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Interspecies Differences in the Effects of HCG on Testicular Function Among Rodents

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Adult mice, rats and hamsters were injected with 0 or 0.3 IU hCG/g BW, 24 h before sacrifice. Basal LH receptor concentration was highest in rats and lowest in hamsters (rats > mice > hamsters). Injection of hCG caused LH receptor down-regulation in rats and mice, and up-regulation in hamsters. Basal plasma progesterone was highest in hamsters and lowest in rats (hamsters > mice > rats), however, hCG increased plasma progesterone levels in mice and rats, but not in hamsters. Mice had much higher plasma and testicular testosterone levels than other species, but hCG did not induce a relatively more dramatic increase in any species. When testes fragments were incubated with 0 or 12.5 mIU hCG/ml for 4 h, hCG increased media progesterone levels in rats and control mice, but not in hamsters and hCG-injected mice. Also, hCG elevated media testosterone levels in control but not in hCG-injected animals. Furthermore, addition of hCG *in vitro* partially prevented the elevation of media testosterone induced by *in vivo* hCG. The present results indicate that the mechanisms for the transduction of the gonadotropic signal by the Leydig cells are species-defined.

Key words: LH receptors, Testosterone, Progesterone, Testes, hCG.

Studies in different species of rodents have shown that the testicular LH receptor concentration as well as the circulating levels of testosterone (T) vary greatly among species (1, 3-7, 9). Interspecies differences in basal and hCG-stimulated *in vitro* T production have also been observed (2, 7, 14, 15, 18, 27). Correlating the changes in testicular LH receptor concentration directly with the variations in T levels *in vivo* or *in vitro* has proven generally difficult. As a rule, the changes in these parameters are temporarily dissociated. In a previous study using an *in vitro* system exclusively (7), we have shown that the short-term hCG-induced changes in T production by incubated testicular tissue occurred without measurable changes in LH receptor concentration.

In the present study, we have used an *in vivo-in vitro* system in which long-term

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effects of hCG on LH receptors and on basal as well as hCG-stimulated T synthesis can be analyzed simultaneously. The advantage of this approach is that it allows us to study the variations in several parameters of testicular function that accompany changes in LH receptor metabolism. The study was conducted using laboratory stocks of house mice (*Mus musculus*), Norway rats (*Rattus norvegicus*) and Syrian hamsters (*Mesocricetus auratus*).

Materials and Methods

Adult (> 10 week old) male DF/B mice (*Mus musculus*; from our breeding colony), Sprague-Dawley rats (*Rattus norvegicus*; Hsd: (SD)Br from Harlan-Sprague-Dawley, Indianapolis, Indiana), and Syrian hamsters (*Mesocricetus auratus*; F_2B from Biobreeders, Watertown, Mass.) were housed in polycarbonate cages with free access to food and water. The animal rooms had controlled temperature (22 ± 2 °C) and illumination (12 h light/24 h for mice and rats and 16 h light/24 h for hamsters).

Animals were injected s.c. with either 0 or 0.3 IU hCG/g BW, and were sacrificed by decapitation 24 h later. Trunk blood and testes were collected. Plasma was stored frozen at −20 °C until assayed for steroid levels. Testes were decapsulated and weighed. A testis fragment (one hemitestis for mice and a 200-400 mg piece for rats and hamsters) was rapidly frozen in a dry ice: acetone mixture and stored at −70 °C until assayed for LH receptors. Another fragment of similar size was stored frozen at −70 °C until it was homogenized in distilled water at 10,500 rpm with a Tekmar Tissuemizer, and the homogenate was stored at −20 °C until assayed for testosterone levels. Two other testes fragments of similar weights to the previous ones were incubated with either 0 or 12.5 mIU hCG/ml media for 4 h at 32 ± 1 °C in Krebs Ringer bicarbonate with glucose and in a 95 % O_2: 5 % CO_2 atmosphere (12, 31, 32). Media was then stored frozen at −20 °C until assayed for steroid levels.

Testicular LH receptors were measured by radioreceptorassay as previously described for each species (9, 16, 17). The ^{125}I-hCG (CR-121, NIH) used as a tracer had a maximum binding ability of 28.0 % and a specific activity of 11.5 μCI/μg. The protein concentration in the membrane preparation used was determined by a modification of Lowry's procedure (19) using BSA as a standard.

Table I. *Characterization of solid-phase radioimmunoassays.*
Comparison between the statistical parameters of the curves derived from sample pools and those of the standard curves they were assayed with. m = slope, Y^I = Y intercept and r = correlation coefficient.

Hormonal sample	m	yI	r
a) Media Progesterone			
Mouse	—1.640	0.159	1.000
Rat	—1.641	0.163	1.000
Hamster	—1.637	0.166	1.000
Standard Curve	—1.635	0.158	0.994
b) Plasma Progesterone			
Mouse	—1.637	0.152	1.000
Rat	—1.642	0.173	1.000
Hamster	—1.633	0.160	1.000
Standard Curve	—1.635	0.158	0.994
c) Plasma Testosterone			
Mouse	—1.756	0.431	1.000
Rat	—1.773	0.433	1.000
Hamster	—1.749	0.424	1.000
Standard Curve	—1.755	0.430	0.995
d) Testes Testosterone			
Mouse	—1.844	0.701	1.000
Standard Curve	—1.839	0.692	0.994
e) Testes Testosterone			
Rat	—1.757	0.434	1.000
Hamster	—1.751	0.452	1.000
Standard Curve	—1.755	0.430	0.995

Incubation media T levels were determined by a liquid-phase radioimmunoassay (RIA) (32). Plasma T and progesterone (P), testicular T, as well as P, levels were determined by solid-phase radioimmunoassays using kits (Diagnostic Products Corporation, Los Angeles, Ca) with ^{125}I-T or ^{125}I-P tracers, and anti-T or anti-P antibody-coated polypropylene tubes. Because these kits use standard curves based on human serum, parallelism between each respective standard curve and curves made up by different volumes from pools of each type of sample from each species was determined and confirmed (table I).

Data were obtained using the RRAPLOT and RRADOSE programs for the radioreceptorassay, and the RIAPLOT and RIADOSE programs for the radioimmunoassays (8). Data were then analyzed by two —or three— way analysis of variance using the SPSS-X software on an IBM mainframe (20-22). The data were tested for normality of distribution by the Kolmogorov-Smirnov test and for homogeneity of variance by Bartlett's test, and log —or square— root transformed as needed (22, 28).

Results

Basal LH receptor levels were highest in rats, intermediate in mice and lowest in Syrian hamsters (fig. 1a). When animals were injected with hCG (0.3 IU/g BW), 24 h before sacrifice, negative autoregulation (down-regulation) of LH receptors was observed in mice and rats, whereas in hamsters, hCG caused a positive autoregulation (up-regulation) of LH receptors (fig. 1a). Basal plasma P levels were the highest in hamster, intermediate in mice and the lowest in rats (fig. 1b). Injection of hCG caused a dramatic elevation in the plasma P levels of rats. In mice, hCG produced a slight but significant increase in plasma P levels; which was not seen in hamsters (fig. 1b). Basal plasma T levels were significantly higher in mice than in rats or Syrian hamsters (fig. 1d). Injected hCG produced a large increase in circulating T levels in all three species. However, in rats and hamsters the hCG-stimulated T levels only reached the magnitude of basal levels in mice (fig. 1d). The differences between mice and rats or Syrian hamsters with respect to basal testicular T levels were even more pronounced (fig. 1c). Injection with hCG caused a very dramatic elevation of the testicular T levels in rats and hamsters, while in mice the increase was statistically significant but numerically modest (fig. 1c). Nevertheless, hCG-stimulated testicular T levels in rats and hamsters did not reach the basal levels measured in mice.

When incubation media steroid levels were measured, further interspecies differences were revealed. Basal media P levels were significantly higher in incubation of testes fragments from animals injected with hCG than in controls (fig. 2a). When testes fragments were incubated with hCG (12.5 IU/ml media), media P levels were increased in incubations of testicular tissue from control mice and rats, and from hCG-injected rats, but not in the incubations of testicular tissue from hamsters or hCG-injected mice (fig. 2a). Species differences in media T levels were very pronounced since the range of levels of media T were ten-fold higher in rats than in hamsters, and a further ten-fold greater in mice than in rats. In control mice, exposure to hCG *in vitro* increased T levels by eleven-fold. In hCG-injected mice basal media T levels were four times higher than in control mice, but incubation with hCG caused a 50 % decrease in media T versus its respective basal levels. These reduced levels were, however, higher than the basal levels measured in incubations from control mice (fig. 2b). In control rats, hCG treatment *in vitro* produced a six-fold increase in media T levels. Rats injected with hCG had basal T levels similar to the hCG-stimulated levels from

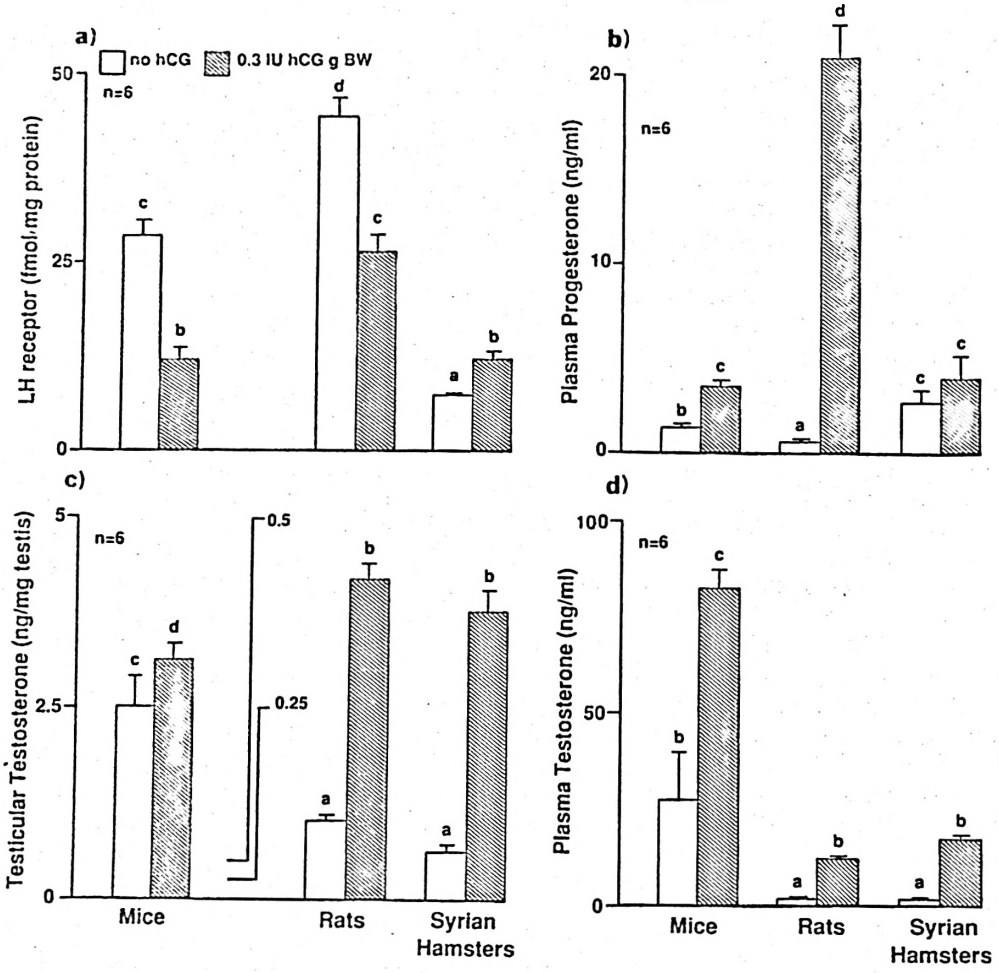

Fig. 1. *Testicular LH receptors (a), plasma progesterone (b), testicular testosterone (c) and plasma testosterone (d) levels as a function of species and treatment with hCG.*
Values are expressed as mean ± sem for the number of animals indicated. Points with the same letter in superscript are not significantly different ($p > 0.05$; Student-Newman-Keuls procedure of the multiple range test). Please note that in panel (d) a different scale for testicular testosterone levels was used depending on the species to allow a better visualization of the data.

control mice, and *in vitro* hCG exposure was unable to produce further elevation of media incubation media T levels (fig. 2b). A similar pattern and magnitude of changes in incubation media T levels were observed also in incubation of hamster testes fragments (fig. 2b).

Discussion

The present results support previous studies that have shown that the basal concentration of testicular LH receptors varies among species (1, 3-7, 9-11, 15). When mice rats and Syrian hamsters, were

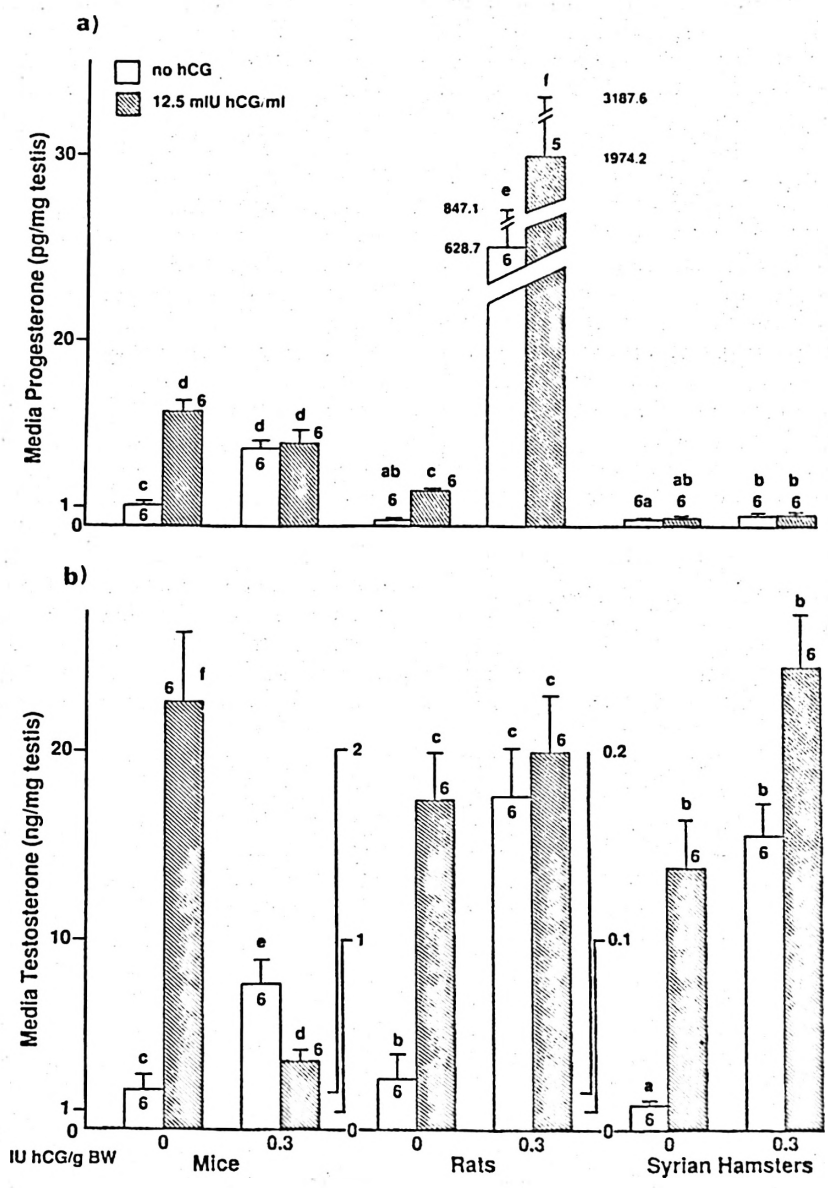

Fig. 2. *Incubation media progesterone (a) and testosterone (b) levels as a function of species and* in vivo *and* in vitro *administration of hCG.*

Values are expressed as mean ± sem for the number of animals indicated. Points with the same letter in superscript are not significantly different ($p > 0.05$; Student-Newman-Keuls procedure of the multiple range test). Please note that in panel (b) a different scale for testosterone levels was used for each species to allow a better visualization of the data.

previously compared, no significant differences between rats and mice could be detected in LH receptor levels, and the values reported here for each species differ somewhat from the ones presented before (7). This is probably related to the fact that in our previous study we have used rats and hamsters from different suppliers (Charles River) and a different stock of mice (DW/B). Intraspecies differences are not unexpected since it has been repeatedly demonstrated that there is a considerable genotype-determined variability in LH receptor levels (3, 6, 29). The current results document also the differences in autoregulation of LH receptors among species, with the mice and rats used here having negative autoregulation (down-regulation), and Syrian hamsters having positive autoregulation (up-regulation), in response to injection of the same doses of hCG. Different responses of LH receptor levels to hCG in different species had been implied by data on each species obtained in separate studies (1, 3, 5, 6, 30).

Circulating T levels tend to vary greatly among stocks of animals within each species, especially mice (3, 6, 9, 10). This variability notwithstanding, the previously published data tended to indicate that basal plasma T levels were, on average, higher in mice than in other species, and that mice responded better, in terms of T production, to stimulation by hCG (3, 5, 6, 9, 10). In the present study we confirm these observations and present data that might explain some of these differences. It should also be noted that mice are more efficient than other species in transducing the gonadotropic signal to the Leydig cell. Namely, they produce more T per LH receptor than do other species (2, 7). The present results indicate that having more LH receptors does not necessarily imply a more active T production, since rats have more LH receptors than mice, but produce less T. Furthermore, Syrian hamsters which have fewer LH receptors are actually more efficient than rats in producing T (7).

Comparison of plasma P levels provided evidence that hamsters have the highest P levels, which, however, were not increased by hCG treatment. This could indicate that, in comparison with other species, hamsters have a passive or gonadotropin-reversible blockage of the enzymatic conversion of P to T. Under basal conditions they convert P to T relatively inefficiently, but when stimulated by hCG, hamster testes convert all the excess P to T. It is also possible that much of the peripheral P levels in the male hamster is of adrenal rather than of testicular origin. Rats on the other hand, seem to convert P to T under basal conditions very effectively, but when stimulated by hCG they suffer a blockage of this conversion mechanism(s), with a consequent accumulation of most of the excess P. In contrast, mice seem to be able to convert P to T under both basal and gonadotropin-stimulated conditions, while conserving adequate P reserves. These differences could be a result of interspecies variation in the sensitivity to the inhibition of the synthesis of steroidogenic enzymes by high levels of estradiol induced by hCG administration (13, 23-26).

The measurement of testicular T gives further insight into the handling of steroids by each species. Basal testes T levels correspond closely to those in the circulation. However, in mice most of the T produced in response to hCG stimulation is released into circulation, whereas in rats and Syrian hamsters the release of T into the blood is directly correlated with the testicular levels of this steroid.

The second part of this experiment gives an insight on how testes react to gonadotrop,in stimulation once all extra-testicular influences have been removed. Also, it allowed us to compare the responsiveness of the testes, in each species, after *in vivo* pretreatment with hCG. As in a previous study, basal and hCG-stimulated

media T levels from control animals were highest in mice and lowest in Syrian hamsters (mice > rats > hamsters). The ranges of T levels did not overlap, with the hCG-stimulated T levels of the less active species barely reaching the basal T levels of the next active species. Basal media T levels in hCG-injected animals reflected testicular T levels measured for each species. When testes from hCG-injected animals were incubated in the presence of hCG, it was observed that exposure to hCG *in vitro* did not cause a further increase in media T levels from rat and hamster testes. In mice, addition of hCG *in vitro* actually produced a partial inhibition of the *in vivo* hCG-induced elevation in media T levels. This could indicate the existence of stimulatory thresholds set at different levels in different species.

Basal media P levels were also higher in mice than in rats and hamsters, however the differences in media P levels among species were much smaller than those observed for media T. The *in vivo* but not the *in vitro* administration of hCG caused an increase in media P. This set of data supports the possibility that in hamsters hCG can overcome their relative blockage of P to T conversion under normal conditions. In rats, *in vivo* hCG causes a great increase in basal media P levels, whereas *in vitro* hCG causes a slight but significant elevation in media P levels from testes of both control or hCG-injected rats. This reinforces our previously stated observation that *in vivo* hCG, at the dose administered here causes a subsequent blockage of P to T conversion. Also, it should be noted that the *in vitro* dose of hCG did not affect P to T conversion. Rats were the species which had the largest increase in media P in response to *in vivo* hCG, but mice had the best response to *in vitro* hCG. When testes from hCG-injected mice were incubated with hCG, media P levels were similar to the basal media P levels in these animals and to the hCG-induced levels in control mice. Therefore, in mice it would seem that the toxic effects of excess hCG include not only a blockage of the conversion of P to T, but also suppression of P production.

Comparative study in different species of laboratory rodents reveals that the relationship between changes in LH-R and in steroidogenesis is different in each species. Also, the efficiency of each steroidogenic step, as well as its regulation, varies depending on the species. Thus the mechanisms for the transduction of the gonadotropic signal received by the Leydig cell are species-defined, albeit all species achieve similar reproductive success.

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Resumen

Se inyecta a ratones, ratas y hamsters adultos 0 o 0,3 UI/g de peso corporal, 24 h antes de matarlos. La concentración basal de receptores a LH más alta es la de los testículos de rata y la más baja la de hamster (ratas > ratones > hamsters). La inyección de hCG produce, en ratas y ratones, una autorregulación negativa de los receptores a LH, mientras que en los hamsters causa una autorregulación positiva. Los niveles basales más altos de progesterona plasmática se dan en hamster y los más bajos en rata (hamster > ratones > ratas); sin embargo, la administración de hCG aumenta los niveles plasmáticos de progesterone en ratones y ratas, pero no en hamsters. Los niveles de testosterona plasmática y testicular observados, son más altos en ratones que en las otras dos especies. La inyección de hCG produce un aumento relativo en los niveles de testosterona plasmática y testicular de similar magnitud, en las tres especies. Cuando se incuban fragmentos de testículos con 0 ó 12,5 mUI hCG/ml durante 4 h, se elevan únicamente los niveles de progesterona en el medio de incubación de todas las ratas y ratones control.

Además, la hCG aumenta los niveles de testosterona en el medio de incubación de los animales control, pero no en el de los animales inyectados con hCG. De hecho, el uso de hCG *in vitro* previene parcialmente el aumento de testosterona en el medio de incubación resultante de la administración *in vivo* de hCG en ratones. Del presente estudio se deduce que los mecanismos de transducción de la señal gonadotrópica en la célula de Leydig están específicamente definidos por y en cada especie animal.

Palabras clave: Receptores de LH, Testosterona, Progesterona, Testículo, hCG.

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