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TRANSIENT ACTIVATION OF C-MYC PROTOONCOGENE EXPRESSION IN LEYDIG CELLS BY HUMAN CHORIONIC GONADOTROPIN

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Summary: This study evaluated the effects of in vivo administration of human chorionic gonadotropin (hCG) on c-myc oncogene expression in Leydig cells. Sprague-Dawley rats (46-50 days old) were treated with hCG (10 units, i.p.), and purified Leydig cells were isolated 1-24 h later. HCG caused a transient elevation of c-myc mRNA in 4 h and returned to normal or lower than normal levels at 24 h. There was no change in c-fos or β-actin mRNA levels. Our results suggest that the growth promoting effects of hCG on Leydig cells may be mediated by the transient expression of c-myc protooncogene.

Steroidogenesis of Leydig cells is primarily controlled by LH/hCG (1). However, LH/hCG has many effects other than stimulating Leydig cell steroidogenesis. The number and size of Leydig cells increased after chronic hCG treatment (2,3). Hypophysectomy caused Leydig cell atrophy and reduced the number of Leydig cells. Treatment with LH prevented Leydig cell atrophy and loss of steroidogenic function (4). The mechanism responsible for this growth promoting effect of LH/hCG remains unclear.

Recent evidence supports a central role of protooncogenes in cell proliferation (5-7). Elevated protooncogene expression has been demonstrated in embryonic and neonatal tissues, in cultured cells stimulated with growth factors, in regenerating liver tissue after partial hepatectomy and in a number of malignant tumors (8-14). The products of one group of oncogenes which include myc, fos and myb are believed to exert their effects on the nucleus of eukaryotic cells and seem likely to be involved in the regulation

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of growth and replication (7-9,15,16). In the present study, we have evaluated the effect of hCG on Leydig cell c-myc and c-fos mRNA expressions.

MATERIALS AND METHODS

Male Sprague-Dawley rats (46-50 days old) were obtained from Zivic-Miller Laboratories (Allison Park, PA). Animals were kept under a 12-h light, 12-h dark cycle and were fed rat chow and water ad libitum. For each experiment, a group of 8-10 rats was treated with hCG (A.P.L., Ayerst Laboratories, New York, NY) 10 units i.p. and sacrificed 1 to 24 h later. The testes were decapsulated, and crude interstitial cells were prepared using collagenase digestion (17). Purified Leydig cells were obtained by 0-32% Metrizamide gradient centrifugation (17). Using 3β-hydroxysteroid dehydrogenase staining to identify Leydig cells, more than 80% of the cells stained positively.

Total cellular RNA was extracted with proteinase K (1 mg/ml) plus sodium dodecyl sulfate as previously described (18). For Northern blot analysis, 10 μg of total cellular RNA was denatured with 6% formaldehyde and 50% formamide, run on a 1% agarose gel containing 2.2 M formaldehyde, and transferred onto Nytran (0.45 μm, modified Nylon-66 membrane, Schleicher and Schuell). The membranes were then baked at 80°C for 2 h under vacuum and were prehybridized at 55°C for at least 1 h in a mixture containing 5X SSC, 5X Denhardt's solution (50X Denhardt's, 1% Ficoll, 1% bovine serum albumin, 1% polyvinyl-pyrrolidine), 50% formamide, 20 mM sodium phosphate, pH 6.8, 2.5 mM EDTA and 0.1 mg/ml denatured salmon testis DNA (19). Hybridization was carried out in the same solution at 55°C overnight with 10% dextran sulfate, 1-5 X 10^7 cpm of c-myc probe and 5 X 10^5 cpm of β-actin probe labeled by using a Random Primers DNA Labelling System (Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, MD) with (α-^{32}P)-deoxycytidine 5'-triphosphate (dCTP; 3000 Ci/mmol; Amersham, Evanston, IL). Beta-actin probe was used to insure that equal amounts of RNA were loaded in each lane. The filters were washed and then exposed to Kodak XAR-5 film with intensifying screens at -70°C. The intensity of the autoradiographic signal was quantified using a computer-based measurement of the integral of area and density by laser densitometric scanning. After hybridization with c-myc and β-actin, filters were stripped of the probe by heating to 74° C in deionized formamide for 1 h and then rehybridized with a probe for v-fos.

The c-myc 3rd exon, v-fos (mouse FBJ osteosarcoma virus proviral DNA, 1,000 b.p.) and chicken actin (770 b.p.) cDNA probes were obtained from Oncor (Gaithersburg, MD). Ribosomal RNA 23S and 16S (E. coli R13), and ribosomal RNA 28S and 18S (calf liver) were obtained from Pharmacia (Piscataway, N.J.). Proteinase K was a product of Boehringer Mannheim Biochemicals (Indianapolis, IN). All other reagents were obtained from Fisher Scientific or Sigma.

RESULTS

Northern blot analysis of total cellular RNAs isolated from purified Leydig cells of control and hCG-treated rats is shown in Fig. 1. It is evident that c-myc mRNA is expressed in control normal Leydig cells (Fig. 1A). The 2.4 kb size of the major transcript is consistent with the reported size of c-myc mRNA in many other tissues (20). The relative abundance of c-myc mRNA did not change at 1 and 2 h (data not shown), but increased markedly 4 h after the i.p. treatment with hCG (Fig. 1A and 1C). By 24 h, c-myc mRNA expression had decreased lower than basal control (in a total of five separate experiments, c-myc mRNA levels were decreased more than 50% of the control

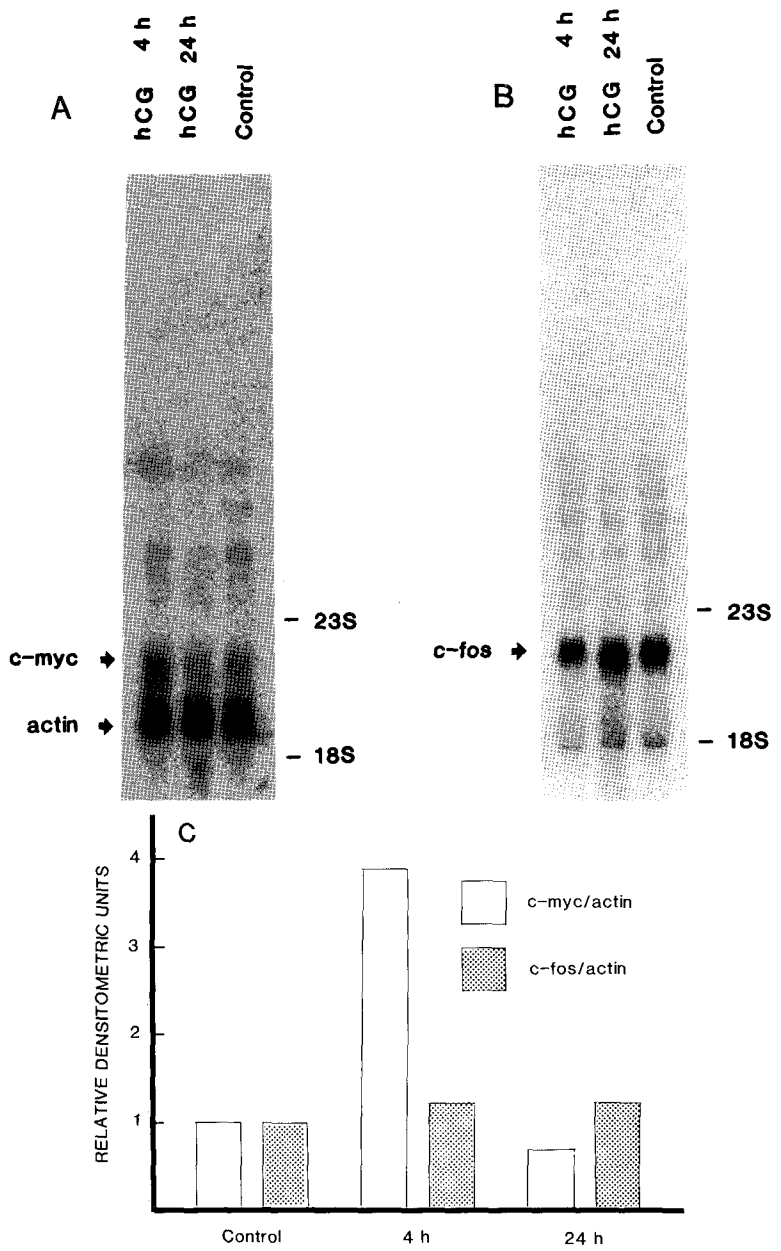

Fig. 1. The effect of in vivo administration of hCG (4 and 24 h) on c-myc (A) and c-fos (B) expression. Samples (10 µg) of total cellular RNA from Leydig cells were electrophoresed, transferred to Nytran membrane and hybridized with ^{32}P-labeled c-myc, v-fos and β-actin probes. Densitometric quantification of the autoradiograph of (A) and (B) are shown in (C).

levels at 24 h). When the same blot was rehybridized with v-fos, there was no significant change of c-fos mRNA (Fig. 1B and 1C). The relative abundance of c-fos mRNA also did not change 1 and 2 h after hCG administration (Fig. 2). This indicates that hCG causes a specific induction of c-myc mRNA in Leydig cells.

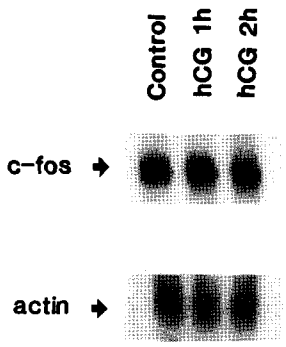

Fig. 2. The effect of in vivo administration of hCG (1 and 2 h) on c-fos expression. Total cellular RNAs of purified Leydig cells (10 μg) were electrophoresed, transferred to Nytran membrane and hybridized with ^{32}P-labeled v-fos and β-actin probes.

DISCUSSION

The c-myc gene was first identified as the transforming sequence within the avian retrovirus MC29, and has been highly conserved throughout evolution (21). The c-myc protooncogene encodes a 65-68 kd phosphoprotein with a high proline content and is localized in the nucleus (22,23). The isolated c-myc protein binds DNA in vitro and has some structural similarity with the E1A-transforming protein of adenovirus (22-25). Elevated expression of the c-myc oncogene is often associated with cell proliferation (26,27). The c-myc construct transfected into cells activates the transcription of an exogenously introduced heat-shock protein gene upon expression of the c-myc protein (28). It has also been reported recently that a c-myc antisense oligodeoxynucleotide inhibits entry of cells into S phase (29,30). These data suggest that c-myc is involved in nuclear regulation aimed at activating the cell to grow (16). In the present study we evaluated the effect of hCG on c-myc and c-fos mRNA expressions in purified Leydig cells. We found that in vivo administration of hCG caused a transient induction of c-myc mRNA at 4 h, while c-fos mRNA levels remained unaltered.

Expression of c-myc has been studied in crude interstitial cells, Sertoli cells and a germ cell line of testes. Stewart et al. (31) reported levels of c-myc transcription in CD-1 mouse testes of various ages. Following a brief collagenase treatment, the developing testes were separated into two fractions: the interstitial tissue and the seminiferous tubules (comprised of Sertoli cells and the differentiating germ cells). During the first several days of postnatal development, the interstitial cells actively divide. At the time of late puberty, cell division drops to a low rate. The pattern of interstitial cell division correlated closely with the level of myc transcription. The amounts of myc RNA in the isolated seminiferous tubules

also exhibited age-dependent changes, being highest at day 3 and then gradually diminishing until day 30 when the myc transcript was no longer detectable. The result correlated with the developmental changes in the proliferation of Sertoli cells, which divide actively only during late gestation and early postnatal life. Hall et al. (32) investigated the expression of the c-fos in Sertoli cell primary cultures. They found that FSH treatment increased c-fos mRNA transiently with a maximal stimulation reached in 1 h and which returned to basal level within 4-6 h. Dibutyryl cAMP and forskolin mimicked the effects of FSH and caused induction of c-fos expression. C-myc mRNA levels were found to be very low in Sertoli cells and seminiferous tubules and there was no apparent changes after FSH stimulation (31,32). In our present study, we found that hCG treatment markedly increased c-myc mRNA expression in purified Leydig cells, while c-fos mRNA levels remained unchanged. In most cell systems studied so far, both c-myc and c-fos mRNA are induced by serum or growth factors. In NIH 3T3 cells, serum and purified growth factors cause a dramatic induction of expression of c-fos and protein in a few minutes, followed by activation of c-myc (33). C-fos induction seems to be the primary event and the earliest effect on gene expression by growth factors. Therefore, it is interesting that FSH induced c-fos mRNA expression in Sertoli cells, while hCG induced c-myc mRNA expression only in Leydig cells.

In conclusion, we have provided evidence that hCG induces a transient expression of c-myc mRNA, while c-fos mRNA remains unchanged in Leydig cells. Growth promoting effects of hCG may be mediated by increased expression of c-myc protooncogene.

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