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EARLY hCG-INDUCED DESENSITIZATION IN LEYDIG CELLS [1]

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SUMMARY

The early mechanism of hCG induced down regulation of its own receptor as well as steroidogenesis refractoriness of rat Leydig cells to gonadotropin stimulation have been investigated. A single injection of 5, 12, 25, 50 and 100 IU of hCG in rats induced within 8 hours, Leydig cells desensitization. However, apparent receptor loss was significantly lower only in the rats who received 50 and 100 IU of hCG. Cycloheximide inhibits hCG-induced receptors loss but had no effect on hCG-induced desensitization. The most likely explanation for desensitization in the presence of binding sites and a normal adenylate cyclase, is a defective coupling between the receptor sites and the catalytic subunit.

INTRODUCTION

Self regulation of membrane receptors by their own hormone has been demonstrated for several types of tissues (1-3). LH or hCG injections induce a "loss" of its own receptor in Leydig cells and a refractoriness of the cell to gonadotropin stimulation (4-12). It could therefore be suggested that LH-hCG induced desensitization of Leydig cells is mainly due to receptor loss. However, several observations suggest that the refractoriness of Leydig cells following hCG administration is a complex phenomenon which cannot be explained simply by diminution of hormone receptors. First, within 12 hours following hCG injection, Leydig cells become insensitive to further hormone stimulation, at a time when they apparently still have enough "free" receptors (8, 11) ; second, within 24 hours, hCG-desensitized Leydig cells present also a partial refractoriness to stimulation by cAMP (8) or DbcAMP (11), indicating an abnormality of some step in steroidogenesis beyond cAMP formation ; third, full response to DbcAMP reappears 48 to 72 hours before the hCG receptor and responsiveness to the hormone have recovered (11) ; fourth, no clear correlation is observed between number of membrane binding sites and hormonal responsive-

(1) Abbreviations = LH : luteotropin ; hCG : human chorionic gonadotropin ; cAMP : adenosine 3':5'-cyclic monophosphate ; DbcAMP : N^6,O^2-dibutyryl adenosine 3':5'-monophosphate.

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ness of isolated Leydig cells in the process of desensitization and recovery (8, 11).

In order to clarify the mechanism of the early desensitization we have investigated within the first 12 hours the effect of *in vivo* administration of hCG, at several dose levels, on receptor loss and hormone-refractoriness of Leydig cells. The effect of cycloheximide on both phenomena was also investigated.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 55 to 80 days old were injected intramuscularly with saline, hCG (Pregnyl) or cycloheximide. Leydig cell preparations were obtained after collagenase dissociation of the testes as previously described (14). Particulate fractions containing the plasma membranes were prepared from isolated Leydig fraction (9, 11). Iodination of hCG (15,000 IU/mg from Dr. Bosh, the Netherlands) was achieved by the lactoperoxidase method (15).

Binding of $[^{125}I]$hCG. Binding was performed at three different dilutions of interstitial cell particles in the presence of saturating concentrations of $[^{125}I]$hCG ($\simeq 5 \times 10^{-9}$ M). The incubation conditions and separation of bound and unbound hormone were achieved as described (9, 11). All binding experiments were performed in triplicate. Three additional samples were also run in the presence of a 100 fold excess of unlabelled hCG for estimation of the non-specific binding.

Testosterone and cAMP testicular levels. Testes were removed, decapsulated and immediately immersed in 30 ml of ethanol at - 60°C containing $[^{3}H]$cAMP ($\simeq$ 2000 cpm) and $[^{3}H]$testosterone and kept at this temperature for 4 hours. Thereafter the testes were homogenized in the same solvent. After centrifugation at 3000 x g for 10 min the supernatant was saved and the pellet extracted again by 30 ml of ethanol. The pool of supernatants was evaporated. Half of the extracts was chromatographed on a Dowex 1-X2 column (0.6 x 6 cm, 200-400 mesh Cl^- form) using the method described by Mao *et al* (16) and the fraction containing the $[^{3}H]$cAMP was saved and evaporated. After acetylation (17) the cAMP content was measured by radioimmunoassay (18). The testosterone content of the other half was estimated by specific radioimmunoassay after purification in a microcelite column (19).

In vitro testosterone production by isolated Leydig cells. Cells were incubated at 33°C for 3 hours in MEM medium pH 7.4 containing 20 mM Hepes (N-2-hydroxyethyl piperazine-N^1-2-ethane-sulfonic acid), bovine serum albumin (0.5 %) and 0.1 mM MIX (3-isobutyl-1-methylxanthine). After incubation, the tubes were centrifuged and testosterone in the supernatant was estimated by a specific radioimmunoassay (19).

RESULTS

Effects of several doses of hCG on receptor loss and Leydig cell refractoriness. (Fig. 1). Since these two processes seem to be hCG dose-dependent (4-10), we have investigated the early effects of different doses of hCG on both phenomena. Rats received a first injection of either saline (control) or hCG (5 to 100 IU). Eight hours later half of the animals of each group received a second

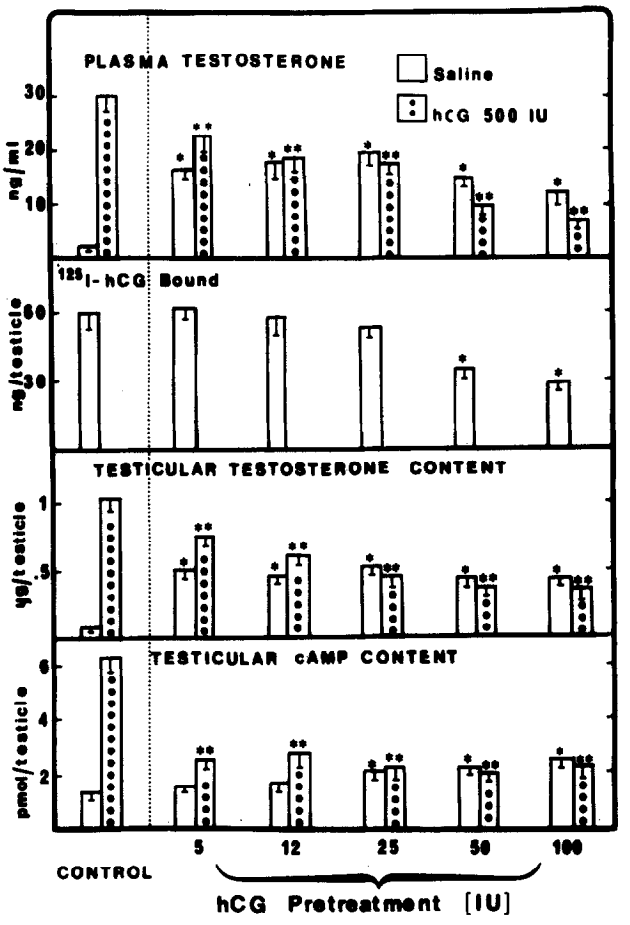

Figure 1 : Effect of the *in vivo* administration of different doses of hCG on plasma testosterone levels (upper panel), binding of ^{125}I-hCG to interstitial cell particles (second panel), testosterone (third panel) and cAMP (lower panel) testicular contents. Male rats 57 days old were injected with the doses of hCG indicated in the bottom of the figure and 8 hours later, half of the animals (6 rats of each group) were injected with saline (open column) while the other half (dotted column) received 500 IU of hCG. All rats were killed 2 hours later. Vertical bars represent ± 1 SD.

* $p < 0.05$ compared to control group (0 IU of hCG)

** compared to hCG (500 IU) injected control group.

injection either of saline or of 500 IU of hCG, and were killed two hours later. Plasma testosterone levels in all animals who received the first injection of hCG were significantly higher than that of controls (Fig. 1, upper panel, open columns). However the response to the second injection of hCG was significantly lower than that of controls which received only one injection of hCG (Fig. 1, upper panel, dotted columns) indicating that within 8 hours desensitization was clearly established.

TABLE 1

Effects of a single injection of hCG (100 IU), cycloheximide (800 µg/100 g) or both, on plasma testosterone, *in vitro* binding of [^{125}I]hCG by interstitial cell particles and *in vitro* testosterone production by interstitial cells. Rats were killed 6 hours after injection

Treatment *in vivo*	Plasma testosterone (ng/ml)*	[^{125}I]hCG bound % of control	*In vitro* testosterone production by interstitial cells* pg per µg DNA per 3 hours			
			Basal	hCG (1 µg/ml)	DbcAMP 10^{-3} M	CHolera toxin (2 µg/ml)
Saline	1.8 ± 0.3	100 ± 7	115 ± 14	457 ± 30☆	477 ± 27☆	487 ± 46☆
hCG	19.5 ± 1.9	38 ± 4▲	560 ± 45	537 ± 37	645 ± 31☆	667 ± 52☆
Cycloheximide	0.31 ± 0.1	105 ± 8	52 ± 7	426 ± 25☆	430 ± 32☆	451 ± 27☆
hCG + Cycloheximide	1.8 ± 0.2	89 ± 6	321 ± 37	341 ± 33	651 ± 45☆	666 ± 39☆

* Mean ± SD of four rats
* Mean ± SD of triplicate replicates of a pool of four rats.
▲ $p < 0.05$ compared to saline of the same column.
☆ $p < 0.05$ compared to basal of the same treatment.

Indeed, only the rats who received 5 IU of hCG in the first injection responded to the second dose of hCG, while in the animals who received 50 and 100 IU of hCG, the second administration of hCG induced (by unknown mechanism) a significant decrease in plasma testosterone. Nevertheless the apparent number of hCG receptors was significantly lower only in animals who received 50 and 100 IU of hCG at the first injection (Fig. 1, second panel). It must be noted that the binding affinity of hCG for its receptors was similar in all groups (data not shown).

Refractoriness to hCG following the first injection of the hormone was further demonstrated by the fact that, 2 hours after the second injection of hCG, cAMP and testosterone contents were significantly lower than those of rats receiving only the second injection of hCG (hCG pretreatment = 0) (Fig. 1, third and lower panel). Indeed, only animals who received 5 and 12 IU of hCG in the first injection, responded to the second injection of hCG with an increase of both testosterone and cAMP testicular content. Thus, this group of experiments shows a dissociation between desensitization, present in all groups treated with hCG, and the loss of receptors that was seen only with high doses of hCG.

Effects of cycloheximide on hCG-induced receptor loss and Leydig cell refractoriness. In order to investigate if both process are dependent on new protein synthesis, the effects of cycloheximide were studied. With the doses of cycloheximide used (800 µg/100 g of body weight), about 85 % of testicular protein

TABLE 2

Effects of a single injection of testosterone propionate (10 mg i.m.) on plasma testosterone and [^{125}I]hCG binding to interstitial cell particles

Hours after injection	Plasma testosterone	[^{125}I]hCG bound
	ng/ml	ng/testicle
0	1.6 ± 0.2*	45 ± 6*
6	265 ± 40	40 ± 4
12	125 ± 20	42 ± 7
24	92 ± 18	43 ± 5

* Mean ± SD of four rats.

synthesis were inhibited (data not shown). Cycloheximide alone decreased plasma testosterone levels without any significant change in the level of hCG receptors (Table 1). As expected, when both hCG and cycloheximide were administered, the antibiotic blocked completely the steroidogenic effect of hCG. On the other hand, the receptor loss induced by hCG was blunted by cycloheximide. These results could suggest that testosterone itself is responsible of the hCG-induced loss of its own receptors. Table 2 shows that this does not seem to be the case, since very high levels of plasma testosterone during a 24 hour period did not modify significantly hCG receptor levels.

In vitro, Leydig cells isolated from cycloheximide rats responded normally to different stimuli (Table 1), indicating that most of the cycloheximide was washed out during the preparation of isolated Leydig cells (four washings with 100 ml of medium). On the contrary, the isolated cells from hCG-cycloheximide pretreated animals, did not respond to hCG in spite of the fact, that the binding capacity was only 11 % lower than that of control animals. Nevertheless both DbcAMP and cholera toxin stimulated testosterone production, indicating that the steroidogenic refractoriness was specific only for hCG.

DISCUSSION

These studies show that early hCG-induced Leydig steroidogenic refractoriness to gonadotropin stimulation is not directly related to the number and/or affinity of receptors, since desensitization is induced by low doses of hCG and is not blocked by cycloheximide, while receptor loss appears only with high doses of hCG and is blocked by cycloheximide. In addition, the observation that cycloheximide inhibited the negative regulation of gonadotropin receptor

tRNA molecules. The discovery of the existence of hydrophilic character beyond the normal binding region, and the proper exploitation of this information has provided the first active puromycin analogue possessing a hydrophilic amino acid R-group.

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