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# LH/hCG Responsive Adenylyl cyclase in Rat Testis and Testes from Testicular Feminized Male (Tfm) Rats and Mice: Desensitization by Homologous Hormone

By

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Testes of testicular feminized (tfm) rats and mice, as well as of normal male rats contain an LH/hCG responsive adenylyl cyclase. Basal, as well as hCG stimulated activities were higher in tfm rats and mice than in normal rats. The presence of an LH/hCG responsive adenylyl cyclase in the testis of tfm rats and mice shows that the greatly elevated LH levels present in males having this syndrome, giving 80–90 % reduction in LH/hCG receptors, do not cause an uncoupling of the remaining receptors from the adenylyl cyclase. It also shows that androgens are not essential for coupling of the LH/hCG receptors to the adenylyl cyclase.
Injection of 200 IU of hCG into adult normal rats and tfm rats caused, after 48 h, a complete loss of LH/hCG stimulated adenylyl cyclase, whereas the FSH responsive adenylyl cyclase in both animal preparations was maintained. Desensitization of the LH responsive adenylyl cyclase by hCG in normal rats, confirms previous studies showing lack of hCG stimulated cyclic-AMP secretion after a comparable dose of hCG *in vivo*. Similarly, hCG (50 IU) caused a transient loss of LH/hCG responsive adenylyl cyclase in tfm mice, with a complete disappearance of response after 24 h. At 48 and 72 h after injection of hCG the response gradually returned to normal. The fact that hCG caused a complete desensitization of the LH/hCG re-

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sponsive adenylyl cyclase in both tfm rats and mice, proves that androgen receptor mediated events are not involved in hCG desensitization of the adenylyl cyclase in Leydig cells.

*Key words:* LH-receptors – testis – adenylyl cyclase – desensitization – testicular feminization.

It is currently assumed that luteinizing hormone (LH), and hCG are activating the Leydig cells by binding to specific membrane receptors, which triggers adenylyl cyclase and results in increased intracellular concentrations of the second messenger. cyclic-AMP (Cooke et al. 1972: Dorrington & Fritz 1974; Braun & Sepsenwol 1974, 1976; Dufau et al. 1973). With few exceptions (Braun & Sepsenwol 1974, 1976) the involvement of the adenylyl cyclase in LH activation of Leydig cells stems from measurements of cyclic-AMP secretion into the medium after incubation of living cells.

It has recently been shown that elevation of circulating LH or hCG by exogenous administration of hCG causes a time and dosedependent reduction in LH receptors with a concomittant desensitization of the Leydig cells measured either by androgen secretion or cyclic-AMP secretion into the incubation medium (Sharpe et al. 1976; Dufau et al. 1978; Purvis et al. 1977b, 1978b). In two recent publications we have reported that Leydig cells of testicular feminized male (tfm) rats exhibit a greatly decreased number of LH receptors and also a decreased responsiveness to hCG as measured by *in vitro* production of testosterone (Purvis et al. 1977a), and androstenedione and cyclic-AMP (Purvis et al. 1978a). The marked reduction in LH receptors on tfm Leydig cells, is probably due to the very high levels of circulating LH found in animals with this syndrome (Purvis et al. 1977c), since a comparable decrease in LH receptors was observed in normal rats treated with LHRH or LHRH-agonists (Labrie et al. 1978). It has previously been shown that the LH sensitive adenylyl cyclase of the ovary (both follicles and corpus luteum) is rapidly desensitized after injections of an ovulatory dose (100–200 IU) og hCG *in vivo* (Hunzicker-Dunn & Birnbaumer 1976a,b). In the present study we explored, using direct determination of the LH responsive adenylyl cyclase in the testis, whether the decrease in steroid and cyclic-AMP secretion reported previously (Dufau et al. 1978; Purvis et al. 1978c), is due to a direct effect on the adenylyl cyclase system. Furthermore, since Leydig cells from tfm rats and mice already are desensitized (at least as judged from the number of LH receptors and by the increased dose of LH required for androgen production), it was of interest to examine whether we could detect an LH responsive adenylyl cyclase in tfm rats and mice. Finally, if that were the case, it would be of interest to see whether comparable doses of hCG would cause desensitization of the adenylyl cyclase in tfm Leydig cells.

Results from these experiments should answer the question whether or not androgen receptor mediated events are involved in hCG desensitization of the adenylyl cyclase.

## Materials and Methods

*Animals:* Adult (70 day old), male Sprague Dawley rats were used as controls. Tfm rats and their normal male littermates were purchased from the International Foundation for the Study of Rat Genetics and Rodent Control, Oklahoma City, USA. Tfm mice were bred locally and the breeding animals were kindly supplied by Dr. Mary Lyon. The tfm rats and mice were used at an age of 12–16 months old, since Leydig cell hyperplasia is progressing with age. In most instances, tissues were processed immediately after sacrifice, but freezing either of tissues or membrane particles at –70°C allowed the later determination of LH responsive adenylyl cyclase.

*Materials:* hCG (CR 119) was a gift from the NIH-NIAMDD, Bethesda Maryland. Human LH (ca. 20 000 IU/mg by RIA) and hFSH (ca. 15 000 IU/mg by RIA) were gifts from Peter Torjesen, Oslo. A commercial hCG preparation (Physex, LEO, Denmark) was used for injection *in vivo*, and was dissolved in 0.9 % NaCl. Hormones used in the *in vitro* studies were also dissolved in 0.9 % NaCl (1 mg/ml stock solution) and diluted to the desired concentration (5 $\mu$g/ml), with water containing 0.1 % BSA on the day of assay. Creatine phosphate and creatine kinase were obtained from Cal Biochem [$\alpha$-$^{32}$P]ATP was obtained from Amersham, England. Myokinase Tris-ATP, c-AMP, EDTA and sodium dodecyl sulfate (SDS) were from Sigma Chemical Co. All other reagents were of analytical grade.

*Adenylyl cyclase assay:* Testis tissue was homogenized in 20 vol. (w/v) of 10 mM Tris-HCl buffer containing 1 mM EDTA, pH 7.4 (Tris/EDTA buffer), using an Ultra-Turrax homogenizer (Janke-Kunkel, K.G. Germany) at rheostat setting 7 for 2 x 15 seconds. The homogenates were filtered through a nylon cloth and centrifuged at 27 000 x g for 30 min at 0–4°C. The pellet was resuspended in the same volume of Tris/EDTA buffer, rehomogenized and recentrifuged at the same speed and time. The final pellet was resuspended in 10 vol of Tris/EDTA buffer containing 0.1 % BSA. Adenylyl cyclase activity was determined in 20 $\mu$l aliquots of resuspended membrane particles (20–80 $\mu$g protein/assay tube) in a final volume of 50 $\mu$l containing 0.2–2 mM ATP (2–10 x $10^6$ CPM of [$\alpha$-$^{32}$P]ATP), 2.0–3.5 mM $MgCl_2$ (0.3–1.6 mM in excess of ATP EDTA), 1 mM EDTA, 1 mM c-AMP (with ca. 10 000 CPM of [$^3$]c-AMP), 2 x $10^{-5}$M of GTP or GMP P(NH)P (guanyl-5$^1$yl-imidophosphate), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.2 mg/ml myokinase and 25 mM Tris-HCl pH 7.4. In-

cubations were carried out at 30 or 35°C for 10 min. The reaction was stopped with 0.1 ml of a solution containing 10 mM c-AMP, 40 mM ATP, and 1 % SDS followed by immediate cooling to 0°C. The [$\alpha^{32}$P]c-AMP formed and [$^3$H] c-AMP (ca. 10 000 CPM) added to monitor recovery were isolated by a combination of Dowex 50-X4 and alumina oxide chromatography (Salomon et al. 1974; Birnbaumer et al. 1976). Overall recovery 60–75 %, and reaction blanks were 4–10 CPM/$10^6$ CPM of ATP added. Occasionally, the efficiency of the regenerating system was determined by thin layer chromatographic analysis of the [$\alpha^{32}$P] in ATP at the end of the incubation. Thin layer plates (PEI cellulose on plastic sheets, Brinkmann) were developed with 1 M Tris-HCl. More than 70 % of [$\alpha^{32}$P] was always in ATP.

*Other analytical techniques.* Protein was determined by the method of Lowry et al. (1951) and radioactivity, in 4 ml of eluant from the alumina columns, was measured in a Nuclear Chicago, Mark I liquid scintillation counter using 5 ml Insta gel (Packard). Statistical analysis was done using Student *t*-test.

## Results

As shown in Table 1, membrane particles from testicular homogenates obtained from normal adult rats or from tfm rats both contained an LH/hCG responsive adenylyl cyclase. Basal adenylyl cyclase activity was approximately 1.6 times higher in tfm rats compared to the normal rats. (21 pmol cAMP/mg min and 13 pmol cAMP/mg min, respectively). As shown in the same Table, tfm rats also contained an FSH responsive adenylyl cyclase. Forty-eight h after the injection of 200 IU of hCG to normal and tfm rats, a complete desensitization of LH/hCG responsive adenylyl cyclase was observed, whereas the FSH responsive adenylyl cyclase could still be stimulated (Table 1). As seen from the same table, hCG appeared to cause an elevation of basal activity both in normal and tfm rats. However, since the assays were done on tissue pools rather than individual animals, the statistical significance of this could not be assessed.

Fig. 1 shows that an adenylyl cyclase which is responsive to LH and hCG, is also present in membrane particles of testicular homogenates from tfm mice. The $ED_{50}$ for cyclase activation by hCG and human LH (PT) were approximately 15 ng/ml (150 mIU/ml) and 45 ng/ml, respectively. Membrane particles from tfm mice did not show any specific FSH response and activation of adenylyl cyclase by high doses of hFSH (PT-1) shown in Fig. 1 was due to contamination by LH (2 %) in the FSH preparation. When FSH-PT1 was added to dispersed Leydig cells, it stimulated androgen production in a dose-dependent manner. The dose-response curve was parallel to that of hLH and hCG, and

*Table 1.*
Hormone responsive AC activities in normal adult and tfm rats (mean ± SD)

| | AC activities (pmol/mg prot. min) | | |
|---|---|---|---|
| | Basal | hCG | FSH |
| Normal | 13.2 ± 0.02 | 27.4 ± 2.0* | 33.68 ± 0.22* |
| Tfm | 20.9 ± 0.02 | 33.6 ± 0.44* | 31.28 ± 0.56* |
| Normal + 200 IU hCG | 17.1 ± 0.3 | 18.8 ± 1.7ns | 41.9 ± 0.8* |
| Tfm + 200 IU hCG | 31.2 ± 0.9 | 30.9 ± 0.2ns | 41.8 ± 0.3* |

Pooled tissue from 5 adult male rats and 3 adult tfm rats were homogenized and membrane particles prepared as described in Materials and Methods.
Conditions for assay were: [$\alpha$-$^{32}$P]ATP 1.7 mM, GMP-P(NH)P $4 \times 10^{-5}$M, $Mg^{2+}$ 0.4 mM in excess of ATP and EDTA, pH 7.40 at 30°C and 10 min.
*$P < 0.01$.
ns not significant.

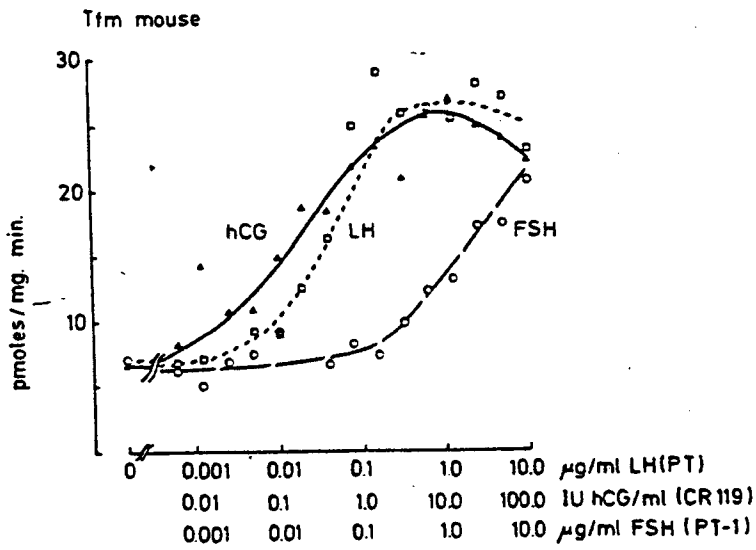


*Fig. 1.*
LH/hCG responsive adenylyl cyclase (pmol cAMP/mg prot. min) in testis particles of tfm mice. Testis particles (pooled tissue from 12 tfm mice) were prepared as described in Materials and Methods and incubated for 10 min at 30°C with 1.71 mM ATP, EDTA 1.4 mM, pH 7.0, and with varying concentrations of hCG, hLH (PT), or hFSH (PT-1). Each point represents single determinations.

relative content of biologically active LH was 2.1 % (Jahnsen et al., to be published). Furthermore, when 10 μg/ml of hFSH-PT1 was added to membrane particles from tfm mice together with a saturating amount of hCG (10 μg/ml) no additive effect was seen ($P > 0.05$) (Table 2).

Fig. 2 shows time course studies of the LH responsive adenylyl cyclase using membrane particles from tfm mice. As shown in the figure, enzymatic activity was constant with either GTP or the GTP-ase resistant GTP analogue, GMP-P(NH)P, and the degree of hormonal activation was of the same magnitude regardless of the nucleotide used. The time course curve for GTP was linear going through the origin, whereas the basal curve for GMP-P(NH)P showed a typical delay of approximately 1–2 min, after which a more rapid but linear rate of c-AMP accumulation was observed. It should also be noted from the same figure that this delay was eliminated when saturating amounts of hLH were added.

A detailed study of hCG induced desensitization of LH/hCG responsive adenylyl cyclase was performed in tfm mice. Fig. 3 presents results from a study of the time course of desensitization by hCG in tfm mice in which the dose response curves of hCG stimulated adenylyl cyclase in membrane particles were determined, 2–72 h after hCG injection. As seen in the figure, there is a dramatic reduction in hCG responsiveness of the adenylyl cyclase already 2 h after the injection of 50 IU of hCG. After 24 h, hCG responsiveness is completely abolished. However, after 48 h (right panel), the adenylyl cyclase in tfm mouse testicular particles starts to reappear and by 72 h after injection of hCG response is normalized.

*Table 2.*
Lack of additivity between hFSH and hCG in stimulating adenylyl cyclase activity in membrane particles from tfm mice. Membrane particles were prepared as described in Materials and Methods (pooled tissue from 12 animals), and assayed as described in the legend to Fig. 1.

| | Adenylyl cyclase activity (pmoles cAMP/mg prot. min) | Relative stimulation |
|---|---|---|
| | Mean SD | |
| Basal | 7.0 ± 0.05 | – |
| hCG (10 μg/ml) | 23.8 ± 0.14 | 3.40 |
| FSH (10 μg/ml) | 17.5 ± 0.17 | 2.50 |
| hCG + FSH | 24.1 ± 0.19 | 3.44 |

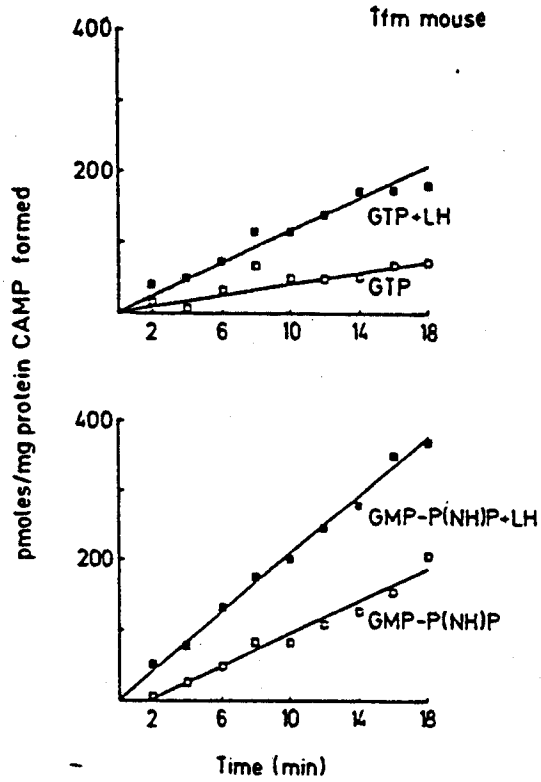


*Fig. 2.*

Time-course of adenylyl cyclase activity (pmol cAMP/mg prot. min) in testis particles from tfm mice (pooled tissue from 12 animals) in the presence or absence of saturating concentrations of hLH (10 $\mu$g/ml) and using either GTP ($2 \times 10^{-5}$M) or GMP-P(NH)P ($2 \times 10^{-5}$M). Conditions for assay were otherwise as in Fig. 1. Each point represents single determinations.

## Discussion

The present study shows the presence of an LH/hCG responsive adenylyl cyclase in tfm rats and mice as well as an FSH responsive adenylyl cyclase in tfm rats. Furthermore, hCG is capable of causing desensitization of the LH/hCG responsive but not of the FSH responsive adenylyl cyclase in normal and tfm rats, as well as of the hCG responsive adenylyl cyclase in tfm mice.

An ovulatory dose of hCG causes a rapid desensitization of the ovarian adenylyl cyclase and luteolysis (Hunzicker-Dunn & Birnbaumer 1976a,b; Conti et al. 1976). In the rat ovary the responsiveness of hCG stimulated adenylyl cyclase reappears approximately 7–10 days after injection of hCG, however, this is probably due to ovulation and to the occurence of new corpora lutea (Hunzicker-Dunn et al. 1979). In the ovary it is shown that desensitization of

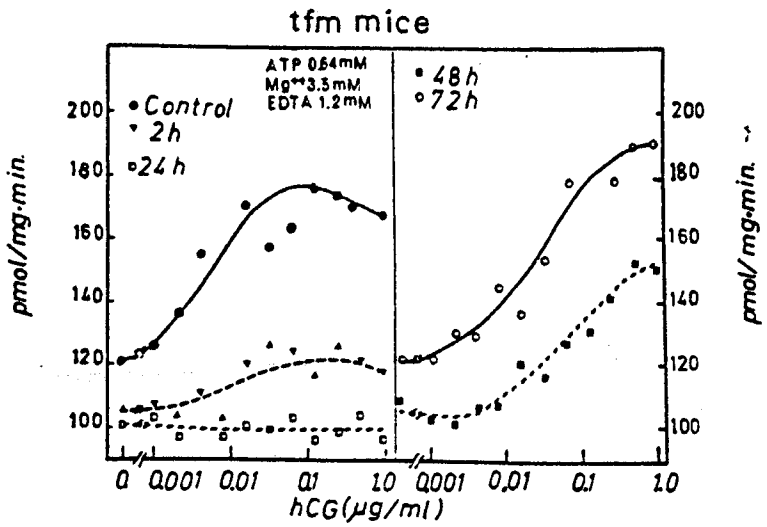


*Fig. 3.*

LH/hCG 1–1000 ng) responsive adenylyl cyclase (pmol cAMP/mg prot. min) in testis particles of tfm mice at varying time intervals (2–72 h) following a sc injection of 50 IU of hCG Physex). Each group consists of pooled tissue from 20 animals. Assay condition were: ATP 0.64 mM. EDTA 1.2 mM. $Mg^{++}$ 1.56 mM in excess of ATP + EDTA. pH 7.4, time 10 min, temperature 30°C. Each point represents single measurements of adenylyl cyclase activity.

the adenylyl cyclase occurs after a few hours (Hunzicker-Dunn & Birnbaumer 1976a,b) and precedes the decrease in LH/hCG receptors by several hours or days (Conti et al. 1976). We have recently (Jahnsen T., Gordeladze J., and Hansson V., in preparation) examined the time course of adenylyl cyclase desensitization and down-regulation of LH receptors shown that the time kinetics of receptor loss and adenylyl cyclase desensitization is very similar to that in the ovary. Furthermore Dufau et al. (1978) have also shown that, following a desensitizing dose of hCG, cyclic-AMP secretion from Leydig cells in suspension is reduced prior to any loss of LH receptors.

The fact that the tfm rat testis shows FSH and LH responsive adenylyl cyclases is interesting, and indicates that coupling between receptors and cyclase can occur in an animal preparation in which the androgen receptors are deficient. The tfm mouse is believed to constitute an absolute androgen insensivity syndrome, and even in this situation the LH responsive adenylyl cyclase was detectable. The lack of any FSH responsive adenylyl cyclase in the tfm mouse testis may be due to the extensive Leydig cell hyperplasia (frequently with micro-adenomas) resulting in a relative predominance of Leydig cells in these testes. In future studies we will examine the FSH responsive adenylyl

cyclase in younger tfm mice in which the Leydig cell hyperplasia has not progressed to the same extent.

It has recently been shown (Day & Birnbaumer 1979) that steroids (oestradiol) can cause a loss of the LH responsiveness of adenylyl cyclase in the rabbit corpus luteum. The question is therefore raised whether desensitization by hCG in the testis is due to hCG as such or due to a subsequent steroid response. For that reason we tested whether hCG induced desensitization of the LH/hCG responsive adenylyl cyclase would occur in tfm rats and mice. The fact that both tfm rats and mice can be desensitized by hCG strongly indicates that hCG desensitization of the LH/hCG responsive adenylyl cyclase does not require a functional androgen receptor. It is also interesting to note that tfm animals, which to some extent are already desensitized *in vivo* (due to elevated levels of circulating LH), still show an LH/hCG responsive adenylyl cyclase. This means that the small number of LH/hCG receptors still present on tfm Leydig cells are functioning and coupled to the catalytic subunit of adenylyl cyclase.

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