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Regulation of Leydig Cell Function by Prolactin, Growth Hormone and Luteinizing Hormone

By

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The effects of prolactin (PRL), growth hormone (GH) and luteinizing hormone (LH) on testicular LH receptor concentration and on testosterone synthesis in response to LH (testicular responsiveness) was studied in mature intact and hypophysectomized rats. Hypophysectomy reduced LH receptor concentration by 80 % and testicular responsiveness to LH by 70 %, 7 days after surgery. Daily treatment with LH initiated immediately following surgery resulted in a further dose-dependent decrease in LH receptors and a dose-dependent increase in testicular responsiveness. Loss of LH receptors was not due to occupancy of the receptor by exogenous LH. PRL (150 μg/day) or GH (150 μg/day) partially prevented the loss of LH receptors in hypophysectomized saline-treated rats. The effect of PRL plus GH on LH receptor concentration was additive. The combination of LH (5 μg/day), PRL and GH prevented any loss of LH receptors after hypophysectomy. A positive effect of LH on its receptor occurred in the presence of PRL. Treatment of hypophysectomized rats with 5 μg LH + 150 μg PRL enhanced the effect observed with PRL alone on maintenance of LH receptors. PRL, administered together with higher doses of LH (25 or 50 μg/day) prevented LH from exerting a negative effect on LH receptor concentration. Similar treatment with GH plus LH neither allowed a positive effect of LH nor prevented higher doses of LH from exerting a negative effect on LH receptor concentration. Loss in testicular LH receptors was also demonstrated in intact rats which received a single administration of LH. Administration of 150 μg PRL for 3 days partially prevented the LH-induced loss in LH receptors, while treatment with 150 μg GH had no effect on LH-induced loss of testicular LH receptors.

Despite the ability of PRL to increase LH receptor concentration in hypophysectomized rats, PRL treatments did not enhance testicular respon-

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siveness to LH. Only when LH was administered together with either PRL or GH was testicular responsiveness to LH maintained at intact control values. These data indicate that 1) maintenance of testicular LH receptor concentration in adult hypophysectomized rats is dependent on the combined effects of PRL, GH and LH; 2) PRL not only prevents LH from exerting a negative effect, but allows LH to have a positive effect in the hypophysectomized rat on the testicular LH receptor; 3) PRL and GH appear to act at different sites and by different mechanisms; and 4) hormonal regulation of LH receptor concentration appears to be distinct from hormonal regulation of testicular responsiveness to LH.

We previously reported that hypophysectomy of adult rats resulted in an 80 % loss of testicular LH receptors 7 days after surgery. Associated with this receptor loss was a 70 % reduction in testicular responsiveness to LH (Hauger et al. 1977a). Treatment of hypophysectomized rats with FSH, LH, FSH plus testosterone, testosterone, dihydrotestosterone or estradiol had no effect on maintenance of testicular LH receptor concentration. In fact, daily treatment of hypophysectomized rats with LH resulted in a further decrease in LH receptor concentration. In a subsequent study (Hauger et al. 1977b) we demonstrated that treatment of intact rats with anti-LHRH, estradiol or testosterone markedly reduced FSH and LH concentrations; however, these treatments did not result in a loss of LH receptors. These observations suggested that pituitary hormones other than gonadotropins are essential for maintenance of testicular LH receptors in the mature rat. It has been reported that prolactin treatment increases LH receptors in gonads of dwarf male mice (Bohnet & Friesen 1976) and in atrophic testes of light-deprived hamsters (Bex & Bartke 1977a). In addition, Aragona et al. (1977) demonstrated that in immature male rats, inhibition of prolactin (PRL) release by administration of 2α-Bromo-ergocryptine resulted in a decrease in testicular LH receptors. Specific receptors for PRL have been demonstrated in Leydig cells of rat testes (Charreau et al. 1977; Aragona et al. 1977). These observations suggest that PRL may be essential for maintenance of LH receptors in the rat Leydig cell. The present study was undertaken to evaluate the effects of PRL, growth hormone (GH) and luteinizing hormone (LH) on maintenance of testicular LH receptor content in adult hypophysectomized rats and on LH-induced loss of LH receptors in intact rats. We also investigated the relationship of changes in LH receptor concentration as a result of pituitary hormone treatments with changes in testicular responsiveness to LH as measured by serum testosterone concentration 2 h after a stimulatory dose of LH.

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Materials and Methods

Adult male Sprague-Dawley rats 70–90 days old were hypophysectomized between 08:00 and 16:00 h under ether anesthesia by a transauricular approach (Gay 1967). Sham operations were performed by furrowing into the sphenoid bone without entering the sella turcica. All animals were killed by decapitation between 08:00 and 12:00 h; testes, ventral prostates and seminal vesicles including the fluid were immediately dissected and weighed.

Hormones were administered subcutaneously either as a single dose or as twice daily injections as indicated in the figure legends. Hormone treatments in hypophysectomized rats were instituted within 6 h of surgery. Control animals received equal volumes of saline on the same injection schedule as treated groups. LH receptor concentration was determined by measuring specific binding of [^{125}I]hCG to aliquots of 20 000 × g testicular preparations as previously described (Hauger et al. 1977a; Chen & Payne 1977).

To determine testicular responsiveness to LH, serum testosterone was measured 2 h after administration of 25 μg LH intraperitoneally (ip). This amount of LH had no appreciable effect on [^{125}I]hCG binding capacity at 2 h in either intact or hypophysectomized rats. Trunk blood was collected after decapitation and serum testosterone was measured by a modified radioimmunoassay (Hauger et al. 1977a). In one experiment testicular testosterone concentration was also determined in order to establish that serum testosterone concentrations in response to LH stimulation, reflect new synthesis of testicular testosterone and not just release of testosterone. The means of results for different groups were tested for significant difference by Student's t-test and one way analysis of variance.

Results

The effects of hypophysectomy and LH treatment on LH receptor concentration and testicular responsiveness to LH are presented in Fig. 1. Seven days post hypophysectomy, LH receptor concentration was 20 % and testicular responsiveness to LH was 28 % of that found in saline-injected control rats. Treatment with LH at 5, 25 or 50 μg/day within 6 h of hypophysectomy caused a further dose-related decrease in LH receptor concentration and a dose-related increase in testicular responsiveness to LH. At 50 μg/day a 90 % loss in hCG binding capacity was observed. In a previous study we demonstrated that gentle homogenization of testes in 4 M $MgCl_2$ dissociates bound LH from its receptor without altering either the binding capacity or the binding affinity of the receptor (Chen & Payne 1977). To establish that the additional loss in LH receptor concentration in the hypophysectomized, LH-treated rats was not a
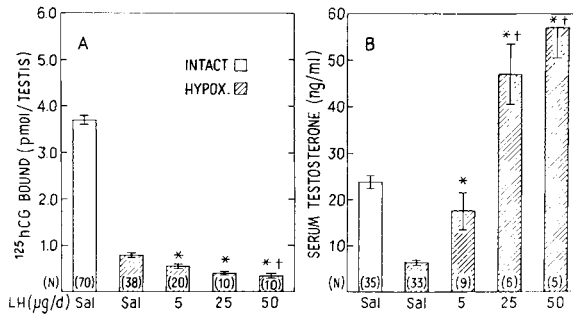
Fig. 1.

Effect of LH treatment on testicular LH receptors and responsiveness to LH.
Hormone treatment was administered sc twice daily for six days. Animals were
killed on the 7th day.

A. LH receptor concentration was measured by [^{125}I]hCG binding capacity (pmol/testis)
of aliquots of resuspended 20 000 × g testicular pellets.

B. Testicular responsiveness was determined by measuring serum testosterone (ng/ml)
2 h after ip injection of LH (25 μg).

Each value represents mean ± SE. (N) = number of rats. * significantly different from
hypophysectomized (hypox)-saline (sal) treated rats, $P < 0.01$. † significantly different
from hypox-LH (5 μg/d) treated rats, $P < 0.01$ (from Zipf et al. 1978b).

result of occupancy, LH receptor concentration was determined in contralateral
testes after bound LH had been dissociated with 4 M MgCl$_2$. LH receptor con-
centration in hypophysectomized, LH (50 μg/day) treated rats was 0.39 ± 0.1
pmol/testis in the MgCl$_2$ treated testes compared to 0.36 ± 0.1 pmol/testis in
the testes homogenized in buffered sucrose.

Table 1.

Effects of LH on weights of androgen-dependent organs from hypophysectomized
(hypox.) adult rats. Values expressed as mean ± SE.

Treatment group	(N)	Ventral prostate (mg)	Seminal vesicles (mg)
Sham operation + saline	(35)	310 ± 20	889 ± 40
Hypox. + saline	(44)	110 ± 10	252 ± 10
Hypox. + LH (5 μg/day)	(20)	200 ± 20	442 ± 30
Hypox. + LH (25 μg/day)	(10)	190 ± 20	554 ± 70
Hypox. + LH (50 μg/day)	(10)	339 ± 50	993 ± 70

Table 2.

Effect of hypophysectomy and daily LH treatment on LH receptor concentration and testicular responsiveness to LH*.

Treatment group	[125I]hCG binding pmol/testis	Testosterone			
		Serum (ng/ml)		ng/testis	
		–LH	+LH	–LH	*LH**
Sham operated + saline	4.07 ± 0.26	9.05	39.4	316	853
Hypophysectomy	0.63 ± 0.09	1.03	9.3	8.7	273
Hypophysectomy + 100 μg LH/day	0.32 ± 0.06	1.45	47.5	58.7	1090

* Adult rats were hypophysectomized and injected sc twice daily with saline or 50 μg LH for 6 days. Rats were killed 48 h after the last sc injection.

** Stimulation with 25 μg LH ip 2 h before killing.

The dose-related increase in testicular secretion of testosterone in response to LH was also reflected in the effects observed on the androgen-dependent organ weights as illustrated in Table 1. Seven days after hypophysectomy, ventral prostate and seminal vesicle weights had decreased to approximately $1/3$ of control values. An increase in the weights of seminal vesicles and ventral prostates was observed in all the hypophysectomized LH-treated rats.

The effect of hypophysectomy and LH treatment on LH receptor concentration, serum and testicular testosterone concentration and testicular responsiveness to LH is presented in Table 2. The data presented in this table illustrate that in hypophysectomized LH-treated rats, 48. h after the last daily administration of LH, intratesticular testosterone was less than 20 % and LH receptor concentration was less than 10 % of that found in intact control rats. Even though LH receptors were markedly reduced in these rats, a single ip injection of 25 μg LH resulted in higher intratesticular testosterone concentrations 2 h post injection than was observed in intact rats injected ip with the same dose of LH. This increase in testicular testosterone was reflected by a parallel increase in serum testosterone concentration. These data demonstrate that changes in serum testosterone concentration reflect an increase in testosterone synthesis in response to LH (testicular responsiveness).

The effect of PRL on LH receptor concentration as measured by the binding capacity of [125I]hCG is presented in Fig. 2 A. Administration of either 75 (not illustrated) or 150 μg PRL/day partially prevented the loss in LH receptors, which was observed in hypophysectomized saline-treated rats ($P < 0.05$).
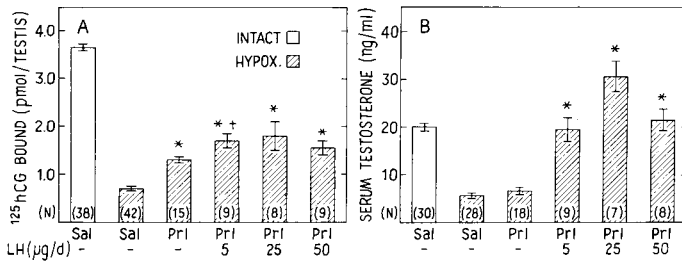
Fig. 2.

Effects of PRL (150 µg/d) and LH treatments on testicular LH receptors and responsiveness to LH. Hormone treatments were administered separately sc twice daily for six days. Animals were killed on the 7th day.

A. LH receptor concentration was measured by [^{125}I]hCG binding capacity (pmol/testis) of aliquots of resuspended 20 000 × g testicular pellets.

B. Testicular responsiveness was determined by measuring serum testosterone (ng/ml) 2 h after ip injection of LH (25 µg).

Each value represents mean ± SE. (N) = number of rats. * significantly different from hypophysectomized (hypox)-saline (sal) treated rats, $P < 0.01$. † significantly different from hypox-PRL treated rats, $P < 0.05$ (from Zipf et al. 1978b).
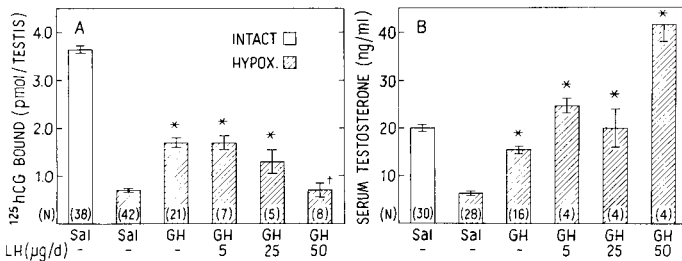

Fig. 3.

Effects of GH (150 µg/d) and LH treatments on testicular LH receptors and responsiveness to LH. Hormone treatments were administered separately sc twice daily for six days. Animals were killed on the 7th day.

A. LH receptor concentration was measured by [^{125}I]hCG binding capacity (pmol/testis) of aliquots of resuspended 20 000 × g testicular pellets.

B. Testicular responsiveness was determined by measuring serum testosterone (ng/ml) 2 h after ip injection of LH (25 µg).

Each value represents mean ± SE. (N) = number of rats. * significantly different from hypophysectomized (hypox)-saline (sal) treated rats, $P < 0.01$. † significantly different from hypox-GH treated rats, $P < 0.05$ (from Zipf et al. 1978b).
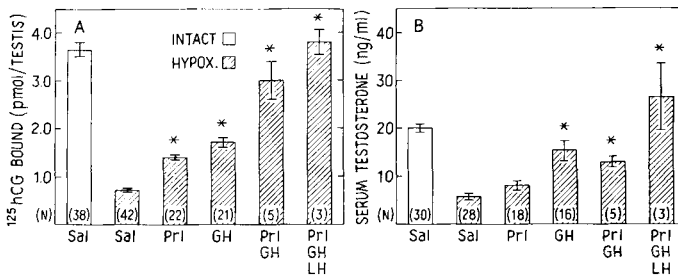
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Fig. 4.

Effects of PRL (150 μg/d), GH (150 μg/d) and LH (5 μg/d) treatments on testicular LH receptors and responsiveness to LH. Hormone treatments were administered separately sc twice daily for six days. Animals were killed on the 7th day.

A. LH receptor concentration was measured by [125I]hCG binding capacity (pmol/testis) of aliquots of resuspended 20 000 × g testicular pellets.

B. Testicular responsiveness was determined by measuring serum testosterone (ng/ml) 2 h after ip injection of LH (25 μg).

Each value represents mean ± SE. (N) = number of rats. * significantly different from hypophysectomized (hypox)-saline (sal) treated rats, $P < 0.01$ (from Zipf et al. 1978b).

No significant difference was observed between 75 μg/day and 150 μg/day. When 5, 25 or 50 μg LH/day was given with 150 μg PRL/day, PRL prevented the decrease in [125I]hCG binding that occurred when LH alone was administered daily to hypophysectomized rats as illustrated in Fig. 1 A. LH, 5 μg/day, significantly enhanced [125I]hCG binding capacity in the presence of PRL, $P < 0.05$ (Fig. 2 A).
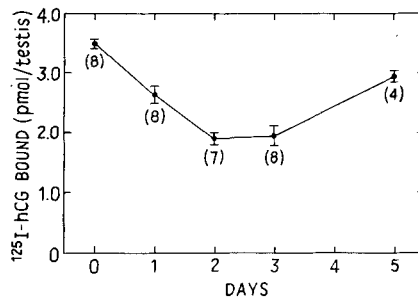

Fig. 5.

Effect of a single dose of 50 μg LH to intact adult rats on LH receptor concentration as measured by [125I]hCG binding.

Each point represents mean ± SE. ( ) = number of rats. LH receptor concentrations on all days after LH administration are significantly different from 0 day controls $P < 0.001$.
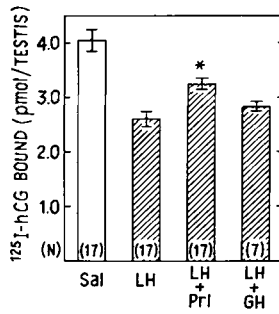
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Fig. 6.

Effect of PRL and GH treatment on LH-induced loss of LH receptors in intact adult rats. LH (50 µg) was administered sc between 8–9 a. m. Saline, PRL (150 µg/d) or GH (150 µg/d) was administered twice daily for 3 days. Animals were killed in the morning of day 4.

Each value represents mean ± SE. (N) = number of rats. * significantly different from LH + saline injected rats, $P < 0.01$.

Table 3.

Effect of increasing single dose of LH on LH receptor concentration as measured by [125I]hCG binding and testicular responsiveness to LH in adult rats.

Values expressed as mean ± SE.

Dose injected (µg)	[125I]hCG binding		Testicular responsiveness to LH	
	pmol/testis	per cent of control	serum testosterone ng/ml	per cent of control
0	3.5 ± 0.1	100	22.7 ± 2.2	100
30	2.04 ± 0.08*	58	18.2 ± 1.4	80
50	1.55 ± 0.23*	44	24.7 ± 4.1	109
100	1.64 ± 0.19*	47	10.7 ± 1.8*	47
200	1.44 ± 0.07*	41	13.3 ± 1.8**	59
1000	1.33 ± 0.17*	38	12.5 ± 1.2*	55

* significantly different from controls at $P < 0.01$.
** significantly different from controls at $P < 0.025$.

The effect of PRL with and without LH on testicular responsiveness to LH are presented in Fig. 2 B. Prolactin alone had no effect on testicular responsiveness despite its positive effect on hCG binding capacity. When 5 μg LH/day was administered together with 150 μg PRL/day, the observed increase in testicular responsiveness to ip LH was not different from that observed with 5 μg LH/day administered alone. However, PRL plus 25 or 50 μg LH/day resulted in significantly lower testicular response to LH compared to hypophysectomized rats treated with LH alone.

The effect of GH administration on LH receptor concentration is presented in Fig. 3 A. Administration of GH had a similar positive effect on maintenance of testicular hCG binding capacity in hypophysectomized rats as was observed with PRL. No significant difference between the effect of 75 μg/day (not illustrated) and 150 μg/day of GH was observed on the maintenance of [125I]hCG binding capacities. Addition of 5 μg LH/day to 150 μg GH/day did not enhance the effect observed with GH alone on hCG binding capacity (Fig. 3 A). However, 25 or 50 μg LH with 150 μg GH resulted in a dose-related decrease in hCG binding capacities compared to GH alone (Fig. 3 A). Treatment with GH resulted in an increase in testicular responsiveness to LH compared to saline-treated hypophysectomized rats (Fig. 3 B). Addition of LH to GH treatments increased responsiveness further.

The combined effect of GH (150 μg/day) plus PRL (150 μg/day) on maintenance of [125I]hCG binding capacity was additive as illustrated in Fig. 4 A. Addition of 5 μg/day of LH did not significantly enhance the effect of GH plus PRL. PRL plus GH or PRL, GH plus LH prevented any loss in [125I]-hCG binding capacity after hypophysectomy. Even though GH plus PRL maintained hCG binding capacities at values not different from intact saline-treated controls, responsiveness to LH was still below controls. The addition of 5 μg LH/day resulted in maintenance of testicular responsiveness at intact control values (Fig. 4 B).

Administration of a single 50 μg dose of LH to intact adult rats resulted in a 25 % loss of LH receptors ($P < 0.001$) 24 h after the injection. A further decrease to approximately 54 % of intact controls was observed at 2 days. No additional loss in LH receptors occurred between 2 and 3 days post injection. Five days after a single 50 μg dose of LH, testicular LH receptor concentration had not yet returned to intact control values (Fig. 5). The effect on LH receptor concentration and testicular responsiveness to LH 3 days after single doses of LH administered to intact rats is presented in Table 3. Increasing the dose of LH from 50 to 1000 μg resulted in very little additional decrease in LH receptor concentration. Although 30 or 50 μg LH resulted in 42 and 56 % loss in LH receptors, respectively, at 3 days no loss in testicular responsiveness to LH was observed (Table 3). Larger doses of LH which yielded a similar loss in LH receptor concentration to that seen with 50 μg of LH, resulted in

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approximately a 50 % loss in testicular responsiveness to LH. No relationship was observed between dose of LH (100–1000 μg) and extent of loss of testicular responsiveness at 3 days after a single dose.

The effect of daily PRL or GH treatment on the LH-induced loss of LH receptors is presented in Fig. 6. Intact rats treated with a single injection of 50 μg LH plus PRL (150 μg/day) for 3 days exhibited LH receptor concentration significantly higher than LH plus saline-treated rats. In contrast GH (150 μg/day) for 3 days had no effect on LH-induced loss of testicular LH receptors.

Discussion

Our studies on the role of pituitary hormones in Leydig cell function demonstrate that maintenance of testicular LH receptors in adult hypophysectomized rats is dependent on PRL, GH and LH. However, only LH appears to be necessary for maintenance of hormonal testosterone synthesis. As reported earlier (Zipf et al. 1978a) a direct relationship between LH receptor concentration and testicular responsiveness to a standardized stimulatory dose of LH was not observed.

It has previously been reported that the absence of pituitary hormones results in a loss of LH receptors (Hauger et al. 1977a; Hsueh et al. 1976; Thanki & Steinberger 1976) and in a decrease in testicular responsiveness to LH (Hauger et al. 1977a). In this study we demonstrate that daily treatment with LH initiated within 6 h after hypophysectomy results in a dose-related increase in responsiveness despite a further decrease in LH receptors. This indicates a dissociation between the negative regulation of LH receptors by LH and testicular responsiveness to LH. The studies in intact rats on LH-induced loss of LH receptors with increasing doses of LH, also indicate a dissociation between loss of LH receptors and loss in testicular responsiveness at concentrations of LH below 100 μg. At the higher doses of LH a parallel decrease in receptors and loss in testicular responsiveness to LH was observed. This is in agreement with a previous report from this laboratory (Zipf et al. 1978a). The decrease in testicular responsiveness observed at the higher doses of LH cannot be attributed solely to the decrease in LH receptor concentration, since a single injection of 50 μg LH results in a similar loss of LH receptors, but was not accompanied by a loss in steroidogenic responsiveness. This observation suggests that a single high dose of LH has a negative effect on steroidogenesis by a mechanism other than decreasing LH receptor concentration. This hypothesis is supported in recent studies reported by Sharpe (1977) and Tsuruhara et al. (1977). These investigators reported that testes or Leydig cells obtained from rats which had received a single administration of hCG were unable to respond to dibutyryl cyclic AMP and to hCG. This is consistent with the sug-

gestion that the lesion in testosterone synthesis as a result of *in vivo* adminis-
tration of hCG or high doses of LH is beyond cyclic AMP. Our studies differ
with those reported by Tsuruhara et al. (1977), in that loss of testicular LH
receptors induced by the administration of LH to intact rats does not exceed
60 % irrespective of dose administered and approximately the same loss in re-
ceptors is observed with a single 50 μg dose as is with a 1000 μg of LH.
Tsuruhara et al. (1977) reported that 10 μg hCG almost completely abolished
LH receptors. This difference is probably due to the large difference in half-
life of LH compared to hCG. It has been reported by Ascoli et al. (1975) that
the majority of iv administered oLH to male rats is cleared from the circula-
tion with a half-life of 5 min and this is independent of the injected amount
of hormone over a wide dose range. This is in contrast to hCG which was
reported to have a half-life of about 24 h in male rats (Hsueh et al. 1976).
Thus investigations which involve *in vivo* administration of hCG or high doses
of LH need to be interpreted with caution. From the studies in our laboratory
(Chen & Payne 1977; Zipf et al. 1978) and in other laboratories (Tsuruhara
et al. 1977; Sharpe 1977; Haour & Saez 1977) it can be concluded that once
LH binds to its specific receptor, that receptor and probably additional LH
receptors are lost. However, desensitization of steroidogenesis is not a result
of this loss of receptors and does not occur except with very high non-physio-
logical doses of LH or with the LH analogue, hCG. Therefore loss of receptors
caused by homologous hormone does not appear to provide a mechanism by
which testosterone synthesis in the Leydig cell becomes unresponsive to further
stimulation by LH.

It has previously been reported that PRL increases testicular LH receptors
in atrophic testes of light-deprived hamsters (Bex & Bartke 1977a), in dwarf
male mice (Bohnet & Friesen 1976) and in rats treated with 2α-bromo-ergo-
cryptine (Aragona et al. 1977). In hamsters, maintained in a short photoperiod,
treatment with GH also resulted in an increase in testicular LH binding (Bex
& Bartke 1977b). The present study demonstrates that both PRL and GH treat-
ment partially prevent the loss of testicular LH receptors in hypophysecto-
mized rats. The effects of PRL and GH are additive which suggests that PRL
and GH act at different sites in the testis. Specific receptors for PRL have
been demonstrated in Leydig cells of rat testes (Aragona et al. 1977; Charreau
et al. 1977; Costlow & McGuire 1977). Specific receptors for ovine GH in rat
testes have not been demonstrated to date. It is not known if the observed effect
of GH on LH receptor concentration and testicular responsiveness to LH is a
direct effect of GH on Leydig cells or is mediated by somatomedin. GH and
PRL, in addition to acting at different sites, appear to influence Leydig cell
function by distinct mechanisms. PRL treatment in hypophysectomized rats
allowed low doses of LH to have a positive effect on its receptor. Further-
more, PRL prevented or partially prevented the LH-induced loss of LH recep-

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tors in hypophysectomized or intact rats. In contrast GH was neither able to unmask the positive effect of LH on the LH receptor nor prevent the LH-induced decrease in LH receptors in hypophysectomized (Fig. 3 A) or intact rats (Fig. 6).

GH and PRL could affect testicular LH receptor concentration in the hypophysectomized rat either by maintenance of Leydig cells or by a specific effect on LH receptors. In support of maintenance of Leydig cells is a study in which it was reported that both GH and PRL (when given in mg amounts daily) caused the growth of Leydig cell tumors in mice (Yang et al. 1974). In the present study, no attempt was made to quantitate the number of Leydig cells per testis. However, it has been reported that Leydig cell number does not begin to decline until 2 weeks after hypophysectomy (Desjardins et al. 1975). Since LH receptors begin to decline with in 48 h after hypophysectomy (Hauger et al. 1977a) and since we studied the effect of various hormone treatments 7 days after hypophysectomy, our data suggest that PRL and GH regulate the number of LH receptors per Leydig cell.

Testicular responsiveness to LH as measured by testosterone production appeared to depend mostly on LH. We did not observe an increase in testicular responsiveness to LH in rats treated with PRL as has been reported to occur in hypophysectomized rats with ectopic pituitary homografts (Bartke & Dalterio 1976). No difference in testicular responsiveness to LH was observed in rats which received 5 μg LH + 150 μg PRL compared to rats which received only 5 μg LH; and rats which received 25 or 50 μg LH together with 150 μg PRL actually exhibited a lower testicular response to a stimulatory dose of LH compared to hypophysectomized rats treated with this amount of LH alone. A positive effect of GH on testicular responsiveness to LH was observed. It appears unlikely that the increase in testicular responsiveness to LH after treatment with GH was due to LH contamination since GH alone did not increase the weights of androgen-dependent organs (Zipf, Payne & Kelch, unpublished data) as was demonstrated with 5 μg LH (Table 1). Odell & Swerdloff (1976) and Swerdloff & Odell (1977) reported similar effects of GH and PRL on LH-stimulated testosterone secretion in immature hypophysectomized male rats as was observed in the present study. Our studies indicate that even though GH and PRL appear to be necessary for maintenance of normal LH receptor concentration, only LH can maintain normal testosterone synthesis.

In conclusion our studies demonstrate 1) maintenance of testicular LH receptor concentration in adult hypophysectomized rats is dependent on the combined effects of PRL, GH and LH; 2) PRL not only prevents LH from exerting a negative effect, but allows LH to have a positive effect in the hypophysectomized rat on the testicular LH receptor; 3) PRL and GH appear to act at different sites and by different mechanisms; and 4) hormonal regulation of LH receptor concentration appears to be distinct from hormonal regulation of testicular responsiveness to LH.

Acknowledgments

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DISCUSSION

Swerdloff: While our data and yours are in general agreement, I think we must all be cautious in interpreting data demonstrating effects of pharmacologic amounts of various partially purified pituitary hormones (given to hypophysectomized rats) on testis hCG/LH receptors and LH stimulated testosterone secretion as evidence that these hormones are all required for normal maintenance of testicular function. These reservations seems appropriate based on the increasing complexity of possible regulating factors on receptors. Duration of treatment dose of administered hormone, varying degrees of hormonal contamination and duration of hypophysectomy all seem to influence response. When selected doses of multiple hormones are used together in a study, the difficulties are geometric. These methodological differences may explain the discrepancies between results of different investigations.

Bartke: In response to Dr. Swerdloff's comment, I would like to indicate that the doses of prolactin (PRL) and other pituitary hormones utilized by Dr. Payne and by ourselves are not necessarily pharmacological. To obtain some of the well established biological effects of PRL in the female rat (maintenance of corpus luteum function, lactation) it is necessary to inject several hundred milligram of PRL per day. Thus the doses used in our studies could be regarded as reasonable replacement doses.

Labrie: We have recently found that treatment of adult male rats with CB-154 decrease testicular LH receptor levels while elevated levels of circulating prolactin secondary to transplantation of pituitaries under the kidney capsule increase the level of LH receptors. These data show that physiologically acceptable changes of circulating prolactin levels can modulate testicular LH receptors.

Troen: Drs. Hsu, Stratico, Oshima and myself observed the presence of receptors for ^{125}I-labelled human chorionic gonadotropin ($[^{125}I]$hCG) in adult human testis. The specific binding of $[^{125}I]$hCG to testicular membrane protein is temperature dependent and is a saturable process with respect to added receptor protein and hormone. Scatchard analysis revealed a dissociation constant, Kd, of 5.0×10^{-10} M, and 6.2 fmole of binding site/mg protein. Intact unlabelled hCG effectively inhibits the specific binding of $[^{125}I]$hCG to human testicular receptors. For inhibition of binding of $[^{125}I]$hCG the α-subunit has 0.04 % of the potency of intact hCG. Specific binding is pH dependent, with an optimum at pH 7.4. Brief exposure to extremes of pH causes irreversible damage to the receptors. Incubation of cell membranes with protease and trypsin results in an almost complete loss of binding activity, while ribonuclease, deoxyribonuclease, phospholipase C or neuraminidase treatment does not significantly alter hormone binding activity. Binding activity was found to be positively correlated to the concentration of intratesticular testosterone.

Sharpe: Dr. Wu and myself in Edinburgh have some preliminary findings on *in vitro* testosterone production by testicular biopsy tissue from men. We find that, basally, there is a very high production of testosterone – of the order of 5 times that seen with adult rat tissue. However, we find that testosterone production following addition of hCG is not greatly higher than the basal testosterone production.

Troen: Our data suggest that a higher capacity for peptide hormone binding is reflected in a higher level of testosterone production as indicated by endogenous steroid levels. This correlation may indicate the early steroidogenic response to different degrees of endogenous LH stimulation resulting from the pulsatile nature of circulating LH in adult men.

Bardin: Perhaps there was a difference in the samples used by Drs. Troen and Sharpe. What was the clinical status of Dr. Sharpe's patients?

Nieschlag: Perhaps I can classify the question raised by Dr. Sharpe. We have in collaboration with Dr. Mauss incubated testicular biopsies from 21 infertile men with and without hCG. The histological appearance of the testes ranged from normal, to Sertoli cell only syndrome and to Leydig cell hyperplasia due to inflammatory processes. In men with normal Leydig cells we observed a 2–3 fold increase of testosterone production over basal values after 3 h of incubation with hCG. The response was much greater in tissue with Leydig cell hyperplasia. Thus in contrast to Dr. Sharpe we find a distinct response of human testicular tissue *in vitro* to hCG in a relatively large number of subjects.

Hansson: Could the low hCG binding in testes with low intratesticular testosterone be due to increased proteolytic activity in a regressing testis?

Troen: I do not know. We examined the morphologic appearance of the testis from the patients studied and could detect no gross histologic correlation with the findings we have reported. However, these are testes from older patients and changes may be present which are not reflected in the microscope.

de Jong: Just to comment on Dr. Payne's data on the increase of aromatase after repeated injections of hCG, I would like to quote from our work on estradiol in testicular venous plasma from adult rats: hCG did not have any effect on the secretion of estradiol after 5 daily injections with 100 IU of hCG (de Jong, Hey & van der Molen, J. Endocr. 1973).

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As far as the conversion of radioactive testosterone to estradiol is concerned, we have calculated what the amount of radioactivity in estradiol could be if the androgen production of estradiol in total testis is compared with the specific activity of radioactive plus endogenous testosterone. The result was a total conversion of 10–20 dpm after a 4 h incubation of 100 mg of tissue.

Payne: We incubate cell free homogenate from one testis with 20 μCi of [^{3}H]testosterone for 3 h in buffer containing an NADPH-generating system. After the incubation, [^{14}C]estradiol and [^{14}C]estrone are added to monitor for recovery. Extracts from two incubations are combined and estrogens are separated from neutral steroids and chromatographed by paper and thin layer chromatography and finally recrystallized to constant specific activity. Endogenous testosterone is measured by RIA and the amount of [^{3}H]testosterone is corrected for endogenous testosterone and in turn the [^{3}H]estradiol is corrected for the total testosterone incubated. The final total dpm in the purified estradiol from cell free homogenates from two testes varies between 10 000 and 40 000 dpm depending on endogenous testosterone. We have determined aromatization from 12 rats and between 250 and 300 pg estradiol (calculated from radioactive recrystallized estradiol) is formed per testis during a 3 h incubation.