Rapid in vitro desensitization of the testosterone response in rat Leydig cells by sub-active concentrations of porcine luteinizing hormone

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We have studied in rat Leydig cells, the effect of sub-active concentrations of porcine LH on the subsequent stimulation of the cAMP and testosterone production by a sub-maximal concentration of pLH or hCG. We found that extremely low concentrations of pLH (0.01–2.0 ng/ml) were able to induce rapidly a partial but highly significative desensitization of the testosterone response without affecting the cyclic AMP response. These data indicate that desensitization of the steroidogenic response might be due to some lesion beyond cAMP formation or at the level of one discrete compartment of cyclic AMP, directly involved in the control of steroïdogenesis. Moreover, our data strongly suggest that the basal circulating concentrations of LH can exert an inhibitory control on the testosterone response to LH pulses in vivo.

Testosterone secretion *Cyclic AMP production* *Desensitization* *Luteinizing hormone* *Choriogonadotropin* *Leydig cell*

1. INTRODUCTION

Luteinizing hormone (LH) and human choriogonadotropin (hCG) specifically stimulate Leydig cell steroidogenesis through adenylate cyclase activation [1]. In addition, at high concentrations, they induce down-regulation of LH receptors and desensitization of the cyclic AMP response both in vivo and in vitro [2–5]. Low active concentrations of hCG can promote desensitization of the cAMP response in the absence of prior down-regulation of receptors [6]. Desensitization of the testosterone response has also been reported with hCG but a high concentration of hormone (40 ng/ml) was used [5].

In [14], we showed that non-active FSH concentrations were able to inhibit further stimulation by an active dose of FSH. To determine whether such a desensitization existed in rat Leydig cells, we have measured the effect of a short preincubation (1 h) with non-active concentrations of porcine LH (pLH) on the cyclic AMP and testosterone responses to a subsequent stimulation (3 h) by a sub-maximal concentration of either hCG or pLH.

2. MATERIALS AND METHODS

Porcine LH (pLH) was purified as described [12]. Its activity amounted to 1.2 × NIH LH S1 and its FSH cross-contamination was less than 0.1% by wt. Human choriogonadotropin (hCG CR118) was a kind gift from Dr R. Canfield (New York, USA).

The testes from 10 Wistar O3 rats were removed at 52 days of age and carefully decapsulated. The cells were dispersed by collagenase (0.25 mg/ml) digestion of the testes (2 ml/testis) in L15 medium (Serva) for 20 min at 34°C under continuous shaking. After settling of the tubular tissue in a glass cylinder, the supernatant was recovered and centrifuged for 5 min at 80 × g. The cell pellet was resuspended in 2 ml of L15 medium and placed on-

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to a Percoll (Pharmacia) discontinuous gradient (17, 42, 70%). After centrifugation (80 × *g*, 12 min), the Leydig cells were recovered from the 42% phase and washed twice in L15 medium.

The incubations were performed on aliquots (0.4 ml) of cell suspension (4–6 × 10E6 cells/ml) in 3-ml polystyrene tubes, under continuous shaking. The hormones were added in 0.05 ml of L15 medium and the incubations were carried out for 3 h at 34°C. When applicable, a 1 h preincubation was performed under the same conditions in the presence of subeffective concentrations of pLH. At the end of the preincubation, an effective dose of hCG or pLH was added in 0.05 ml of L15 and the incubation was continued as above. No washing was performed at the end of the preincubation period since the hormone concentrations were largely lower than those used during the incubation period. At the end of the experiments, the cells were centrifuged (80 × *g*, 10 min) and secreted testosterone was measured by radioimmunoassay on an aliquot of the incubation media without previous extraction of testosterone [13]. Cyclic AMP concentration in the media was measured by radioimmunoassay using the Institut Pasteur kit.

The relative affinities of pLH and hCG for LH receptors were determined on testicular membrane preparations from animals identical to those used for the study of testosterone and cAMP secretion. The relative affinities of the hormones were determined from their concentrations giving half-maximal inhibition of ^{125}I-oLH binding.

3. RESULTS

Table 1 shows the relative binding activities of pLH and hCG on rat testicular receptors as well as their relative potencies in the stimulation of cAMP production and testosterone secretion by rat Leydig cells. Although hCG exhibits a binding activity similar to that of pLH, its relative potency is largely higher in both the stimulation of testosterone secretion and that of cAMP production.

To determine the effect of preincubation with LH, Leydig cells were incubated for 1 h with pLH concentrations between 2 pg/ml and 10 ng/ml. Afterwards, a submaximal dose of either hCG or pLH was added and the incubation was carried on for 3 h. Preincubation of the cells with pLH con-

Table 1

Relative activities of human choriogonadotropin (hCG) and porcine LH (pLH) in LH receptor binding, cAMP production and testosterone secretion by rat Leydig cells

	Binding	Cyclic AMP	Testosterone
Porcine LH	1	1	1
Human CG	1.1	75	40

The relative activities were calculated on the basis of the concentrations of each hormone giving half-maximum response (inhibition of ^{125}I-LH binding, cAMP production or testosterone secretion) taking porcine LH as the reference

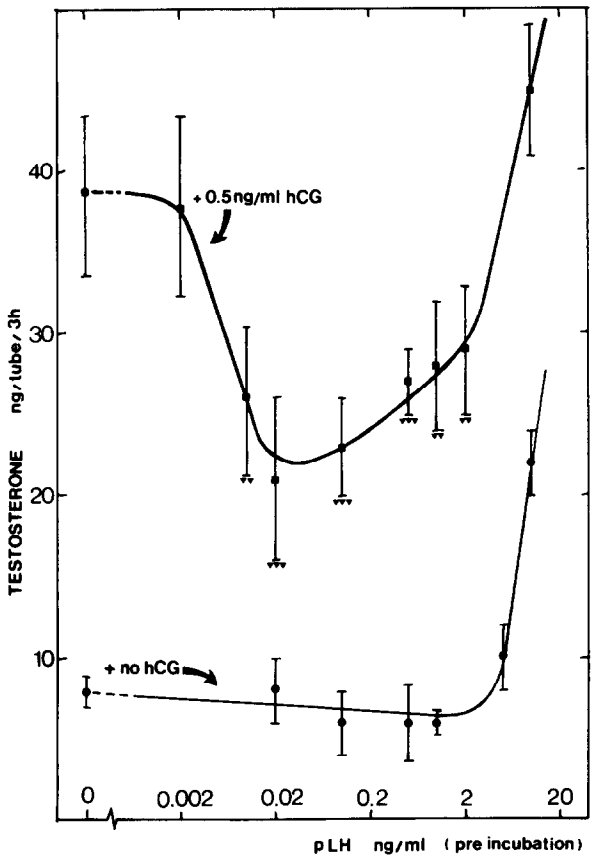

Fig.1. Effect of preincubation of rat Leydig cells with non-active pLH concentrations on the subsequent testosterone response to hCG. Leydig cells were incubated with the indicated pLH concentrations for 1 h, after which time buffer or a sub-maximal concentration of hCG was added for an additional 3 h incubation. Bars denote standard deviations. Significance of the effect related to control preincubated without pLH: (▼ ▼) *P* 95%; (▼ ▼ ▼) *P* 99%.

centrations between 10 pg/ml and 2 ng/ml induced partial but highly significative inhibition of the subsequent stimulation of testosterone secretion by a sub-maximal concentration of either hCG (fig.1) or pLH (fig.2). At the highest concentrations of hormone during the preincubation period, the stimulating effect cumulated with that of the constant dose of gonadotropin.

Fig.3 shows that there was no inhibitory effect of sub-effective concentrations of pLH on the subsequent cyclic AMP response to a sub-maximal concentration of the same hormone. Similarly, preincubation with non-active concentrations of hCG (2–500 pg/ml) was without effect on the response to a subsequent stimulation by a submaximal dose of hCG (1 ng/ml). In this figure, it can also be noted that the testosterone response was obtained at a much lower LH concentration than the cAMP response.

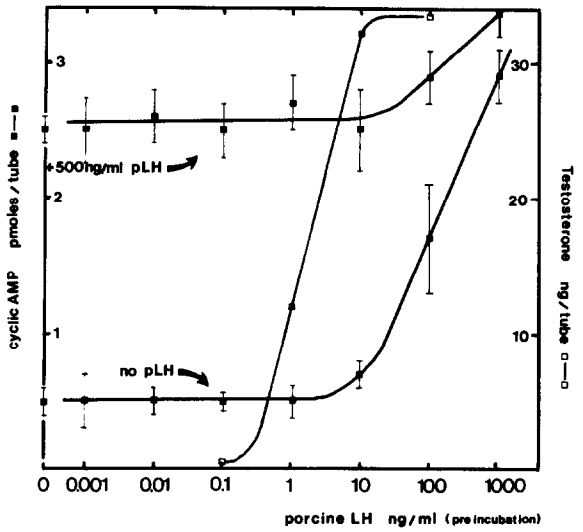

Fig.3. Effect of preincubation of rat Leydig cells with non-active pLH concentrations on the subsequent cAMP response to a submaximal concentration of LH. The dose-related testosterone response is shown for the sake of comparison.

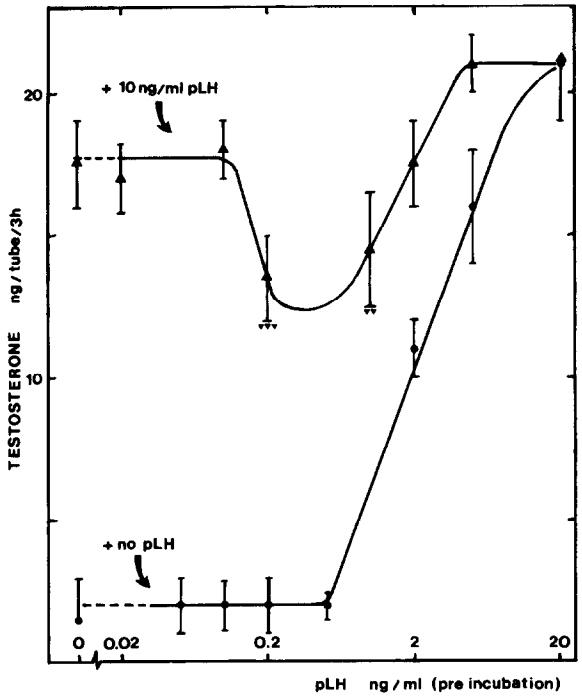

Fig.2. Effect of preincubation of rat Leydig cells with non-active pLH concentrations on the subsequent testosterone response to the same hormone. Conditions similar to those in fig.1 except that pLH was used for stimulation instead of hCG.

4. DISCUSSION

We have shown here that the relative potency of hCG in the stimulation of testosterone secretion by rat Leydig cells was largely higher than that expected from its binding activity (table 1). This behaviour might be related to the prolonged stimulation by hCG even when it is present only during a short time [15]. Indeed, in similar conditions, oLH stimulated testosterone production only for a short period of time. The sustained stimulation by hCG compared to oLH could be due to a lower rate of internalization as in luteal cells [16].

Since hCG was much more effective in stimulating cAMP and testosterone production when bound to the Leydig cell plasma membrane (table 1), it was plausible that pLH could inhibit its activity by competition at the receptor level. Indeed, fig.1 shows that pLH inhibits the testosterone response to a fixed submaximal dose of hCG.

However, it is obvious that this inhibition was not due to competition at the receptor level between pLH and hCG since the response to a submaximal concentration of pLH was also diminished after preincubation with non-effective con-

centrations of the same hormone. Moreover, it had been previously demonstrated [8] that full steroidogenic response in rat Leydig cells was triggered with less than 1% occupancy of the LH receptors. Since inhibition was observed with concentrations of hormone which were even lower, competition at the receptor level cannot be invoked to explain it. Consequently, these data led us to the conclusion that inhibition of the testosterone response by non-stimulating concentrations of hormone was due to Leydig cell desensitization.

At present, the step at which this desensitization occurs cannot be defined with certainty. It is unlikely that it is due to down-regulation of receptors. Indeed, long incubations (12 h or more) of Leydig cells with high concentrations of hCG (10–100 ng/ml) have been shown to result in down-regulation of receptors and reduced steroidogenic response [2–7] but the maximal steroidogenic response to cholera toxin or 8-Br-cAMP was also reduced [5] indicating that receptor down-regulation was not fully responsible for desensitization by high concentrations of hormone. Moreover, at very low concentrations of hormone, no down-regulation of receptors was observed [11].

We demonstrated partial inhibition of the hCG-stimulated testosterone response with pLH concentrations as low as 2–20 pg/ml. These concentrations are about 1000-fold lower than the stimulating concentrations of pLH. Consequently, desensitization cannot be attributed to depletion of the cholesterol pool following testosterone production as proposed by Freeman and Ascoli [5] to explain the desensitization of the testosterone response by high doses of hCG (40 ng/ml).

Interestingly, we did not find any inhibition of the cAMP response to a submaximal concentration of pLH or hCG after preincubation of Leydig cells with non-effective concentrations of these hormones. This result suggested that desensitization by non-effective concentrations of hormone either affects some distal step beyond cAMP formation or one discrete pool of cAMP directly involved in steroidogenesis control. Schumacher et al. [7] reported that the cAMP response of mouse Leydig cells to a high dose of hCG (100 ng/ml) was diminished after preincubation with low but active concentrations of hCG (0.1–1.0 ng/ml). The difference between the data from the two laboratories might be due either to the species studied or to the fact that we followed the modification of the response to a submaximal dose of hCG (1 ng/ml) while these authors studied the inhibition of the maximal response to hCG (100 ng/ml).

From a physiological point of view, it is known that LH secretion in the male rat is pulsatile [10] with a basal concentration around 0.3 ng/ml. We have demonstrated in this paper that extremely low (non-active) LH concentrations (0.01 ng/ml) inhibited the testosterone response to an active concentration of LH or hCG. This observation strongly suggests that the basal circulating concentrations of hormone exert an inhibitory effect on the in vivo testosterone response elicited by LH pulses.

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