Bartke *et al.,* 1978).

* Present address: Department of Biological Science, Oakland University, Oakland, Michigan, U.S.A. Abbreviations used: Lutropin (LH), human chorionic gonadotropin (hCG), Follicle stimulating hormone (FSH), prolactin (PRL), rat PRL (rPRL) Ovine PRL (oPRL); antiserum (a/s).

In the present study, the ontogeny of LH receptors in the normal developing male rat has been examined to explore the possible existence of a temporal relationship among serum LH, PRL, testicular LH-receptor and receptor occupancy. In addition, the effects of PRL administration and its depreviation by specific immunoneutralisation on LH receptors in short-term experiments are described. A preliminary account of the work has been reported earlier (Prasad, 1978).

Materials and methods

Hormones and chemicals

Human lutropin (hLH), hCG, ovine PRL (oPRL), rat PRL (rPRL) and radioimmunoassay (RIA) kit for rat LH used in the present study were obtained through the courtesy of the Hormone Distribution Officer, NIAMDD, NIH, Bethesda Maryland, USA. Sephadex gels were obtained from Pharmacia, Uppsala, Sweden. Carrier-free [Na^{125} I] and [^{3} H]-testosterone (sp. act 90-105 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks, UK. Testosterone-antiserum used in the present study was a kind gift of Prof. N. R. Moudgal, Department of Biochemistry, Indian Institute of Science, Bangalore. All other reagents and chemicals were of reagent grade.

Animals

The male rats of Wistar strain were housed in cages and fed on pelleted diet (Hindustan Lever Products, Bombay), and water *ad libitum.* They were exposed to a 14 h light 10 h darkness schedule.

Antiserum to rat PRL

Specific antiserum (a/s) to rat PRL was raised in rabbits. The hormone (200 μg/0.5 ml) emulsified with an equal volume of Freund's complete adjuvant (Difco) was injected subcutaneously at weekly intervals for 4 weeks into the animal at multiple sites. The rabbits were bled through the ear vein 7 days after the last injection. The presence of antibodies in the serum was tested by Ouchterlony double diffusion analysis (Ouchterlony, 1967). The antiserum gave a single precipitin line with rPRL and did not cross-react with ovine growth hormone or rLH and failed to bind significant amounts of these hormones (iodinated with ^{125} I) as measured by the procedure of Moudgal *et al.* (1979). The titre of the antiserum was high since at equivalence point, 1.0 ml of it could neutralise 400 μ g of rPRL.

Radioimmunoassay of rPRL

The serum PRL concentrations of rats of different ages were determined by the modified radioimmunoassay procedure as described by Moudgal *et al.* (1979). Briefly, the serum samples were first incubated at 37°C for 10-12 h with rPRL antiserum at an appropriate predetermined dilution which would bind 20-40% of [^{125} I] -rPRL. At the end of this period, a known amount of the iodinated hormone (50,000 cpm, 1.0 ng) was added and the incubation continued for another 10-12 h at 37°C. The free and bound labelled hormones were separated by the addition of 1 ml of goat antiserum to rabbit IgG (double antibody) along with 0.1 ml of 1:50 diluted normal rabbit serum added as carrier. The precipitate formed after a further incubation for 10-12 h at 37°C was collected by centrifugation and counted for radioactivity in a Packard Autogamma Spectrometer (model 2002). The serum samples were assayed

in duplecates at two different levels, and PRL levels were expressed as ng/ml in terms of NIAMDD-rat-PRL-RPL Standard. The coefficient of variation for and between assays were 3% and 10% respectively.

Radioimmunoassay of testosterone

The method was based essentially on the procedure described earlier for other steroids (Niswender *et al.,* 1975). Briefly, the serum samples were extracted twice with 5 volumes of diethyl ether and the ether layer aspirated into separate tubes after freezing the lower aqueous (protein) phase in liquid nitrogen. The pooled ether extracts were evaporated in a water bath at 45°C and reconstituted with 1.0 ml of RIA buffer (0.01 M sodium phosphate buffer, pH 7.5 containing 0.9% NaCl+ 1% gelatin). Routinely, RIA for testosterone was carried out in triplicate. In 10×75 cm glass tubes were included, 100 μ 1 of either the steroid fractions extracted from serum samples, or testoerone standards. Then, 100 μ 1 of [^{3}H]-testosterone (10,000 cpm) and 100 μ 1 of 1:4,000 diluted testosterone-a/s were added. The total volume was made upto 500 μ 1 with the RIA buffer and the tubes incubated at 4°C for 6 h. The nonspecific binding of [^{3}H] -testosterone was determined by omitting the a/s, whereas the total binding to the a/s was computed by omitting the non-radioactive testosterone standard.

At the end of the incubation, free and bound testosterone were separated by adding 500 μ 1 of dextran-coated charcoal (0.05% dextran-70 and 0.5% acid-washed charcoal in RIA buffer without gelatin). The contents of the tubes were quickly mixed and incubated at 4°C for exactly 10 min. They were then centrifuged at 5,000 × g for 10 min at 4°C. The radio-activity in supernatants was measured using toluene-triton x-100 scintillation cocktail in a Beckman LS-100 liquid scintillation spectrometer.

The nonspecifically bound radioactivity was always deducted from the total bound radio-activity and the standard curve constructed by plotting pg of testosterone against the % specific binding. The sensitivity of the RIA was in the range of 5 to 10 pg.

Preparation of [^{125} *I*]*-human lutropin for receptor assay*

The chloramine-T method of Hunter and Greenwood (1962) was not suitable for preparing the labelled hLH for receptor assays. However, a slight modification of this method to minimise the damage, reproducibly yielded [^{125} I]-hLH suitable for hormone-receptor binding studies. Briefly, the iodination reaction was performed in a 10×75 mm glass tube kept in crushed ice. To the tube containing hLH (20 μ g) approximately 500 μ Ci of Na^{125}I was added. The iodination reaction was initiated by the addition of 50 μ 1 of 1 mg/ml chloramine-T and allowed to continued for 30 sec with occasional shaking. The reaction was then stopped by the addition of 50 μ 1 of 5 mg/ml sodium metabisulphite solution. The entire reaction mixture was applied to a Sephadex G-75 column (15 ml bed volume), pre-equilibrated in 0.05 M Tris-HCl (pH 7.6) containing 0.15 M NaCl and 0.5% ovalbumin. The column was eluted with the same buffer and the eluent collected in 0.5 ml fractions. The peak fraction containing labelled hormone was used directly for the receptor assays and had a specific activity of 75,000 cpm/ng protein.

Preparation of receptor-rich membranes

The crude membrane used for testicular receptor assays was prepared as follows: rats of different ages (3-4 animals/group) were killed by decapitation, testis excised and weighed. The decapsulated tissue was minced, and homogenised in a ground-glass homogeniser by hand at 0-4°C using 10 vol of (w/v) 0.02 M Tris-HCl (pH 7.6) buffer containing 0.15 M NaCl. Receptor-rich pellets were obtained by centrifugation at 20,000 g for 20 min. The membranes were washed three times with the initial buffer and finally resuspended in 0.02 M Tris-HCl (pH 7.6) buffer containing 0.15 M NaCl and 10 m M $MgCl_2$ such that the membrane concentration corresponded to 100 mg wet weight of testicular tissue per m 1 of the buffer. These preparations were immediately used for the assay (Reichert *et al.,* 1973).

Incubation procedure for the assessment of [^{125}I]*-human lutropin binding*

Incubations were performed at room temperature in 10 mm × 50 mm glass tubes in a total volume of 0.5 ml. Both the labelled hormone and the membrane preparations were diluted using 0.02 M Tris-HCl (pH 7.6) buffer containing 10 mM $MgCl_2$, 0.15 M NaCl and 0.1% ovalbumin. The binding reaction was initiated by adding an aliquot of approximately 1-1.5 ng [^{125}I]-hLH ($7.5\times10^4 - 1\times10^5$ cpm) to the membrane preparations. Incubations were performed for 12 to 16 h in all the experiments. Binding assays were conducted at two different concentrations of the membranes (equivalent to 10 and 20 mg tissue/tube) in triplicate. Reactions were terminated by dilution with the cold (5°C) buffer (1.0 ml) followed by centrifugation at 20,000 g for 30 min. The supernatants were discarded and the radioactivity in the pellets was measured in a Packard Autogamma Spectrometer (Model 2002).

Total binding refers to the radioactivity bound to the pellet in the absence of added unlabelled hormone. Non-specific binding is represented by the radioactivity bound in the presence of 1000-fold excess (1 μg) of unlabelled hormone. Specific binding was obtained by substracting the nonspecific from the total binding, and was expressed as per cent of the total radioactive hormone added to the incubation tubes. The nonspecific binding was 6 to 8%.

In separate experiments, it could be shown that the specific binding of [^{125} I]-labelled LH to the testicular membranes was not influenced by 1 μ g each of oPRL and ovine growth hormone (oGH) further showing the specificity of the binding of labelled hormone. The Scatchard analysis of the binding data with 40 day old rat testis preparation showed that the binding occurred with $K_a = 0.25\times10^{10} M^{-1}$ and the plots were linear.

Measurement of tissue-bound and serum levels of lutropin

Testis-bound LH was measured by radioimmunoassay carried out at the elevated temperature of 37°C (Muralidhar and Moudgal, 1976; Sheela Rani and Moudgal, 1978). Briefly, the decapsulated testes were homogenised in 5 vol (w/v) of 0.05 M sodium phosphate buffer (pH 7.4), containing 0.5 M EDTA and 0.15 M NaCl at 0-4°C. The homogenate was filtered and suitable aliquots were directly used for LH assay. Ally samples were assayed at two different levels and the amounts of labelled LH nonspecifically bound in the absence of any added a s was determined.

LH in the tissue homogenates and serum samples was measured by the modified RIA procedure using NIAMDD rat LH-RIA kit. Incubations at all stages of the RIA were carried out at 37°C. Briefly, the procedure consisted of incubating the tissue

or serum samples with the rat LH a/s for a period of 10-12 h at 37°C. This was followed by the addition of [^{125} I] labelled rat LH and a further incubation at 37°C for 10-12 h; then goat antibody to rabbit IgG (double antibody) was added to precipitate the bound label and the incubation continued for an additional period of 10-12 h. At the end of this incubation, the tubes were centrifuged at 3,000 g for 30 min and the precipitate obtained was used for bound radioactivity measurement. it was independently determined that the intraassay variation was minimal (8%) and non-target tissue homogenates did not interfere in the assay (Sheela Rani and Moudgal, 1978). The validity and reproducibility of the above mentioned modified RIA method to quantitate the tissue bound hormones have been amply demonstrated earlier by others in this laboratory (Muralidhar and Moudgal, 1976; Sheela Rani and Moudgal, 1978). Recovery experiments have revealed that there was no loss of LH during incubation at 37°C.

Results

Influence of time and temperature on the specific binding of [^{125} I]*-hLH to testicular membranes*

As shown in figure 1 the binding of labelled hLH to testicular membranes was dependent on time and temperature of incubation. At 37°C, equilibrium was attained after 4 h of incubation. Although the rate of specific binding was initially lower at

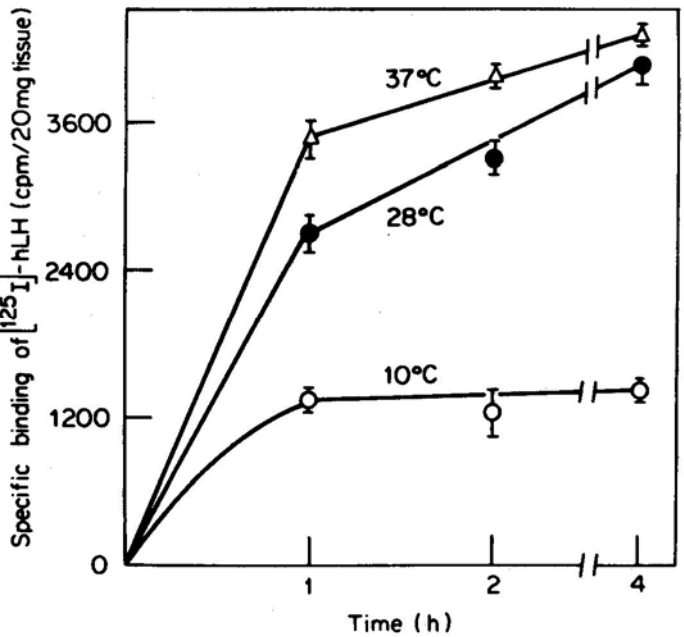

Figure 1, Effect of time and temperature on the specific binding of [^{125} I]-human LH to the testicular membrane preparation. Crude testicular membranes from five animals were prepared and incubated at 0°C, 28°C and 37°C for different periods with [^{125} I]-human LH (50–70×10^3 cpm) in triplicate. Nonspecific binding was determined for each set by including 1 μg of hLH. Data presented are Mean ± S.D. (n=5). The experiments were repeated three times with similar results.

28°C the binding increased with time and ultimately at 4 h approached the values obtained at 37°C. On the other hand, specific binding continued to remain low (~ 50%) at 0°C even after 4 h of incubation. Further studies have shown that incubation at room temperature (28°C) for a period of 12-16 h could give maximum specific binding and therefore most of the studies reported hereafter were carried out by incubating membrane preparatons with [^{125} I]-hormone for 12-16 at 28°C.

Displacement of labelled lutropin by unlabelled hormones

To demonstrate the specificity of hormonal binding, the testicular membrane preparations were incubated with fixed amount of [^{125} I]-hLH with varying amounts of unlabelled hormones. Data presented in figure 2 clearly show that all the three hormones (hCG, ovine LH, and rat LH) could compete for the binding sites and that among these, hCG was the most effective. The unequivocal ability of these hormones to compete effectively for the binding sites testify the physiological relevance of this receptor interaction. Further, as low as 100 ng hCG was sufficient to cause 50% reduction in the specific binding, while a 10-fold excess (1000 ng) of LH was needed for a comparable effect.

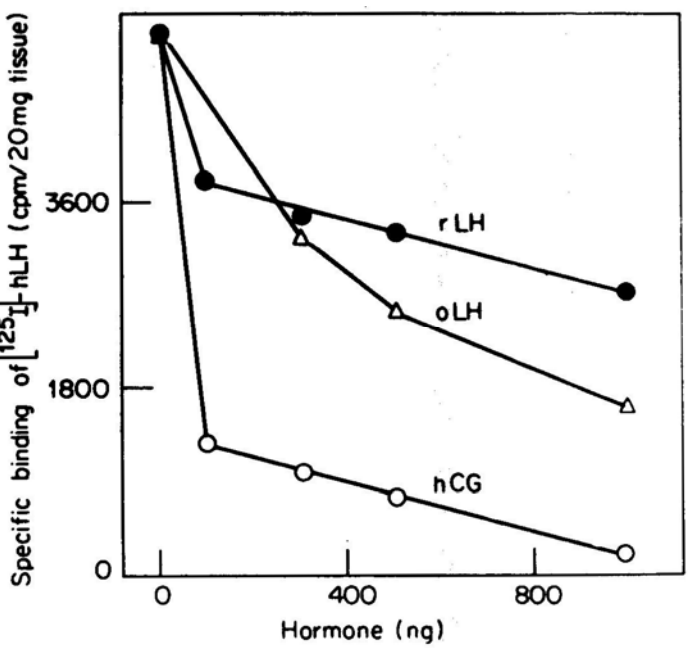

Figure 2. Displacement of [^{125} I]-human LH from testicular receptors by unlabelled hormones. The optimal conditions for hormone binding were described earlier. Binding assay was carried out in duplicate in the presence of known amounts of unlabelled hormones (hCG, ovine LH or rat LH) as competing species. Labelled hormone bound to membrane in the presence of 5 μ g of these hormones was taken as the corresponding nonspecific binding. For further details see text. The experiments were repeated three times employing six animals each time with essentially similar results.

Ontogeny of testicular lutropin receptors–relation to circulatory lutropin, testosterone and occupancy

In order to investigate the developmental changes in the LH receptor content of the testicular tissue, receptor content in testicular membrane preparations from groups of rats of different age were determined. The circulatory and tissue-bound LH levels and serum testosterone concentrations as well as the fresh weights of testis were determined to examine the possible relationship among these parameters. It may be seen (figure 3) that testicular weight increased steeply (nearly 10-fold) between 20 and 40 days of age and continued to increase thereafter, though at a much reduced rate. During this time, there was no significant change in serum LH levels while circulatory testosterone levels (figure 3) showed a mere two-fold increase by day 40 which was followed by a more dramatic increase thereafter.

To gain an insight into this increased ability of the testis (despite exposure to basal levels of circulatory LH) to elaborate enhanced steroid hormone *in vivo* after 40 days of age, it was important to examine the ability of the tissue to bind LH. When the total tissue contents of LH and free LH receptors were measured, the picture

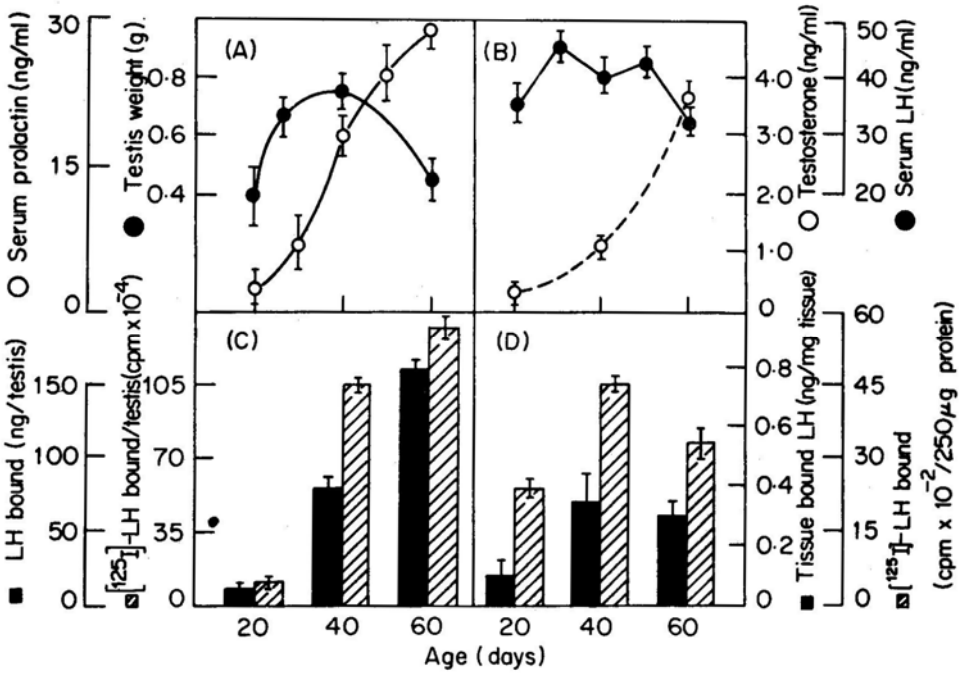

Figure 3. Ontogeny of testicular LH receptors–relation to circulatory LH, T and occupancy. A. Fresh weights of testis and serum PRL levels in 20, 40 and 60-day old rats. Data presented are the Mean ± S. D. of 3 animals/group. B. Serum T and LH levels measured by the specific radioimmunoassays. Data experiments were repeated 4 times with similar results. C. [^{125} I]-hLH total binding capacity/testis from 20, 40 and 60-day old rats. LH bound to testis of 20, 40 and 60-day old rats, measured by the modified RIA. The data represent Mean ± S.D. of 5 animals/group. D. [^{125} I]-hLH binding capacity/unit membrane protein. Amount of LH bound *in vivo* (occupancy) to the testicular membrane protein (250 μ g). The data represent Mean ± S. D. of 5 animals/groups. For further details see text.

that emerged is summarised in figure 3C. It may be seen that with increase in age an almost parallel enhancement in tissue-bound LH as well as total free receptor contents were clearly evident. The increased tissue-bound LH (occupancy) accompanied by corresponding increase in the free receptor content are in excellent agreement with observed increased production of the androgen. Further, a closer examination of figure 3C shows that at 40 days of age, there appears to be a relatively greater number of receptors available for LH binding compared to day 20 or 60. This probably explains the greater capacity of the testis to respond to LH acquired in the course of development of the animal (around day 40). Supporting evidence stems from the results given in figure 3D, pertaining to the capacity of unit testicular membrane protein to interact specifically with [^{125} I]-hLH. It is clear that there was a marked increase in the ability of the membrane preparations to bind LH around 40 days of age to be followed by a marginal decrease there after. Interestingly, parallel changes were observed in the case of tissue-bound LH also.

Modulation of testicular lutropin receptors by prolactin status of the animal

An examination of serum PRL levels during development revealed the existence of peak levels of this hormone around 40-50 days of age (figure 3B) suggesting that the elevated levels of this hormone (PRL) might have altered the ability of the testis to respond to LH at this stage. In order to establish the possible involvement of PRL in the enhancement of free LH receptors in the testis, 60 day-old rats were treated for 3 days with oPRL (1 mg/100 gm body weight) or a potent a/s to rPRL. It is clear from table 1 that treatment with oPRL could infact significantly increase the LH-binding

Table1. Change in LH receptor contents in the testis following treatment with ovine PRL and rat PRL a/s *in vivo* in the male rat.

Treatment	[^{125} I]-hLH bound (cpm)/20 mg tissue (Mean ± S.D.)
None (control)	7239±289
Ovine PRL	11620±321*
PRL a/s	7892±140

Sixty-day old male rats (4 animals/group) were treated for 3 days with PRL (1 mg/day/100 gm body weight) or rat PRL a/s (0.2 ml/day/rat). Specific binding of [^{125} I]-hLH to testicular membranes prepared from each animal was determined as described in the text. Control animals receiving 0.2 ml/animal of normal rabbit serum had their testicular LH receptors equal to untreated controls. The experiment was repeated three times with similar results.

* Significant compared with control ($P < 0.01$) .

capacity of the testis. However, the deprivation of PRL by a/s treatment for 3 days had no marked effect on testicular LH-binding capacity.

The amount (0.2 ml/day) of the potent rPRL a/s administered to each animal would have neutralised 80 μ g of the hormone *in vitro,* and is therefore considered sufficient to neutralise a large proportion of the endogenous hormone whose levels were around 20 ng/ml, the highest during the development (figure 3).

Discussion

The developmental changes in testicular LH receptors, circulatory LH and PRL were determined in the male rat to examine the possible mode of interaction between these two hormones at the receptor levels on the Leydig cells culminating in a dramatic increase in serum testosterone levels after 40 days of age. This incidentally also provided an opportunity to validate several recent claims (Bartke *et al.,* 1978) that PRL directly influences testicular function. Following systematic characterisation of specific LH receptors on testicular membranes, the modulation of their contents during developments in relation to circulatory gonadotropin (LH) levels was examined particularly in view of the recent findings (Lesniak and Roth, 1976) that the primary regulators of the availability of receptors in the respective target tissues are the corresponding hormones themselves. Our data (figure 3) on LH receptor and serum testosterone levels in the developing male rat are of interest in this connection as they show the absence of any correlation between serum LH levels (nearly constant) and the corresponding contents of either the total tissue-bound LH or the free hormonal receptors. The content of both tissue-bound and free LH receptors markedly increased with the age of the animal accompanied by an increase in testicular weight which may be reflection of increased number of Leydig cells during development (Ketelslegers *et al.,* 1978). Low levels of tissue-bound LH as well as the free LH receptors (total as well as per unit weight) thus clearly explain the relative refractoriness of the immature (20 day) rat testis to LH. More intriguing are the data concerning the lack of parallelism between the increase in serum testosterone contents and corresponding dramatic enhancement in testicular weights (> 10-fold; figure 3) during 20-40 days of age (unlike during 40-60 days of age). The most likely explanation for this lag in the steroid hormone output by the testis during the earlier days is provided by the results depicted in figure 3C, which show nearly doubling of the "specific activity" of both the [^{125} I]-LH binding (i.e. per unit tissue weight) and the tissue-bound LH (i. e. per unit membrane protein) around day 40, followed by a slight decrease around day 60. In other words, the marked increase in the capacity of the unit weight of the testis to interact with available LH appears to enable the tissue to harbour enhanced amount of LH from circulation (figure 3C). This greater ability acquired may be one of the factors contributing to the secretion of relatively larger amounts of testosterone after 40 days. This enhanced capacity is, in fact, disproportionate to the testicular weight increments (hence presumably to increase in number of Leydig cells) thereafter. Supporting evidence for this premise stems from the data of figure 3C which show that around day 40, relatiely greater amount of total free LH receptors are available than are occupied (by tissue bound LH) *vis-a-vis* days 20 or 60. But around day 60, a relatively greater proportion of measureable receptors are occupied by the hormone, thus explaining the pronounced increase in circulatory testerone. However, at this time, the capacity of the unit membrane protein to interact with [^{125} I]-LH seems to be slightly reduced. The pattern *viz.,* relatively low levels of total tissue bound LH around day 20, followed by marked increase around day 40, registering further increase thereafter appears to be in concert with the corresponding levels of circulatory testosterone.

One of the likely candidates responsible for initiating this marked change in testicular capacity to bind LH and hence to elaborate increased amount of testosterone appears to be PRL. Presumptive evidence for this postulate is provided by the elevated levels of serum PRL around 40-50 days of age (figure 3). The demonstration that exogenous PRL significantly enhanced both the specific (per unit membrane protein) and total testis LH receptor content is in agreement with the finding that enhanced PRL levels in circulation coincides with augmented testosterone production *in vivo* in 60 days old rat. Pertinent at this juncture are the data of Zipf *et al.,* (1978) showing that in hypophysectomised rats, chronic PRL treatment alone prevented the loss of LH receptor whereas PRL in combination with low levels of LH enhanced the testicular LH receptors. It is realised that enhanced LH receptor content and occupancy alone is not sufficient to stimulate testosterone production by Leydig cells and that biosynthetic processes should also be accelarated. It is relevant to recall that increased responsiveness to exogenous LH during sexual maturation (Odell *et al.,* 1974) coincided with increased LH receptors (Ketelslegers *et al.,* 1978). Also of significance in this context are the findings of others (Hafiez *et al.,* 1971; Musto *et al.,* 1972) that PRL increases precursor availability and stimulates some of the enzymes (3 β and 17-∝-hydroxy steroid dehydrogenases) involved in androgen production.

It is noteworthy that short-time PRL deprivation by immunological neutralization did not significantly alter LH receptor content. Inadequate neutralisation does not appear to be a likely explanation for this negative result since the dosage of the potent PRL a/s would have been adequate to neutralise around 80 μ g of circulatory rPRL/day. Thus it would appear that PRL is dispensable at least for short duration in terms of the maintenance of LH receptor content and this may be related to the turnover rate of the receptors. Also noteworthy is the demonstration of Hauger *et al.* (1977) that selective reduction of plasma gonadotropins for 3-6 days by immunoneutralisation with anti-LHRH serum had no effect on testicular receptor. But Argona *et al.* (1977), employing 2-bromoergocryptine (CB-154) to inhibit PRL release in immature rats have shown significant reduction in testicular LH receptors. The reasons for this apparent discrepancy between our data and those of Aragona *et al.* (1977) are not clear at present and may be related to such factors as the age/strain of the animals and/or the duration and modes (drug vs antibody) of PRL deprivation employed.

However, even though the testis of PRL-treated rats displayed higher LH receptor contents, their responsiveness *in vitro* to high level of LH (10 μg/ml) was not significantly different from the control tissue in terms of steroidogenic capacity (0.4±0.025 ng/h/ml medium/g wet tissue, control vs 0.45±0.03 ng/h/ml medium/g wet tissue, PRL-treated). Since only a small portin (1%) of total available LH receptors need be occupied for maximal steroidogenic response (Dufau *et al.,* 1978) this could have been easily accomplished under *in vitro* conditions employing supraphysiological doses of LH. While a strict correlation between receptor contents and testicular responsiveness to LH has not been demonstrated with an intact tissue, it is conceivable that under *in vivo* conditions, PRL-induced elevated LH receptor content may be of physiological relevance as a possible mechanism enabling the testis to harbour more of LH and hence respond progressively to a greater extent (Dufau and Catt 1978) in the absence of any major surges in the circulatory LH levels. The demonstration of specific PRL receptors on Leydig cells (Aragona *et al.,* 1977) on which LH acts to stimulate testosterone production is of relevance in this context.

Since there is no evidence for an effect of PRL on Leydig cell numbers, it appears that PRL increases the number of LH receptors per Leydig cell.

The data presented here, however, do not exclude other physiological factors exerting coordinated influence in enhancing testicular responsiveness to available LH. For example, Odell and Swerdloff (1976) have suggested that FSH has a significant role to play in this regard especially during sexual maturation. High circulatory levels of FSH (Nandini *et al.,* 1976; Ketelslegers *et al.,* 1978) during days 21 or 32 in the male rat appear to be an essential factor to be reckoned with in this context. More recent demonstration that chronic FSH administration to 15-day old immature rats significantly enhanced total testicular LH receptors which was not strictly proportional to increase in testicular weight suggests that the FSH is involved in the induction of LH receptors (Chen *et al.,* 1977; Marah, 1976). But there was no temporal relationship between LH receptor content arid *in vitro* testicular responsiveness to LH under these conditions, and hence the relevance of these observations to the data described herein is not clear at present. It is conceivable that during early developmental period of the male rat, multiple hormones interact coordinately at the testicular level to enhance the tissue capacity to secrete the androgen by increasing either the number of total Leydig cells, LH receptor content or the capacity of the tissue to bind and respond to LH.

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References

Aragona, C., Bohnet, H. G. and Friesen, H. G. (1977) *Endocrinology,* **99,** 1011.
Catt, K. J. and M. L. Durfau (1976) *Biol. Reprod.,* **14,** 1.
Chen, Y. D. and A. H. Payne (1977) *Biochem. Biophys. Res. Commun.,* **74,** 1589.
Chen, Y. D., Shan, M. J. and Payne A. H. (1977) *Mol. Cell. Endocrinol.,* **8**, 291.
Chen, Y. D., Payne, A. H. and Kelch, R. P. (1976) *Proc. Soc. Exp. Biol. Med.,* **153**, 473.
Dufau, M. L., Hsuch, A. J., Cigorranga, S., Baukal, A. J. and Catt, X. J. (1978) *Int. J. Andorlogy.,* Suppl. **2** Part 1, p. 193.
Dufau, M.L. and Catt, K. J. (1978) *Vitam. Horm.,* **36**,461.
Frowein, J. arid Engel, W. (1975) *J. Endocrinol.,* **64,** 59.
Hauger, R. L., Kelch, R. P., Chen, Y. D. I. and Payne, A. (1977) *J. Endocrinol.,* **75,** 23.
Hsueh, A. J. W., Dufau, M. L. and Catt, K. J. (1976) *Biochem, Biophys, Res. Commun.,* **72,** 1145.
Hunter, W. M. and Greenwood, F. C. (1962) *Nature* (*London*)*,* **194,** 495.
Hafiez, A. A., Philpott, J. E. and Bartke, A. (1971) *J. Endocrinol.,* **50,** 619.
Ketelslegers, J. M., Helzel, W. D., Sherins, R. J. and Catt, K. J. (1978) *Endocrinology,* **103,** 212.
Lesniak, M. A. and Roth, J. (1976) *J.Biol. Chem.,* **251,** 3720.
Marsh, J. M. (1976) *Biol. Reprod.,* **14,** 30.
Moudgal, N. R., Muralidhar, K. and Madhwaräj, H. G. (1979) *Pituitary gonadotropins in methods of homone radioimmunoassay,* 2nd edition, eds. B. M. Jaffe and H. R. Behrman (New York: Academic Press) p. 173.
Muralidhar, K. and Moudgal, N. R. (1976) *Biochem. J.,* **160,** 603.
Musto, N., Hafiez, A. A. and Bertke, A. (1972) *Endocrinology,* **91**,1106.
Nandini, S. G., Lipner, H. arid Moudgal, N. R. (1976)*Endocrinology,* **98,** 1460.
Niswender, G. D., Akbar, A. M. arid Nett, T. M. (1975) *Methods Enzymol.,* **36,** 16.
Odell, W. D. and Swerdloff, R. S. (1976) *Recent Prog. Horm. Res.,* **32,** 245.

Odell W. D., Swerdloff, R.S., Bain, J., Wollesen, F. and Grover, P. K. (1974) *Endocrinology,* **95,** 1380.
Ouchterlony, O. (1967) Immunodiffusion and Immunoelectrophoresis: in *Handbook of experimental immunology,* ed. D. M. Weir (Oxford: Blackwell Scientific Pub.) p. 655.
Prasad M. S. K. (1974) *J. Steroid Biochem.,* **9,** 829.
Purvis, K., Torjesen, P. A., Haug, E. and Hanson, V. (1977) *Mol. Cell. Endocrinol.,* **8,** 73.
Raff M (1976) *Nature* (*London*)*,* **259,** 265.
Reichert, L. E, Leidenberger, F. and Trowbridge, C. G. (1973) *Recent Prog. Horm. Res.,* **29,** 497.
Saez, J. M., Morers, A. M., Haour, F. and Evain, D. (1977) *Endocrinology,* **101,** 1256.
Sharpe R. M. (1976) *Nature* (*London*)*,* **264,** 644.
Sheela Rani, C. S. and Moudgal, N. R. (1978) *Mol. Cell. Endocrinol.,* **11,** 293.
Thanki, K. H. and Steinberger, A. (1976) *Endocr. Res. Commun.,* **3,** 49.
Zipf, W. B. Payne, A. H. and Kelch, R. P. (1978) *Endocrinology,* **103,** 595.

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