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HORMONAL REGULATION OF RAT LEYDIG CELL CYTOCHROME P-450$_{17\alpha}$ mRNA LEVELS AND CHARACTERIZATION OF A PARTIAL LENGTH RAT P-450$_{17\alpha}$ cDNA

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Summary: We have isolated and characterized a P-450$_{17\alpha}$ cDNA fragment from a rat testis library. The partial length rat P-450$_{17\alpha}$ cDNA (1Kb) has high overall nucleotide and deduced amino acid similarity with human and bovine P-450$_{17\alpha}$ cDNA's and contains the conserved tridecapeptide and heme regions, the termination codon and polyadenylation site. Using this rat testis cDNA probe we measured P-450$_{17\alpha}$ mRNA levels of rat Leydig cells from animals treated with hCG. Temporal studies with a low hCG dose showed an early increase in mRNA levels returning to control values at later times, while a higher desensitizing dose caused a marked reduction in the mRNA (24 h) and a small recovery at 48 h. Fetal rat Leydig cells maintained in the presence of LH treated with estradiol showed a 70% decrease in P-450$_{17\alpha}$ mRNA levels and testosterone production followed closely the changes in P-450$_{17\alpha}$ mRNA. These studies suggest that gonadotropin stimulation and desensitization of P-450$_{17\alpha}$ dependent enzymes in the adult rat testis as well as estradiol induced desensitization in fetal Leydig cells are related to levels P-450$_{17\alpha}$ mRNA.

The episodic secretion of luteinizing hormone (LH)[3] supports the steroidogenic function of the Leydig cell through interaction with LH receptors on the cell surface, and subsequent stimulation of cyclic AMP dependent events. In addition to the positive regulation of membrane receptors and steroidogenesis caused by physiological increases in endogenous hormone, major elevations in circulating gonadotropin can cause down-regulation of homologous LH receptors and desensitization of steroid responses in the target cell (1).

The regulation of steroidogenesis by LH/hCG in the adult rat [2-4] has been shown to include a prominent estrogen mediated steroidogenic lesion at the site of conversion of

Abbreviations: hCG, human chorionic gonadotropin; LH, luteinizing hormone; E_2, estradiol-17ß; P-450$_{17\alpha}$, 17α-hydroxylase cytochrome P-450, the product of P450XVII gene (13).

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progesterone to androgen [4] leading to a decreased testosterone response to hCG in vitro [1-4]. Desensitization of steroidogenic enzymes of the androgen pathway (17α-hydroxylase/17,20-desmolase) was found to be preceded by a cAMP mediated-activation of aromatase activity with increased estrogen production [5] and estradiol receptor mediated responses including the synthesis of a 27,000 Mr protein [6-8]. The similarity of estrogen lesions to those produced by gonadotropin treatment have indicated involvement of endogenous estrogen in the development of microsomal enzymatic lesions (4).

The inability of fetal and immature Leydig cells to be desensitized by gonadotropin [9,10], a characteristic of the adult cell [1-8], is attributed to low aromatase activity with lack of consequent receptor-mediated estrogen regulation [11,12]. Estradiol (E_2) treatment of functional fetal Leydig cell cultures caused induction of a steroidogenic lesion resembling that observed in gonadotropin desensitized adult Leydig cells, with reduction of the microsomal enzyme activities 17α-hydroxylase/17,20 desmolase that resulted in decreased androgen production [12]. It was of immediate interest to assess whether hormonal modulatory actions related to changes in P-$450_{17\alpha}$ mRNA levels could account for steroidogenic stimulation and desensitization. For this study we have characterized and identified a partial length rat P-$450_{17\alpha}$ cDNA clone. The 1 Kb cDNA insert, displaying high similarity with the previously isolated P-$450_{17\alpha}$ cDNA structures from human (14,15), bovine (16) and porcine (14) species, was employed to evaluate the hormonal regulation of mRNA levels in adult and fetal Leydig cells.

EXPERIMENTAL PROCEDURES

Leydig cells were prepared by collagenase dispersion of testes from control and hCG treated (subcutaneous 0.2-10 μg) adult 50 day old male rats as previously described (17). For testosterone production, 10^6 Leydig cells were incubated in suspension for 3 h at 35 C in the presence or absence of 100 ng hCG. Testosterone in the media analyzed by immunoassay (1). Studies in cultured fetal Leydig cells were performed with cells from 20.5 day fetuses prepared as previously described (12). Treatment of fetal cell cultures with ovine LH (1 μg) every third day (with media change) was previously found to be optimal to maintain steroidogenic function without induction of the desensitization observed in the adult cells (12). Cultures maintained in the presence of LH were also treated with E_2 (600 ng/day), to induce steroidogenic desensitization in fetal cells similar to that observed in adult cells (12). At the end of the culture period media was removed and total RNA from Leydig cells was prepared as described below. In parallel wells, cells were incubated with or without hCG (100 ng/ml) for an additional 3 h to monitor acute maximal testosterone responses to hCG (2-4).

Total RNA was prepared from different tissues (bovine, rat adrenal, rat spleen and from adult and fetal Leydig cells) as described by Chirgwin et al. (18). Poly(A)$^+$ RNA was separated from rRNA with oligo (dT)-cellulose. For Northern blot analysis, 10-20 μg of poly (A)$^+$ RNA was denatured with glyoxal, resolved in a 1% agarose gel and subsequently RNA was transferred to nylon filters (19). Also, Poly A$^+$ mRNA (0.2-2 μg) or total RNA (10 μg) were loaded on slot blot followed by hybridization and quantitation of signals by densitometry of X ray film and counting of filters, the latter correlated well with values obtained by direct desitometric reading. Significance of changes were obtained from slot blots by paired T-test and sign test.

Bovine and rat P-$450_{17\alpha}$ cDNA fragments were nick translated using α[^{32}P]dCTP and a nick-translation kit (New England Nuclear, Cambridge, MA). Hybridizations to bovine and rat P-$450_{17\alpha}$ were performed as described by Church and Gilbert (20). Subsequent hybridization to an actin probe (21), (kindly provided by Dr. Narayana Battula, NCI, Bethesda, MD), was performed on the same filters. A rat testis λ gt 11 cDNA library (Clontech Laboratories, Inc., CA) was used to isolate rat P-$450_{17\alpha}$ cDNA. The nicked-translated bovine P-$450_{17\alpha}$ (Pst I-Pst I, 1.2 Kb) restriction fragment (15) was employed to screen the rat testis cDNA library Southern blot analysis of plaque-purified phage, digested by Eco R1, showed a 1 Kb cDNA

insert upon hybridization with ^{32}P labeled bovine P-450$_{17\alpha}$ cDNA Pst I restriction fragment. The positive cDNA fragment was digested by several restriction enzymes, subcloned into M13mp18 and M13mp19 and used to transform E coli strains JM101. Sequencing of restriction fragments was performed by dideoxy chain termination sequencing (22).

RESULTS AND DISCUSSION

Northern blot analysis of rat Leydig cell mRNA using rat P-450$_{17\alpha}$ probe revealed a single 1.9 Kb mRNA species. The rat testis probe hybridized less effectively with P-450$_{17\alpha}$ mRNA of bovine adrenal while no hybridization was observed to the rat adrenal consistent with the known absence of P-450$_{17\alpha}$ enzyme activity in this tissue (Fig. 1, A).

Restriction enzyme mapping (Fig. 1, B) and nucleotide sequence analysis were performed to characterize the 1 Kb P-450$_{17\alpha}$ DNA fragment. This insert contains an open reading frame of 720 bases, a termination codon and 149 bases of the 3' untranslated region. The nucleotide and amino acid sequences exhibit considerable overall similarity with that from human, 79 and 75% (14,15), bovine, 73 and 68% (16) and porcine (14) species, (Fig. 2). Computer alignment of the rat testis P-450$_{17\alpha}$ DNA fragment with the human P-450$_{17\alpha}$ cDNA verifies the presence of the heme-binding region (where 20 out of 21 amino acids are identical), the conserved tridecapeptide region (24) associate with all cytochromes P-450 and the termination codon. Similarity begins at amino acid position 291 of the human P-450$_{17\alpha}$ and continues to the termination codon at amino acid 509. The rat P-450$_{17\alpha}$ polyadenosine addition signal (ATAAA) residing 136 bases 3' from the stop codon was aligned to the human P-450$_{17\alpha}$ polyadenosine signal (14). This alignment was achieved with the introduction of three gaps; between positions 752-753 (two bases), 814 and 815 (3 bases), and 817-818 (10 bases) (Fig. 2, Table 1).

In previous studies we have shown that treatment in vivo with a single high dose of hCG caused desensitization of microsomal 17α-hydroxylase/17-20 desmolase activities leading to decreased testosterone response to gonadotropin stimulation in vitro (2-4), while lower doses caused no changes or even stimulation of androgen production. Thus it is of immediate interest to determine the modulatory influences of gonadotropin on P-450$_{17\alpha}$ mRNA levels. Treatment with 10 μg hCG appears to cause an initial modest but significant stimulation of mRNA levels

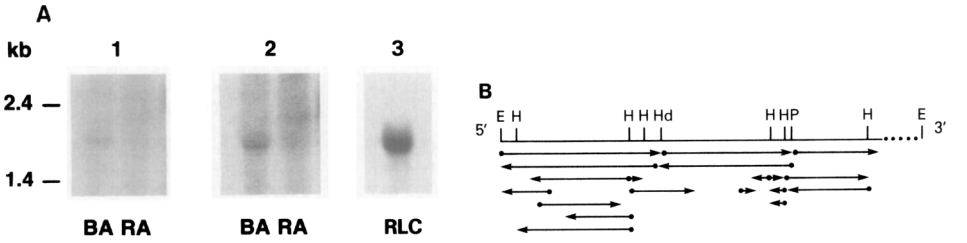

Fig. 1. A. Hybridization of poly (A)+ mRNA from testis, bovine and rat adrenal with rat P-450$_{17\alpha}$ probe, 20μg of poly (A)-enriched mRNA from rat Leydig cell (RLC), bovine adrenal (BA) and rat adrenal (RA) were electrophoresed and hybridized to nick translated rat P-450$_{17\alpha}$ cDNA probe at 55 °C in 0.5M NaH_2PO_4 (pH 7.0), 1 mM EDTA, 7% SDS, 0.5% BSA buffer and washed with 0.5% BSA, 5% SDS, 100 mM NaH_2PO_4, 1 mM EDTA at 65 °C. Autoradiographs were developed at 16 hours (1) and 36 hours (2) and 5 hours (3). Fig. 1. B. Restriction Map for rat testes P450$_{17\alpha}$ insert. Arrows indicate the direction and extent of sequence analysis. Restriction enzymes are as follows: E= EcoR I; Hd=Hind II; P=Pst I; H=Hae III.

at 3 h ($p<0.05$) followed by a reduction at 6 h that continues to decline at 12 h and to near undetectable levels at 24 h with a small observable recovery at 48 h (Fig. 3-left). The expected marked reductions of testosterone production in vitro (1-6, 17) were observed with gonadotropin treatment (not shown). In contrast, administration of a non-desensitizing dose of hCG (0.2 μg) showed an increase in mRNA levels at 12 h ($p<0.05$) while at 24 and 48 h levels returned to near control values (Fig. 3-right). It is possible that the lack of a sustained

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        271                                 280                                     290
pAA                                                                  Leu Leu Ser Asp Arg
hAA         Asn Ser Asp Asn Gly Asn Ala Gly Pro Asp Gln Asp Ser Glu Leu Leu Ser Asp Asn
hADNA       AAC TCA GAT AAT GGC AAT GCT GGC CCA GAT CCA GAT TCA GAG CTG CTT TCA GAT AAC
bAA             Ala                 Ala Gly Pro Asp Gln     Ser Lys Leu Leu     Asn
                             *       *   *   *   *   *       *   *   *   *       *   *
rTAA        Asn Ser Asp Asn Asn Asn Ser Cys Glu Gly Arg Asp Pro Asp Val Phe Ser Glu Arg
rTDNA   -G  AAT TCA GAC AAC AAC AAC AGC TGT GAA GGC CGG GAC CCA GAT GTG TTT TCA GAT AGG
        291                                 300                                     310
pAA                                                     Ala Cys Val Glu Thr Ser Val Ser Val
hAA     His Ile Leu Thr Thr Ile Gly Asp Ile Phe Gly Ala Gly Val Glu Thr Thr Thr Ser Val
hADNA   CAC ATT CTC ACC ACC ATA CGG GAC ATC TTT GGG GCT GGC CTG GAG ACC ACC ACC TCT GTG
bAA         Met             Ile                             Val                 Ser
                     *       *                               *                   *
rTAA    His Ile Leu Ala Thr Val Gly Asp Ile Phe Gly Ala Gly Ile Glu Thr Thr Thr Thr Val
rTDNA   CAC ATC CTT GCC ACG GTG GGA GAC ATC TTT GGG GCG GGC ATA GAG ACA ACT ACC ACT GTG
        311                                 320                                     330
pAA     Phe Ile Trp                                                         Ile Gln Glu
hAA     Val Lys Trp Thr Leu Ala Phe Leu Leu His Asn Pro Gln Val Lys Lys Lys Leu Tyr Glu
hADNA   GTT AAA TGG ACC CTC GCC TTC CTG CTG CAC AAT CCT CAG GTG AAG AAG AAG CTC TAC GAG
bAA     Ile             Val     Tyr     Leu     His     Ser Leu         Arg         Asp
         *           *                   *               *                   *   *   *
rTAA    Leu Lys Trp Ile Leu Ala Phe Leu Val His Asn Pro Glu Val Lys Lys Lys Ile Gln Lys
rTDNA   CTC AAG TGG ATC CTG GCT TTC CTG GTG CAC AAT CCT GAG GTG AAG AAG AAG ATC CAA AAG
        331                                 340                                     350
pAA     Ala Ile Glu Gln Asn Ile Gly Phe Asn Arg Ala Pro Ser Ile Ser Asp Arg Asn Gln Leu
hAA     Glu Ile Asp Gln Asn Val Gly Phe Ser Arg Thr Pro Thr Ile Ser Asp Arg Asn Arg Leu
hADNA   GAG ATT GAC CAG AAT GTG GGT TTC AGC CGC ACA CCA ACT ATC AGT GAC CGT AAC CGT CTC
bAA     Asp             Ile Ile         Asn                 Ile Ser         Asn Arg
                         *                                   *   *           *   *
rTAA    Glu Ile Asp Gln Tyr Val Gly Phe Ser Arg Thr Pro Thr Phe Asn Asp Arg Ser His Leu
rTDNA   GAG ATT GAC CAG TAC GTA CGC TTC AGC CGA ACA CCA ACT TTC AAT GAC CGG TCT CAC CTC
        351                                 360                                     370
pAA     Val Leu Leu Glu Ala Thr Ile Arg Glu Val Leu Arg Phe Arg Pro Val Ser Pro Thr Leu
hAA     Leu Leu Leu Glu Ala Thr Ile Arg Glu Val Leu Arg Leu Arg Pro Val Ala Pro Met Leu
hADNA   CTC CTG CTG GAG GCC ACC ATC CGA GAG GTG CTT CGC CTC AGG CCC GTG GCC CCT ATG CTC
bAA     Val Leu                                                                 Thr
             *                                           *
rTAA    Leu Met Leu Glu Ala Thr Ile Arg Glu Val Leu Arg Ile Arg Pro Val Ala Pro Met Leu
rTDNA   CTC ATG CTG GAG GCC ACT ATC CGA GAA GTG CTG CGT ATC AGG CCG GTG GCT CCC ATG CTC
        371                                 380                                     390
pAA     Ile Pro His Arg Ala Ile Ile Asp Ser Ser Ile Gly Glu Phe Thr Ile Asp Lys Ala Thr
hAA     Ile Pro His Lys Ala Asn Val Asp Ser Ser Ile Gly Glu Phe Ala Val Asp Lys Gly Thr
hADNA   ATC CCC CAC AAG GCC AAC GTT GAC TCC AGC ATC GGT GAG TTT GCT GTG GAC AAG GGC ACA
bAA                             Val Ile                 Asp Leu     Ile Asp     Gly
                                                                 *       *       *
rTAA    Ile Pro His Lys Ala Asn Val Asp Ser Ser Ile Gly Glu Phe Thr Val Pro Lys Asp Thr
rTDNA   ATC CCC CAC AAG GCT AAC GTC GAC TCC AGC ATT GGA GAG TTT ACT GTC CCC AAG GAC ACA
        391                                 400                                     410
pAA     Asp Val Val Val Ser Leu Phe Ala Leu His His Asn Glu Lys Glu Trp His Arg Pro Asp
hAA     Glu Val Ile Ile Asn Leu Trp Ala Leu His His Asn Glu Lys Glu Trp His Gln Pro Asp
hADNA   GAA GTT ATC ATC AAT CTG TGG GCG CTG CAT CAC AAT GAG AAG GAG TGG CAC CAG CCG GAT
bAA     Asp                                         Ser     Lys         Gln His
         *       *   *                                       *           *   *
rTAA    His Val Val Val Asn Leu Trp Ala Leu His His Asn Glu Asn Glu Trp Asp Gln Pro Asp
rTDNA   CAT GTG GTC GTC AAT CTC TGG GCA CTG CAT CAC GAT GAG AAT GAA TGG GAC CAG CCA GAT
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Fig. 2. P-$450_{17\alpha}$ amino acid sequences: hAA= derived from human adrenal cDNA (14,15), rTAA= derived from rat testes cDNA (this study) and bAA=bovine aminoacids (16) that differ from the rat sequence. P-$450_{17\alpha}$ nucleotide sequences: rTDNA=rat cDNA, hADNA= human adrenal cDNA.* represents rat testes amino acids that differ from human residues. Underlined segments represent the conserved peptide region and heme region termination codon and polyadenylation.

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        411                                                 420                                                 430
pAA     Leu Phe Met Pro Glu Arg Phe Leu Asp Pro Thr Gly Thr Gln Leu Ile Ser Pro Ser Leu
hAA     Gln Phe Met Pro Glu Arg Phe Leu Asn Pro Ala Gly Thr Gln Leu Ile Ser Pro Ser Val
hADNA   CAG TTC ATG CCT GAG CGT TTC TTG AAT CCA GCG CGG ACC CAG CTC ATC TCA CCG TCA CTA
bAA     Leu                                                 Thr Gln          Ser      Ser Leu
                                                    *       *   *   *        *        *   *
rTAA    Gln Phe Met Pro Glu Arg Phe Leu Asp Pro Thr Gly Ser His Leu Ile Thr Pro Thr Gln
rTDNA   CAG TTC ATG CCT GAA CGC TTC TTA GAT CCA ACG GGA AGC CAT CTC ATT ACA CCC ACG CAG
        431                                                 440                                                 450
pAA     Ser Tyr Leu Pro Phe Gly Ala Gly Pro Arg Ser Cys Val Gly Glu Met Leu Ala Arg Gln
hAA     Ser Tyr Leu Pro Phe Gly Ala Gly Pro Arg Ser Cys Ile Gly Glu Ile Leu Ala Arg Gln
hADNA   AGC TAT TTG CCC TTC GGA GCA GGA CCT CGC TCC TGT ATA GGT GAG ATC CTG GCC CGC CAG
bAA                                                         Val          Met
                                                                         *
rTAA    Ser Tyr Leu Pro Phe Gly Ala Gly Pro Arg Ser Cys Ile Gly Glu Ala Leu Ala Arg Gln
rTDNA   AGT TAC TTG CCC TTC GGA GCT GGT CCC CGA TCC TGC ATC GGA GAG GCT CTG GCC CGT CAG
        451                                                 460                                                 470
pAA     Glu Leu Phe Leu Phe Thr Ala Gly Leu Leu Gln Arg Phe Asp Leu Glu Leu Pro Asp Asp
hAA     Glu Leu Phe Leu Ile Met Ala Trp Leu Leu Gln Arg Phe Asp Leu Glu Val Pro Asp Asp
hADNA   GAG CTC TTC CTC ATC ATG GCC TGG CTG CTG CAG AGG TTC GAC CTG GAG GTG CCA GAT GAT
bAA                 Leu     Met Ser Arg                         Asn      Glu Ile Pro
                     *   *   *    *                                       *    *
rTAA    Glu Leu Phe Val Phe Thr Ala Leu Leu Leu Gln Arg Phe Asp Leu Asp Val Ser Asp Asp
rTDNA   GAG CTC TTT GTC TTC ACG GCC TTG CTA CTG CAG AGG TTT GAC TTG GAT GTG TCA GAT GAT
        471                                                 480                                                 490
pAA     Gly Gln Leu Pro Cys Leu Val Gly Asn Pro Ser Leu Val Leu Gln Ile Asp Pro Phe Lys
hAA     Gly Gln Leu Pro Ser Leu Glu Gly Ile Pro Lys Val Val Phe Leu Ile Asp Ser Phe Lys
hADNA   GGC CAG CTG CCC TCC CTG GAA GGC ATC CCC AAG GTG GTC TTT CTG ATC GAC TCT TTC AAA
bAA     Gly Lys          Ser             His Ala Ser Leu      Leu Gln      Lys
         *                *               *                                       *
rTAA    Lys Gln Leu Pro Arg Leu Glu Gly Asn Pro Lys Val Val Phe Leu Ile Asp Pro Phe Lys
rTDNA   AAA CAA CTG CCC CGC CTG GAG GGT GAT CCC AAG GTA GTC TTT CTG ATC GAC CCT TTC AAA
        491                                                 500                                                 510
pAA     Val Lys Ile Lys Glu Arg Gln Ala Trp Lys Glu Ala His Thr Glu Gly Ser Thr Ser
hAA     Val Lys Ile Lys Val Arg Gln Ala Trp Arg Glu Ala Gln Ala Glu Gly Ser Thr ---
hADNA   GTG AAG ATC AAG GTG CGC CAG GCC TGG AGC GAA GCC CAG GCT GAG GGT AGC ACC TAA
bAA                 Glu                 Lys Glu                 Gly          Pro ---
                     *                   *  *                    *
rTAA    Val Lys Ile Thr Val Arg Gln Ala Trp Met Asp Ala Gln Ala Glu Val Ser Thr ---
rTDNA   GTA AAG ATC ACG GTG CGC CAG GCA TGG ATG GAT GCA CAG GCT GAG GTT AGC ACC TAG AGG
rTDNA   CCACAACTAACATCCCCGATCCATACCTCAACACCCACAGTACAATCTTAGAGGTGCTACTCCCAGTGCCTCCTAC
        GGTCCTCCTCCCTCCTACCCATTTCTAGTTGGCAGCAATGGGGGGTGATACACATAAATTAAAGTT
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Fig. 2 - Continued.

TABLE 1. SEQUENCE SIMILARITY OF RAT P-450$_{17\alpha}$ cDNA FRAGMENT WITH HUMAN, BOVINE AND PORCINE P450$_{17\alpha}$

Parameter	Percent Similarity		
	Human[a]	Bovine[a]	Porcine[b]
1) Nucleotide			
overall similarity	79	73	
heme*	83	81	
conserved peptide region**	80	78	
2) Amino Acid			
overall similarity	75	68	66
heme*	95	90	90
conserved peptide region**	83	83	74

[a] Amino acids derived from nucleotide sequence analyses (14,15,16) and [b] from peptide sequence (14) % similarity is defined as (100 - # mismatches) /100. There are no gaps in the region depicted in Fig 2 as verified by Wilbur-Lipman computer alignment using PC/gene (Intelligenetics, Inc., Mountain View, CA) (23). Amino acid position: *453-473 **346-368 (see Fig. 2).

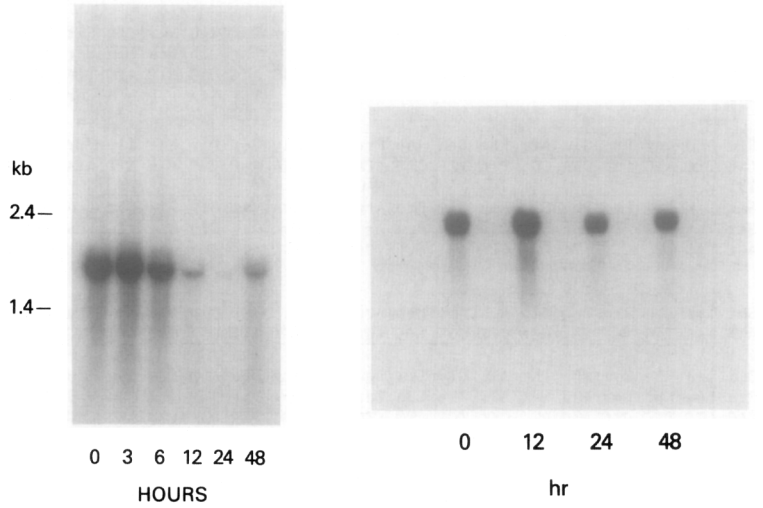

Fig. 3. (Left) Time study of regulation of P-450$_{17\alpha}$ mRNA levels in adult Leydig cells by a desensitizing dose of hCG (10 μg). Poly (A)$^+$ RNA (10 μg) were electrophoresed on 1% agarose/glyoxal gel. The RNA was transferred in Fig. 1 to nylon filter, hybridized to nick translated rat P-450$_{17\alpha}$ cDNA probe as described in Fig. 1. (Right) Time study of regulation of P-450$_{17\alpha}$ mRNA levels in adult Leydig cells by a non-desensitizing dose of hCG (0.2 μg). These are representative results of 4 experiments.

increase in mRNA beyond the 12 h time point could be reflective of some degree of desensitization even at the lower dose. This may somewhat dampen the effect of the gonadotropin treatment on testosterone basal levels and subsequent responses to the acute hormonal stimulus in vitro. The corresponding testosterone production showed a two-fold increase in basal testosterone production at 12 hr and a decrease at later times to slightly above control levels (not shown). This early increase in basal steroidogenic levels could be related in part to the observed increase in P-450$_{17\alpha}$ mRNA.

Unlike the adult Leydig cell, the fetal and immature Leydig cells are refractory to this desensitizing process and maintain up-regulated LH receptors and steroidogenic function (9-12); their resistance to desensitization by gonadotropin is attributed to the absence of an estrogen-mediated regulation of the androgen pathway (10-12). E_2 treatment of functional Leydig cell cultures caused an estrogen-mediated inhibition of 17α hydroxylase/17-20 desmolase activities resembling that observed in gonadotropin desensitized adult cells (4,6,12). Estradiol treatment of fetal cultures for 6 days significantly reduced the levels of P-450$_{17\alpha}$ mRNA ($p<0.05$) (Fig. 4-left), decrease of control and acute hCG stimulated testosterone production was observed at 3 and 6 days of culture. The estradiol effect was even more pronounced at 6 days when basal testosterone levels were undetectable and hCG stimulated levels were markedly reduced (Fig. 4-right). Accumulated testosterone production in the media over the 3 day culture period (see methods) demonstrated about 60% reduction of testosterone in the incubation media with the estradiol treatment (from 135 ± 7 ng/ml to 36 ± 6 ng/ml). This marked reduction of androgen by E_2 treatment is reflective of the inhibition of 17α-hydroxylase/17-20 desmolase activity (12) and is likely related to reduction on P-450$_{17\alpha}$ mRNA content.

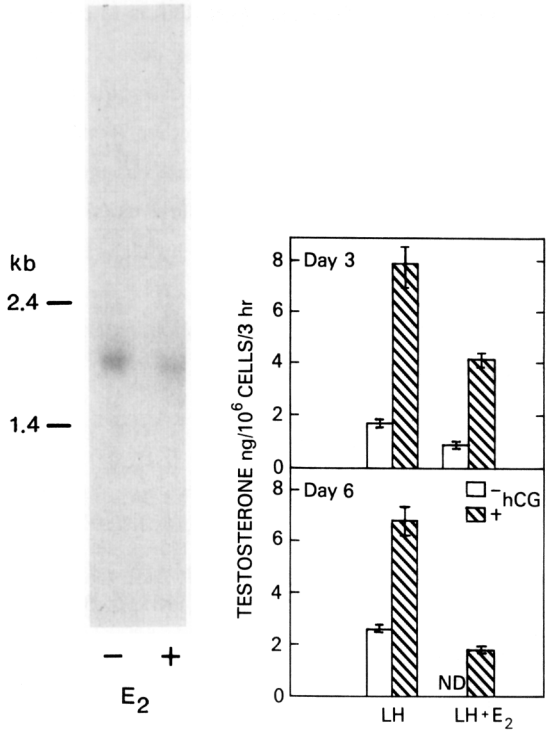

Fig. 4. (Left) E_2 regulation of P-450$_{17\alpha}$ mRNA levels in fetal Leydig cells. Cells were plated at 1 x 10^6 cells/well and treated by 1 μg of oLH every third day in the absence of 17β-estradiol (-), left, and in the presence of 600 ng of 17β-estradiol added every day (+). On day seven total RNA were prepared from cell culture as described in methods. 20 μg of total RNA were electrophoresed on 1% agarose/glyoxal gel, transferred to a nylon filter and hybridized to rat P450$_{17\alpha}$ cDNA probe following conditions described in Fig. 1. (Right) The effect of E_2 on testosterone production by cultured fetal rat Leydig cells. Cells were treated with LH (1 μg) or LH + E_2. At day 3 and 6 after media change, cells were cultured in the presence or absence of hCG for an additional 3 h (acute stimulation). Testosterone was measured in the media. Points are the mean ± SD (n=4). These are representative results of 3 experiments.

The temporal changes in P-450$_{17\alpha}$ mRNA levels in response to gonadotropin followed the steroidogenic desensitization pattern previously described. The early increases in mRNA levels at all dose treatments are consistent with the early increase in steroidogenic activity observed in vivo and in vitro following the gonadotropin stimulus (2-4). Furthermore the marked decrease in mRNA levels induced by estradiol in fetal Leydig cells is consistent with our proposal of E_2 as an inducer of a regulatory function in steroidogenesis, perhaps at the transcriptional level. That the changes in P-450$_{17\alpha}$ are specific were demonstrated by the finding that actin mRNA levels were not significantly changed by the treatments (not shown) and the absence of P-450$_{17\alpha}$ mRNA in rat adrenal (Fig. 1) and spleen (not shown). It is of interest that at the lower dose (0.2 μg) the initial stimulation observed was not sustained. It is likely that lower doses of gonadotropin will provide exclusively a stimulatory pattern.

Characterization of the cDNA insert complementary to rat P-450$_{17\alpha}$ has shown a high degree of homology with the P-450$_{17\alpha}$ of other species and predominantly with the human (14,15), indicating conservation during evolution. The isolated insert containing the

conserved regions and the 3' end of the cDNA has permitted us to determine the levels of mRNA P-$450_{17\alpha}$ in the rat. Results demonstrated an early modest stimulation of mRNA by hCG treatment and marked reductions during gonadotropin and estradiol desensitization.

Further studies will be focused on determining whether P-$450_{17\alpha}$ mRNA levels during hormone exposure are the consequence of transcriptional rate changes or mRNA degradation. Elucidation of the P-$450_{17\alpha}$ genomic structure would allow us to determine the presence of regulatory sequences related to gonadotropin action of the Leydig cell.

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