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Effect of lutropin and cycloheximide on lutropin receptors and cyclic AMP production in Leydig tumour cells *in vitro*

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A system to study lutropin-induced desensitization of tumour Leydig cells *in vitro* has been investigated. Tumour Leydig cells were purified on a Percoll gradient and then incubated for 30 min with lutropin (0–1000 ng/ml). The cells were then washed and incubated in suspension media at 32°C. ^{125}I-labelled human choriogonadotropin binding and basal and lutropin-stimulated cyclic AMP production were determined at various times. Initially the cells showed a dose-dependent decrease in human choriogonadotropin binding (1.18 and 0.13 fmol/10^6 cells respectively) followed by an increase at 1 h (2.32 and 0.87 fmol/10^6 cells respectively). Human choriogonadotropin binding remained elevated in the cells pre-incubated without lutropin, whereas the cells pre-incubated with lutropin showed a dose-dependent decrease over the next 10 h (2.20–0.18 fmol/10^6 cells respectively). Basal production of cyclic AMP initially reflected the pre-incubation conditions (1.17–21.19 ng/10^6 cells per h for 0–1000 ng of lutropin/ml respectively). However, by 1 h there was a marked rise in basal cyclic AMP production which returned to the initial lower values by 4 h. At all time intervals studied, lutropin-induced cyclic AMP production showed a decrease that was proportional to lutropin concentration in the pre-incubated media. The decreases in human choriogonadotropin binding produced by pre-incubations with lutropin (100 ng/ml) was partially inhibited by the presence of cycloheximide in the pre-incubation media and totally prevented by the continuous presence of cycloheximide. These results demonstrate that desensitization of tumour Leydig cells occurs after exposure to lutropin *in vitro*. This desensitization involves both a loss of plasma membrane receptors for lutropin and lutropin-stimulated adenylate cyclase. These events can be prevented by cycloheximide and are therefore probably dependent on protein synthesis.

It is established that rat testis Leydig cells become refractory or desensitized to the steroidogenic action of lutropin *in vitro* after prior *in vivo* exposure to lutropin or human choriogonadotropin (for review see Catt *et al.*, 1979). This loss of sensitivity is accompanied by a loss of plasma-membrane receptors for lutropin and the degree of desensitization is dependent on the initial concentration of lutropin or choriogonadotropin to which the cells are exposed and the length of time of this exposure.

The mechanism of desensitization not only involves lutropin receptor loss but also a decrease in the activity of the plasma-membrane adenylate cyclase (Saez *et al.*, 1978) and of some of the enzymes in the steroidogenic pathway from pregnenolone to testosterone (Tsuruhara *et al.*, 1977). Preliminary studies *in vivo* have indicated that desensitization of the testis Leydig cell may also be dependent on protein synthesis (Sharpe, 1977; Saez *et al.*, 1978). These biochemical events, which have only been investigated after exposure of the Leydig cells to lutropin or choriogonadotropin *in vivo*, may in addition involve other factors, including other hormones such as prolactin, oestrogens and prostaglandins. To investigate these other factors and the detailed mechanisms involved it would be advantageous to study desensitization *in vitro* using a pure preparation of Leydig cells and under defined conditions. In addition if detailed biochemical analysis, including isolation and purification of the components of the system, is to be carried out, then large numbers of Leydig cells are required. The use of rat or mouse testes for this purpose is therefore restrictive as only relatively small numbers of Leydig cells can be isolated. However, a rat Leydig-cell

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tumour, which has been described and characterized (Cooke *et al.*, 1979*b*), has the added advantage of providing large numbers of cells. These tumour cells differ from adult rat testis Leydig cells mainly in the pathways of steroidogenesis. However, in many other respects, e.g. response to lutropin in terms of cyclic AMP, protein kinase activation and phosphorylation of specific endogenous proteins, they were shown to be similar.

In the present paper we report the initial investigations carried out to determine the suitability of the Leydig tumour cells to study desensitization *in vitro*. Suspensions of the cells were prepared by incubation of the tumour with collagenase followed by purification of the cells on Percoll density gradients. Portions of the cells were incubated with various concentrations of lutropin, washed and then incubated in suspension cultures. At various times the binding of ^{125}I-labelled choriogonadotropin and the cyclic AMP production after the addition of maximum stimulating amounts of lutropin were measured. Similar experiments were also carried out in the presence of the protein-synthesis inhibitor cycloheximide.

Materials and methods

Sheep lutropin (NIH S20; 1.9 i.u. NIH SI/mg) and human choriogonadotropin (NIH CR115) were gifts from the Endocrinology Study Section, National Institutes of Health, Bethesda, MD, U.S.A. Na^{125}I (carrier-free) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The antiserum for the cyclic AMP radioimmunoassay was a gift from Dr. H. Cailla, Centre D'Immunologie, Marseille, France. Crude collagenase (180 units/mg) was purchased from Worthington Biochemical Corporation, Freehold, NJ, U.S.A. Methylcellulose, Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and cycloheximide {4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]piperidine-2,6-dione} were purchased from Sigma Chemical Co. Percoll was purchased from Pharmacia Fine Chemicals and 3-isobutyl-1-methylxanthine was purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Foetal calf serum was purchased from Gibco-Biocult.

Choriogonadotropin was radioiodinated by the lactoperoxidase method and purified by Sephadex G-25 chromatography essentially as described by Thorell & Johansson (1971). The specific radioactivity of the ^{125}I-labelled choriogonadotropin was determined by a self-displacement radioreceptor assay to be 29000 d.p.m./ng at the time of preparation.

The 2-*O*-succinyltyrosine methyl ester derivative of cyclic AMP was radioiodinated by using the chloramine-T method and purified by Sephadex G-100 chromatography essentially as described by Cailla & Delaage (1972).

The Leydig tumour used for these studies has been previously described and characterized (Cooke *et al.*, 1979*b*). The tumour was grown from a subcutaneous implant in 3-month-old Fischer rats. Once the tumour had grown to an adequate size (3–4 cm diameter) the rat was killed by cervical dislocation, the tumour excised and then dissected free from the connective tissue and necrotic areas under sterile conditions. The healthy tumour fragments were finely minced with scissors and then incubated in the dissection media (Dulbecco's modified Eagle's minimum essential medium, 25 mM-Hepes, 5% foetal calf serum and 0.1 mg of kanamycin/ml) containing collagenase (1 mg/ml) at 32°C for 20 min. The dispersed cells and fragments were centrifuged (200 *g* for 10 min) and the supernatant was decanted and the pellet resuspended in fresh dissection medium and the centrifugation step was repeated. The pellet was then resuspended in fresh media and flushed 20 times in and out of a syringe (50 ml) fitted with a kwill tube. The large fragments were allowed to settle and the supernatant containing dispersed cells was decanted and saved. The flushing process was repeated twice with tumour fragments and the combined supernatants were then filtered through nylon gauze (60 μm) and centrifuged (200 *g* for 10 min). The resultant pellet was layered on to a 0–90% (v/v) Percoll gradient and centrifuged (1000 *g* for 25 min). The Percoll purification step has been previously described by Schumacher *et al.* (1978).

The purified Leydig tumour cells (density 1.070 g/ml) were then removed from the gradient with a pasteur pipette and diluted with suspension media [Dulbecco's modified Eagle's minimum essential medium, 5% (v/v) foetal calf serum, 25 mM-Hepes, 0.1 mg of kanamycin/ml and 0.1% (w/v) methylcellulose]. These cells were washed twice by centrifugation (200 *g* for 10 min) and resuspended.

In all experiments cells were pre-incubated with either suspension media alone or suspension media containing lutropin (10, 100 or 1000 ng/ml) for 30 min in a shaking water bath at 32°C. After pre-incubation the cells were washed twice and then resuspended in fresh suspension media and maintained in suspension for up to 12 h at 32°C. At various time points cells were removed and specific ^{125}I-labelled choriogonadotropin binding and basal and lutropin (10 μg/ml)-stimulated cyclic AMP production were determined. When indicated cycloheximide (2.5 μg/ml) was present in the pre-incubation media or throughout the experiment.

^{125}I-labelled choriogonadotropin binding was determined by incubating cells in phosphate-buffered saline [0.01 M-phosphate buffer + 0.9% NaCl + 0.1% (w/v) bovine serum albumin, pH 7.4] for 2 h at 32°C

in the presence of ^{125}I-labelled choriogonadotropin (20ng/ml). Non-specific binding was determined in parallel incubations that also contained excess lutropin (20 μg/ml). All incubations were carried out in triplicate. Non-specific binding never exceeded 2% of the total radioactivity present in the incubations. At the end of the incubation period cold (4°C) phosphate-buffered saline (2ml) was added to each incubation mixture followed by centrifugation (1500*g* for 10min at 4°C). The supernatants were decanted and this washing step was repeated. After decanting the supernatants, the cell-bound radioactivity was determined in an automatic gamma spectrometer (LKB) with 60% counting efficiency for ^{125}I.

Estimation of basal and lutropin-stimulated cyclic AMP production was carried out by incubating the cells in fresh suspension media containing 3-isobutyl-1-methylxanthine (0.5mM) with or without lutropin (10 μg/ml) for 1h at 32°C. The cyclic AMP was then extracted from the cells by addition of $HClO_4$ (final concentration 0.5M), this was then neutralized by adding tripotassium orthophosphate (final concentration 0.23M). A portion (200 μl) of the resultant supernatants was then acetylated by the addition of 5 μl of freshly prepared acetylating reagent consisting of 1 part acetic anhydride and 2.7 parts triethylamine; the cyclic AMP standards for the radioimmunoassay were also treated in the same manner. The radioimmunoassay used for cyclic AMP determinations was essentially as described by Delaage *et al.* (1979).

All cell counting was carried out with a haemocytometer and assessment of cell viability was by the Trypan Blue exclusion test. In all experiments there were no more than 10% non-viable cells.

All values quoted for lutropin-induced cyclic AMP production are calculated after subtraction of the basal cyclic AMP production.

Statistical analysis was by Student's *t* test.

Results

The rat tumour Leydig cells used for these studies were purified by centrifugation on Percoll density gradients. The Leydig cells isolated had a density of 1.070g/ml and were free from erythrocytes and other nucleated cells. In agreement with the results of Cooke *et al.* (1979*b*) they were found to respond in a dose-dependent manner to lutropin and gave a maximum testosterone production of 12.1 ± 0.82ng/10^6 cells per 2h (mean $\pm$ S.E.M., $n = 3$) and cyclic AMP production of 59.2 ± 4.6ng/10^6 cells per 2h (mean $\pm$ S.E.M., $n = 3$). In addition they were found to contain 1626 ± 361 choriogonadotropin-binding sites per cell. As described above the cells were maintained in suspension culture in a shaking water bath for a period of up to 12h; no deterioration in the viability of the cells was detected during this period as judged by the Trypan Blue exclusion test and morphological appearance of the cells.

Effect of pre-incubation of isolated Leydig cells with lutropin

Fig. 1 shows the results of pre-incubating the Leydig cells with different amounts of lutropin. After washing the cells with media free from lutropin it was found that the degree of ^{125}I-labelled choriogonadotropin binding was inversely proportional to the amount of lutropin present during the pre-incubation. This probably reflects the degree of receptor occupation by lutropin during the pre-incubation period. During subsequent incubations there was an apparent increase in the number of lutropin receptors; the binding of ^{125}I-labelled choriogonadotropin to the control cells increased from 1.18 ± 0.28 to 2.70 ± 0.19fmol/10^6 cells ($P < 0.01$) during the first 2h of incubation compared with 0.69 ± 0.21 to 1.84 ± 0.39fmol/10^6 cells ($P < 0.01$) in the cells pre-incubated with 100ng of lutropin/ml. The initial rates of increase in ^{125}I-labelled human choriogonadotropin binding after lutropin pretreatments were similar to that of the saline pretreatment. The increased amount of binding was maintained in the saline-pretreated cells, whereas lutropin pretreatments caused a dose- and time-dependent decline in choriogonadotropin binding (Fig. 1).

Initially the basal cyclic AMP production reflected the pre-incubation conditions in which lutropin caused a dose-related increase in basal

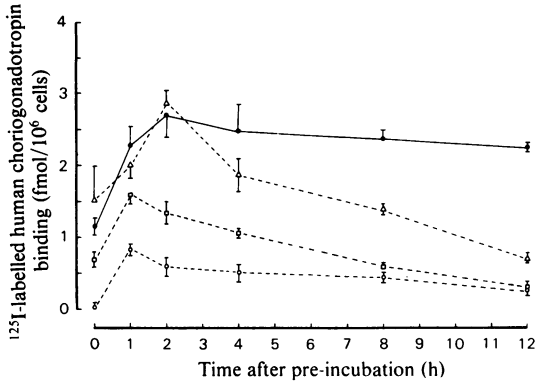

Fig. 1. *Effects of lutropin on the lutropin receptor content of Leydig tumour cells*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated with various concentrations of lutropin (0, ●; 10, △; 100, □; 1000ng/ml, ○) for 30min at 32°C, and subsequently washed and incubated at 32°C for the times indicated and then ^{125}I-labelled human choriogonadotropin binding was determined. The specific ^{125}I-labelled human choriogonadotropin binding values given are means $\pm$ S.E.M. for triplicate determinations.

production. This was followed by a lutropin-independent rise in basal cyclic AMP production that remained elevated for 2h and then declined (Fig. 2). This rise in basal cyclic AMP production occurred at the same time as the lutropin-independent rise in ^{125}I-labelled choriogonadotropin binding (cf. Figs. 1 and 2). The lutropin-stimulated cyclic AMP production of the isolated Leydig cells pre-incubated without lutropin remained constant for 8h after the pre-incubation and started to decline by 12h (Fig. 3). Unlike the ^{125}I-labelled choriogonadotropin binding and basal cyclic AMP production, the cells that had been pre-incubated with lutropin showed immediate dose-related decreases in lutropin-stimulated cyclic AMP production, which further declined by 1h and remained at these decreased values throughout the experiment (Fig. 3).

Effect of cycloheximide on lutropin-pretreated isolated Leydig cells

The presence of cycloheximide (2.5 μg/ml) in both the pre-incubation media, together with lutropin, and in the subsequent incubations partially prevented the initial decrease in ^{125}I-labelled choriogonadotropin binding produced by pre-incubation with lutropin alone (Fig. 4). However, cycloheximide did not inhibit the subsequent lutropin-independent increase in ^{125}I-labelled choriogonadotropin binding and in addition significantly increased the ^{125}I-labelled

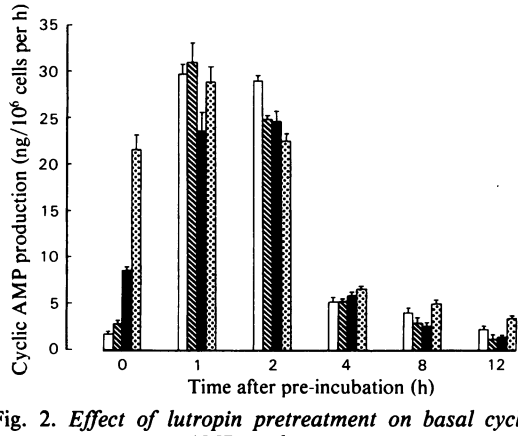

Fig. 2. *Effect of lutropin pretreatment on basal cyclic AMP production*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated with various concentrations of lutropin (0, □; 10, ▨; 100, ■; 1000ng/ml, ⊞) for 30min at 32°C, washed and incubated at 32°C for the times indicated and then the cyclic AMP content was determined as described in the Materials and methods section. Results represent means ± S.E.M. for triplicate determinations.

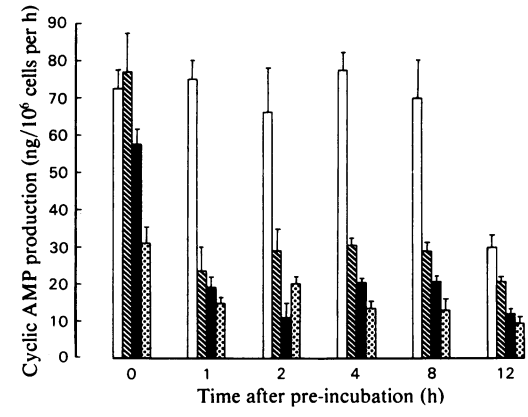

Fig. 3. *Effect of lutropin pretreatment of lutropin-induced cyclic AMP production*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated with saline (□), lutropin (▨; 10ng/ml), lutropin (■; 100ng/ml) or lutropin (⊞; 1000ng/ml) for 30min at 32°C, washed and incubated at 32°C for the times indicated and the lutropin-induced cyclic AMP content was determined as described in the Materials and methods section. Results represent means ± S.E.M. for triplicate determinations. Basal cyclic AMP content has been subtracted from all the values.

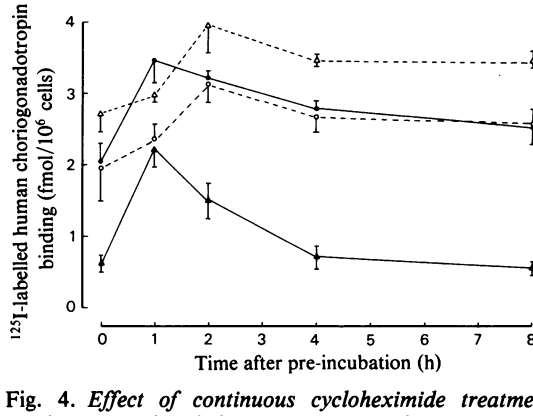

Fig. 4. *Effect of continuous cycloheximide treatment on lutropin-induced lutropin receptor loss in Leydig tumour cells*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated in the presence of saline (●), saline plus cycloheximide (△; 2.5 μg/ml), lutropin (▲, 100ng/ml) or lutropin (○, 100ng/ml) plus cycloheximide (2.5 μg/ml) for 30min at 32°C, washed and incubated at 32°C for the times indicated and then the ^{125}I-labelled human choriogonadotropin binding was determined. Cells that were pre-incubated with cycloheximide also had cycloheximide present in the subsequent incubations. The specific ^{125}I-labelled human choriogonadotropin binding values given are means ± S.E.M. for triplicate determinations.

Table 1. *The effect of cycloheximide pretreatment on lutropin receptor content, basal and lutropin stimulated cyclic AMP production in rat Leydig tumour cells*

Tumour Leydig cells were prepared as described in Materials and methods and then preincubated with saline, cycloheximide (2.5 μg/ml), lutropin (100 ng/ml) or lutropin (100 ng/ml) plus cycloheximide (2.5 μg/ml) for 30 min at 32°C after which time they were washed and incubated at 32°C for the times indicated. Results represent means ± S.E.M. for triplicate determinations. Lutropin-induced cyclic AMP production was calculated after subtraction of the basal cyclic AMP values.

Time after pretreatment (h)	Pretreatment	Specific ^{125}I-labelled human choriogonadotropin binding (fmol/10^6 cells)	Basal cyclic AMP production (ng/10^6 cells/per h)	Lutropin-induced cyclic AMP production (ng/10^6 cells per h)
0	Saline	2.09 ± 0.37	1.83 ± 0.08	67.4 ± 5.42
	Cycloheximide	2.74 ± 0.45	1.11 ± 0.05	79.8 ± 7.81
	Lutropin	0.64 ± 0.12	5.9 ± 0.73	51.6 ± 8.41
	Lutropin + cycloheximide	2.05 ± 0.52	5.4 ± 0.21	43.5 ± 6.82
1	Saline	3.47 ± 0.40	20.4 ± 1.60	93.7 ± 11.74
	Cycloheximide	2.60 ± 0.34	18.4 ± 0.71	107.7 ± 10.53
	Lutropin	2.18 ± 0.33	21.5 ± 0.70	83.2 ± 17.51
	Lutropin + cycloheximide	3.65 ± 0.58	23.7 ± 0.89	75.2 ± 9.83
2	Saline	3.19 ± 0.02	26.4 ± 3.78	98.6 ± 11.41
	Cycloheximide	3.04 ± 0.22	25.3 ± 0.90	93.4 ± 12.93
	Lutropin	1.53 ± 0.56	22.7 ± 1.68	87.1 ± 9.83
	Lutropin + cycloheximide	2.62 ± 0.05	24.5 ± 3.49	125.6 ± 16.51
4	Saline	2.83 ± 0.09	2.98 ± 0.28	88.4 ± 10.31
	Cycloheximide	2.73 ± 0.24	2.70 ± 0.41	92.7 ± 9.31
	Lutropin	0.69 ± 0.21	5.1 ± 0.43	38.3 ± 6.48
	Lutropin + cycloheximide	2.20 ± 0.27	2.24 ± 0.18	66.4 ± 11.76
8	Saline	2.53 ± 0.47	2.3 ± 0.48	78.3 ± 8.31
	Cycloheximide	2.59 ± 0.11	2.9 ± 0.35	86.4 ± 7.90
	Lutropin	0.56 ± 0.05	2.8 ± 0.41	22.4 ± 3.63
	Lutropin + cycloheximide	2.11 ± 0.09	2.6 ± 0.34	67.3 ± 8.75

choriogonadotropin binding of saline-pretreated cells ($P < 0.05$), which was maintained at this increased value throughout the experiment (Fig. 4). The lutropin-dependent decline in ^{125}I-labelled choriogonadotropin binding was also inhibited by the presence of cycloheximide in the pre-incubation medium and subsequent incubations (Fig. 4), but was only partially inhibited when cycloheximide was only present in the pre-incubation media (Table 1).

The presence of cycloheximide in the pre-incubation media or throughout the experiment had no effect on the basal pattern of cyclic AMP production (Table 1 and Fig. 5), but both pretreatment with and the continuous presence of cycloheximide prevented the lutropin-dependent decline in sensitivity to lutropin (Table 1, Fig. 6). In addition the continuous presence of cycloheximide caused a significant increase in lutropin-stimulated cyclic AMP production (Fig. 6). The possibility exists that the cycloheximide present in the pre-incubation media inhibits the initial binding of lutropin to the receptors. However, competitive binding studies with cycloheximide showed that it did not inhibit ^{125}I-labelled choriogonadotropin binding to isolated Leydig cells (results not shown).

Discussion

The results of the present investigation clearly demonstrate that desensitization of the tumour Leydig cells occurs after exposure to lutropin *in vitro*; a dose-dependent loss of both lutropin receptors and lutropin-stimulated cyclic AMP production occurred over a period of 12 h. However, this lutropin-dependent loss of receptors and lutropin-stimulated cyclic AMP production was preceded by a rise in receptor number and basal cyclic AMP production. An increase in ^{125}I-labelled choriogonadotropin binding to homogenates of rat testes after prior exposure *in vivo* to choriogonadotropin has been reported (Hsueh *et al.*, 1977; Huhtaniemi *et al.*, 1978). This receptor rise *in vivo* was choriogonadotropin-dependent, whereas the results of the present study indicated that both the rise in receptor number and basal cyclic AMP production were lutropin-independent. A possible explanation for the lutropin receptor rise is that the collagenase treatment of the tumour fragments may involve some proteinase activity that exposes cryptic receptor sites. However, it has recently been reported that collagenase treatment causes a decrease and not an increase in glucagon receptors in rat liver cells

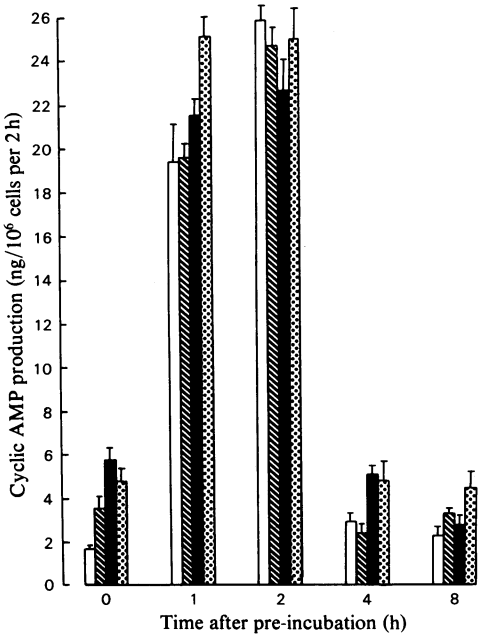

Fig. 5. *Effect of continuous cycloheximide treatment on basal cyclic AMP production*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated with saline (□), saline plus cycloheximide (▨; 2.5 μg/ml), lutropin (■; 100 ng/ml), or lutropin (100 ng/ml) plus cycloheximide (2.5 μg/ml) (▦) for 30 min at 32°C, washed and incubated at 32°C for the times indicated and then the cyclic AMP content was determined as described in the Materials and methods section. Cycloheximide-pretreated cells were incubated throughout the experiment in the presence of cycloheximide. Results are means ± S.E.M. for triplicate determinations.

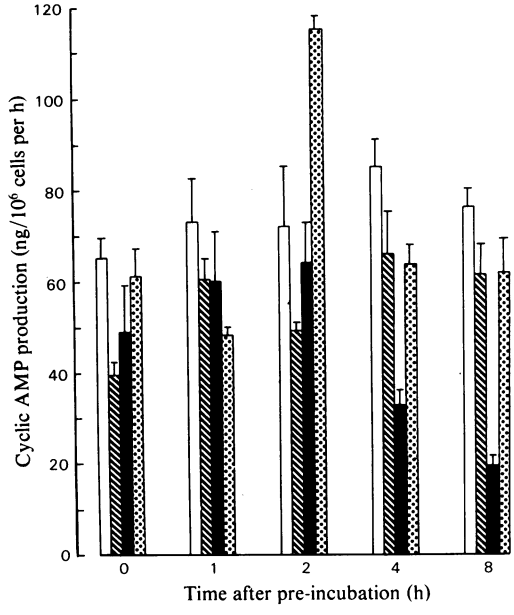

Fig. 6. *Effect of continuous cycloheximide treatment on lutropin-induced cyclic AMP production*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated with saline (□), saline plus cycloheximide (2.5 μg/ml; ▨), lutropin (100 ng/ml; ■) or lutropin (100 ng/ml) plus cycloheximide (2.5 μg/ml (▦) for 30 min at 32°C, washed and incubated at 32°C for the times indicated and the lutropin-induced cyclic AMP content was determined as described in the Materials and methods section. Cycloheximide-pretreated cells also had cycloheximide present throughout the experiment. Results are means ± S.E.M. for triplicate determinations. Basal cyclic AMP content has been subtracted from all values.

(Rouer *et al.*, 1980), and preliminary studies in our laboratory also indicate that a decrease in the number of lutropin receptors in tumour Leydig cells occurs after collagenase treatment (results not shown).

The lutropin-stimulated cyclic AMP production does not follow the same pattern as the lutropin receptors and basal cyclic AMP production; a lutropin dose-dependent decrease in lutropin-stimulated cyclic AMP production was evident from the start and remained so throughout the experiment. Three factors may contribute to this apparent desensitization: first, the occupation of lutropin receptors, which occurs in the pre-incubation with lutropin; secondly, the increased basal cyclic AMP production, which may reflect close to maximal activation of the adenylate cyclase; and finally the eventual decline in lutropin receptor numbers. However, it is possible that uncoupling of the receptor from the adenylate cyclase as reported *in vivo* (Saez *et al.*, 1978) is an early event in lutropin-dependent desensitization and is the cause of this decrease in cyclic AMP production. In support of this is the finding that in ovarian plasma membranes lutropin induces a rapid (10–15 min) decrease in adenylate cyclase activity due to impaired coupling of the enzyme system (Ezra & Salomon, 1980).

The lutropin-dependent decrease in cyclic AMP production and the loss of lutropin receptors are both dependent on protein synthesis and this again agrees with studies *in vivo* (Saez *et al.*, 1978; Sharpe, 1977). However, the initial rise in the number of lutropin receptors is not prevented by cycloheximide and therefore is probably not dependent on protein synthesis *de novo*.

The finding that lutropin receptors and basal cyclic AMP production initially increase may be relevant to previous studies in which it was shown that pre-incubation of isolated rat Leydig cells for several hours *in vitro* causes a decrease in the time the cells take to synthesize testosterone in response to lutropin from 30min to 5min (Cooke *et al.*, 1977). In pre-incubated cells there was also a reproducible and significant stimulation of cyclic AMP-dependent protein kinase activation with all amounts of lutropin that submaximally stimulate testosterone production (Cooke *et al.*, 1976). Also in pre-incubated cells but not in freshly isolated cells, lutropin-stimulated testosterone production is initially independent of RNA synthesis *de novo* (Cooke *et al.*, 1979*a*). These facts and the finding that receptor numbers and basal cyclic AMP increase over the first 2h of incubation suggest that the cells undergo a recovery or re-equilibration period after isolation and purification.

In conclusion we have demonstrated *in vitro* that rat Leydig tumour cells undergo events similar to those described *in vivo* for normal Leydig-cell desensitization. It remains to be seen whether the enzymes in the steroidogenic pathway also show a decrease in activity during this initial desensitization period. With the advantages of well-defined conditions and the ability to prepare large numbers of cells this model should be of great value in the elucidation *in vitro* of the detailed molecular events of desensitization.

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