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Interspecies Differences in Testicular LH Receptors and *in vitro* Testosterone Production Among Rodents

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Testes from rats, mice and hamsters were incubated for 4 h with 0, 3.125 or 12.5 mIU hCG/ml. The LH receptor concentration in incubated testes of rats and mice was higher than that observed in hamsters. Testosterone levels in incubation media were significantly different among species (mice > rats > hamsters). During the incubation, hCG caused an increase in testosterone levels in all three species, but produced no significant changes in LH receptor concentration. Furthermore, a correlation between LH receptor concentration and testosterone only in hamsters is observed. The efficiency of the LH receptor-steroidogenesis interaction was estimated from the ratio of testosterone levels to receptor concentration under basal conditions and was found to differ among species (mice > hamster > rats). The levels of PGE and PGF in incubation media were higher in mice than in rats or hamsters, and hCG did not alter prostaglandin levels in any of the species. The present results indicate that acute *in vitro* hCG stimulation of testosterone synthesis does not involve appreciable changes in testicular LH receptor levels.

Key words: Testosterone, Testicular LH receptors.

Studies in rodents suggest that the testicular concentration of LH receptors as well as plasma testosterone (T) levels vary significantly among species (1, 3, 6, 14). There are also species differences in T production *in vitro,* and its stimulation by hCG (10, 14, 18, 20). Correlating testicular LH receptor concentration changes directly with variations in plasma T has proven difficult. Generally, the changes in these parameters are out of phase, probably due to the influence of other factors, such as other hormones or a specific genotype in the animal studied (2, 4, 5). In this study, we have examined the correlation between LH receptors and T production, using an *in vitro* system in which short-term hCG-induced changes

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in LH receptors and T synthesis can be analyzed simultaneously.

This system allows us to correlate inter-species differences in LH receptor concentrations, their regulations, T levels and responsiveness to hCG *in vitro*. The study was conducted using laboratory strains of Norway rats *(Rattus norvegicus)*, house mice *(Mus musculus)* and Syrian hamsters *(Mesocricetus auratus)* in the same experiment. Since endogenous prostaglandin production may play a role in mediating testicular responses to LH, whereas exogenous prostaglandins have been shown to interfere with the stimulation of testicular steroidogenesis by LH (7, 8, 11, 15, 21) and the regulation of LH receptors (9, 12), testicular production of PGE and PGF was measured in the same experiment.

Materials and Methods

Animals. Adult Sprague-Dewley rats (Crl: CD [SD]BR) and Syrian hamsters (Lak:LVG [SYR]) (> 3 months of age) were obtained from Charles River Lakeview. Adult random bred mice (DW/B) (> 3 months old) were raised in our animal colony. Each species was kept in a separate room, all rooms having controlled temperature of 22 ± 2°C and illumination of 14 h light:10 h darkness. All animals had free access to commercial food (Wayne Breeder Blox) and tap water. Rats and hamsters were sacrificed by decapitation, and mice by cervical dislocation.

In vitro *Incubation.* Testes were decapsulated and cut into fragments of similar size. Three testicular fragments from each animal were incubated in Krebs-Ringer bicarbonate with either 0, 3.125 or 12.5 mIU hCG/ml for 4 h at 32 ± 1°C, following methods used previously (23). Testes fragments were subsequently recovered, snap-frozen and stored at –80°C until assayed for LH receptor levels, whereas incubation media were stored at —20°C until assayed for T and prostaglandin content.

Measurement of hCG Binding and Hormones. Testicular ^{125}I-hCG binding was measured using procedures validates for the rat, mouse and hamsters as described previously (3, 16, 17). The ^{125}I-hCG (CR-121, NIH) used in these studies had a maximum binding of 46 % and a specific activity of 51.4 μCi/μg. The content of protein in testicular membrane preparations used for determination of hCG bindng was measured by a modification of Lowry's method (19), using bovine serum albumin as the standard.

T levels in the incubation media were determined by RIA as described by WOLFE *et al.* (23). Prostaglandin E and F levels in the incubation media were measured by RIA, as described by HARPER *et al.* (13). The antibodies used in this RIA cannot distinguish between PGE_1 and PGE_2, and PGF_{1a} and PGF_{2a}, respectively.

Statistics. The data were evaluated by two-way ANOVA and Pearson's correlation analysis. For the ANOVA, data were tested for normality of distribution by the Kolmogorov-Smirnov test and for homogeneity of variance by Barlett's test, and log- or square-root-transformed as needed (22).

Results

LH receptor concentration was much higher in the two representatives of Murinae (rats and mice) than in Syrian hamsters (Cricetinae). Furthermore, LH receptor levels were numerically although not significantly higher in rats than in mice (table I). However, the presence of hCG in the media did not alter the levels of testicular LH receptors, after 4 h of incubation in any of the species studied.

Table I. *Testicular LH receptor concentration and* in vitro *T synthesis*
Testes fragments of each were incubated with each dose of hCG. Four animals per species were used. Values are expressed as mean ± SEM. Where letters are different, $P < 0.05$ (Student-Newman-Kuel's procedure of the multiple range test).

Species	hCG (mIU/ml)	LH Receptors (fmols/mg protein)	Testosterone (ng/mg testes)	(% increase)
Rat	0	58.84 ± 10.32^{b}	0.288 ± 0.333^{c}	0
	3.125	53.47 ± 8.58^{b}	1.188 ± 0.120^{d}	421.1
	12.5	58.39 ± 9.77^{b}	2.033 ± 0.245^{d}	791.7
Mouse	0	33.05 ± 5.98^{b}	2.298 ± 0.781^{d}	0
	3.125	36.05 ± 6.54^{b}	13.473 ± 0.719^{e}	486.3
	12.5	30.70 ± 4.19^{b}	25.008 ± 2.317^{f}	988.3
Syrian hamster	0	3.48 ± 0.35^{a}	0.035 ± 0.003^{a}	0
	3.125	5.07 ± 0.94^{a}	0.080 ± 0.009^{b}	128.6
	12.5	5.42 ± 0.28^{a}	0.173 ± 0.038^{c}	394.3

In hamsters and mice, hCG caused a dose-related increase in T concentration in the media, while in rats, both doses of hCG used produced similar, statistically significant increases in T production. Great differences in T synthesis were observed among species, with rats and mice exhibiting a greater synthesis than hamsters. T synthesis was 10 times greater in rats than in hamsters, and furthermore, in mice, it was 10 times greater than in rats (fig. 1).

Prostaglandin levels in the incubation media were not significantly affected by hCG, although in hamsters, hCG did cause a slight decrease in prostaglandin levels. The amounts of prostaglandins, and especially PGF, released into the median in the absence of hCG were much higher in mice than rats and hamsters (table II).

The correlation analysis of all parameters studied in mice and rats revealed no significant correlation among any of them

Table II. *Prostaglandin levels in testicular incubation media (pg/mg testes).*
For more detail on statistics, see table I.

Species	hCG	PGE	PGF
Rat	0	3.38 ± 0.50^{a,b}	5.94 ± 1.46^{b}
	3.125	3.91 ± 0.34^{b}	7.09 ± 1.44^{b}
	12.5	4.05 ± 0.71^{b}	6.10 ± 1.52^{b}
Mouse	0	24.38 ± 7.03^{c}	71.12 ± 13.41^{c}
	3.125	20.33 ± 2.77^{c}	57.72 ± 7.60^{c}
	12.5	24.82 ± 0.56^{c}	76.10 ± 5.16^{c}
Syrian hamster	0	3.85 ± 1.30^{a,b}	4.48 ± 1.79^{a,b}
	3.125	2.41 ± 0.31^{a,b}	2.53 ± 0.37^{a}
	12.5	1.77 ± 0.29^{a}	2.43 ± 0.10^{a}

with the exception of PGE vs. PGF ($P < 0.001$) and hCG added vs T ($P < 0.001$). In Syrian hamsters, there was also a significant correlation between LH receptors and T ($P < 0.04$).

Discussion

As expected from previous *in vivo* and vascular perfusion studies (1, 3, 6, 10, 14, 20) differences in basal testicular LH receptor concentration and T production were observed among species, using an *in vitro* system. Both species of Murinae had an order of magnitude higher LH receptor concentrations than did the hamster, a Cricetid rodent. Mice had the highest and hamsters the lowest basal *in vitro* T production. The basal concentration of prostaglandin E and F in the incubation media was much higher in the mouse than in the rat or the hamster. These results indicate that mice have a much higher testicular basal endocrine function than do rats or hamsters.

The efficiency of the LH receptor-steroidogenesis interaction can be assessed by calculating the T : receptor ratio as follows:

T/R ratio = pg testosterone/mg testes / fmols LH receptor/mg protein

Using this approach it can be concluded from the data presented in table I that the mouse has a much more efficient LH receptor-steroidogenesis interaction than does the hamster, and both animals are more efficient than the rat (T/R ratios: mouse, 69.53; hamster, 10.06; rats, 3.87).

Stimulation with hCG did not significantly affect LH receptor concentration, although in hamsters a numerical increase was observed. However, hCG stimulated T synthesis in a dose-dependent manner, indicating that hCG can acutely increase T levels *in vitro* without a measurable variation in the levels of LH receptors. HUHTANIEME *et al.* (14) also failed to see changes in LH receptors in a study using human and rat testes. This phenomenon has been observed also *in vivo* in some mouse strains (4). The lack of changes in LH receptors could be explained by the existence of an equilibrium between the mechanisms that decrease LH receptor levels (i.e., internalization, degradation, occupancy) and those that increase these levels (i.e., *de novo* synthesis, assembly, recycling), in the presence of the hCG doses used in the present study. Whereas the use of correlation coefficients showed no correlation between LH receptor and T levels in Murinae. this correlation was significant in hamsters, although LH receptor changes in these animals were not significant.

The presence of hCG did not alter prostaglandin levels, although suggestive (nonsignificant) decreases were observed in the hamster. These data would suggest that prostaglandins may not have been involved in testicular responses to gonadotropin stimulation and that the effects of prostaglandins on steroidogenesis and on LH receptors that have been reported previously (7, 8, 11, 15, 21) reflect a potential regulatory capacity of prostaglandins to modulate steroidogenesis independently of gonadotropin-regulated mechanisms or perhaps may represent merely a plarmacologic effect of these compounds.

In summary, acute *in vitro* hCG stimulation of T synthesis does not involve appreciable changes in testicular LH receptor levels in the rat, the mouse or the hamster. Also, there are very substantial interspecies differences in all parameters of testicular function examined in this study.

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Resumen

Testículos de rata, ratón y hamster incubados durante 4 h con distintas concentraciones de hCG (0, 3,125 ó 12,5 mIU/ml) muestran concentración mayor de receptores a LH en los testículos de rata y de ratón. Los niveles de testosterona en el medio de incubación son estadísticamente diferentes según la especie (ratón > rata > hamster), sin cambios en la concentración de receptores a LH. Sólo en hamsters se observa una correlación entre la concentración de receptores a LH y los niveles de testosterona. La eficiencia de la interacción entre los receptores a LH y la esteroidogénesis, bajo condiciones basales, se obtiene de la relación niveles de testosterona : concentración de receptores y varía según la especie (ratones > hamsters > ratas). La concentración de prostaglandinas en el medio de incubación es más alta en ratones que en ratas o hamsters, y la incubación con hCG no la altera en ninguna de las especies. Del presente estudio se puede deducir que la estimulación aguda de la síntesis *in vitro* de testosterona por hCG no depende de cambios apreciables en el número de receptores testiculares a LH.

References

1. Amador, A.: *Biol. Reprod.*, **22** (Suppl. 1), 77A, 1980.
2. Amador, A. G. and Bartke, A.: *J. Endocrinol.*, **95**, 301-309, 1982.
3. Amador, A., Bartke, A. and Beamer, W.: *Endocr. Res. Commun.*, **9**, 79-88, 1982.
4. Amador, A., Bartke, A., Parkening, T. A. and Chamness, G. C.: *J. Recept. Res.*, **3**, 495-512, 1983.
5. Amador, A., Parkening, T., Beamer, W., Bartke, A. and Collins, T. J.: *J. Recept. Res.*, **4**, 741-753, 1984.
6. Amador, A., Steger, R. W., Bartke, A., Johns, A., Hayashi, R. H. and Stallings, M. H.: *J. Androl.*, **4**, 67-70, 1983.
7. Bartke, A.: In «Regulation Mechanisms of Male Reproductive Physiology» (Spilman, C. H., ed.). Excerpta Medica, Amsterdam, 1976, pp. 79-86.
8. Bartke, A., Kupfer, A. and Dalterio, S.: *Steroids*, **28**, 81-88, 1976.
9. Diekman, M. A., O'Callaghan, P., Nett, T. M. and Niswender, G. D.: *Biol. Reprod.*, **19**, 1010-1013, 1978.
10. Ewing, L. L., Zirkin, B. R., Cochran, R. C., Kromann, N., Peters, C. and Ruiz-Bravo, N.: *Endocrinology*, **105**, 1135-1142, 1979.
11. Grotjan, H. E. Jr., Heindel, J. J. and Steinberger, E.: *Steroids*, **32**, 307-322, 1978.
12. Haour, F., Mather, J., Saez, J. M., Kouznetzova, B. and Dray, F.: Crastes de Paulet, INSERM Symposia Series. Paris, vol. **91**, 75-88, 1980.
13. Harper, M. J. K., Norris, C. J., Friedrichs, W. E. and Moreno, A.: *J. Reprod. Fertil.*, **63**, 81-89, 1981.
14. Huhtaniemi, I., Bolton, N., Leinonen, P., Kontturi, M. and Vihko, R.: *J. Clin. Endocrinol., Metabol.*, **55**, 882-889, 1982.
15. Joseph, M. M. and Bartke, A.: *Int. J. Androl.*, **2**, 250-256, 1979.
16. Klemcke, H. G. and Bartke, A.: *Endocrinology*, **108**, 1763-1768, 1981.
17. Klemcke, H. G., Bartke, A. and Goldman, B. D.: *Biol. Reprod.*, **25**, 536-548, 1981.
18. Kühn-Velten, N., Schumacher, H., Wolff, J. and Staib, W.: *Cell Biochem. Funct.*, **2**, 26-32, 1984.
19. Markwell, M. A. K., Hass, S. M., Bieber, L. L. and Tolbert, N. E.: *Anal. Biochem.*, **87**, 206-210, 1978.
20. Probst, B.: *Acta Endocrinol.*, **103**, 567-571, 1983.
21. Sairam, M. R.: *Prostaglandins*, **17**, 929-937, 1979.
22. Sokal, R. R. and Rohlf, F. J.: Biometry (2nd), W. H. Freeman and Co., San Francisco, Calif., 1981.
23. Wolfe, H. G., Bartke, A., Amador, A., Van Sickle, M., Dalterio, S. and Brown, D.: *J. Endocrinol.*, **90**, 367-373, 1981.