Cultured Leydig cells exhibited time-dependent decreases in the microsomal cytochrome P-450 enzyme activities, 17α-hydroxylase and C17-20 lyase when maintained under standard culture conditions (95% air, 5% CO2). Inclusion of the hydroxyl radical scavenger dimethyl sulfoxide in the culture medium, or the reduction of oxygen tension from 19 to 1% O2 was effective in preserving these enzyme activities and the combined effects of low O2 and dimethyl sulfoxide were synergistic. Leydig cells in culture were treated with 1 mM 8-Br-cAMP to investigate steroidogenic desensitization which resulted in greater decreases in 17α-hydroxylase and C17-20 lyase activities, as well as a diminished capacity to produce testosterone in response to subsequent acute stimulation with 8-Br-cAMP. Reduction of the oxygen tension from 19 to 1% O2 prevented this enhanced loss of microsomal P-450 activities in desensitized Leydig cells. The activity of Δ5-3β-hydroxysteroid dehydrogenase-isomerase, a microsomal enzyme which is not a P-450 enzyme, was stable in cultures of both control and desensitized Leydig cells under all culture conditions. These data are consistent with the hypothesis that oxygen-mediated damage is responsible for the time-dependent decrease in 17α-hydroxylase and C17-20 lyase activities of control Leydig cells, and is the mechanism by which these microsomal P-450 activities are further decreased in desensitized Leydig cells.

Desensitized Leydig cells exhibited a 50 and 70% decrease at 24 and 48 h, respectively, in their ability to produce testosterone in response to subsequent acute stimulation with 8-Br-cAMP. Reduction of the oxygen tension from 19 to 1% O2 showed no greater loss of enzyme activity than did controls, loss of microsomal P-450 activities is not the cause of the diminished testosterone biosynthetic capacity of desensitized Leydig cells.

It is well established that treatment of animals with a single high dose of gonadotropin results in desensitization of the hormone-sensitive adenylate cyclase activity of Leydig cells (1, 2). Leydig cells from these animals also exhibit desensitization of the steroidogenic pathway which, by definition, results in a diminished capacity to produce testosterone in response to subsequent acute stimulation and is accompanied by decreases of the microsomal P-450 activities, 17α-hydroxylase and C17-20 lyase (1–5). This steroidogenic desensitization is independent of desensitization of the hormone-adenylate cyclase system since exogenous cAMP does not overcome steroidogenic desensitization (1–4). At least two defects in the steroidogenic pathway have been identified, one prior to the production of pregnenolone and one involving decreases in microsomal P-450 activities. The latter has been more thoroughly studied but reports from different laboratories have generally been in disagreement on the mechanism responsible for, and the significance of, the decreases in microsomal P-450 activities of desensitized Leydig cells. One group of studies has supported the hypothesis that the decrease in microsomal P-450 activities is caused by increased production of estradiol which acts via a receptor-mediated process (6–8). Other studies have reported a decrease in cytosolic estrogen receptors accompanying desensitization but have failed to demonstrate a causal relationship between this decline and the desensitization process (9–12). A recent report from this laboratory demonstrated that decreases in 17α-hydroxylase and C17-20 lyase activities following administration of a single high dose of luteinizing hormone were not necessarily associated with a decrease in testosterone biosynthetic capacity (13). In addition, reports from this laboratory and others have indicated that depletion of the precursor cholesterol, due to prolonged stimulation of steroidogenesis is an important factor in the limited steroidogenic response of desensitized Leydig cells (14–16). Thus, the mechanism by which these microsomal P-450 activities are decreased and the relationship of this reduction in enzyme activities to the diminished steroidogenic capacity of desensitized Leydig cells remains unclear.

A recent report by Murphy and Moger (17) provided indirect evidence that the P-450 enzymes of Leydig cells in primary culture are subject to oxygen-free-radical-initiated damage. These authors found that, when the oxygen tension was reduced or antioxidants (MeSO or α-tocopherol) were included in the culture medium, the testosterone production in response to gonadotropin of Leydig cells cultured for 4 days was increased. Recent studies by Hornsby (18) and Crivello et al. (19, 20) which demonstrated that the mitochondrial P-450 activity, 11β-hydroxylase, is rapidly reduced in adrenocortical cell cultures unless protected from oxygen-mediated damage, are consistent with this hypothesis. Evidence is available from these studies and others that the cytochrome P-450

The abbreviations used are: P-450, cytochrome P-450 enzyme; Hepes, N·2·hydroxyethylpiperazine·N·2·ethanesulfonic acid; MeSO, dimethyl sulfoxide.
enzyme complex produces reactive oxygen species during catalysis and that they can damage the P-450 directly or indirectly, by initiating peroxidation of the membrane lipids in which the P-450 is embedded (21-25).

The present study was designed to investigate the role of oxygen-free radical generation in Leydig cell steroid biosynthesis. A primary culture system of mouse Leydig cells was used to determine: 1) whether oxygen-mediated damage causes the time-dependent decrease in the microsomal P-450 activities, 17α-hydroxylase and C17-20 lyase, of cultured Leydig cells; 2) whether oxygen-mediated damage is the mechanism responsible for the accelerated loss of these P-450 activities in desensitized Leydig cells; and 3) whether the decline in these P-450 activities is the major cause of the decreased steroidogenic capacity of desensitized Leydig cells.

MATERIALS AND METHODS

Steroids

[7-(N)-3H]Pregnenolone (10 Ci/mmoll was purchased from American Corp., [1,2-(N)-3H]Progesterone (58 Ci/mmoll), [1,2-(N)-3H]17α-hydroxyprogesterone (40 Ci/mmoll), [4,4′-C]pregnenolone, [4,4′-C]progesterone, [4,4′-C]17α-hydroxyprogesterone, [4,4′-C]androstenedione, and [4,4′-C]Testosterone were purchased from New England Nuclear. Radiochemical purity was estimated by recrystallization of aliquots with authentic steroids. Pregnenolone, progesterone, and 17α-hydroxyprogesterone were obtained from Steraloids Inc. and recrystallized prior to use.

Culture Supplies

Medium 199, Dulbecco’s modified Eagle’s medium, nutrient mixture F-12 (F12), and penicillin-streptomycin were obtained from Grand Island Biological Co. Bovine insulin, Hepes, and 8-Br-CAMP were obtained from Sigma. Tissue culture dishes (35 mm diameter), 100-mm Petri dishes (Gelman Sciences, Inc.), and 100-mm 1 ml Falcon were aseptically removed. All other procedures were carried out under sterile conditions. Testes were decapsulated by a modification of the method of Schumacher et al. (26). Briefly, the tissue was drawn up six times and then gently expelled from a 20-ml plastic syringe with a 6-mm opening. The proximally smaller sizes of Tygon tubing (6-, 4-, and 2-mm diameter) and finally drawn up in a flame polished Pasteur pipette. The resulting material was then filtered through orga to remove seminiferous tubules and other undissociated tissue and cells were collected by centrifugation at 350 X g for 20 min. Cells were resuspended in 3 ml of DMEM and applied to a 15 ml continuous 11-22% gradient of Metrizamide (Accurate Chemical and Scientific Corp.) in H199, which was centrifuged at 3300 X g for 5 min. Fractions were collected from the bottom of the gradient and fractions corresponding to ml6—10 were diluted in 10 volumes of culture medium and cells were collected by centrifugation, as described above. The resulting pellet was resuspended in culture medium in a 35 X 10-mm tissue culture dish to initiate the culture. 1 ml 8-Br-CAMP was added to half of the cultures during the initial 24 h to induce steroidogenic desensitization, and the other half served as controls. All cultures were maintained in a humidified atmosphere at 32 °C and the medium was collected every 24 h and replaced with fresh medium. For standard culture conditions (19% O2) the atmosphere was 95% air, 5% CO2. For low oxygen tension, a smaller incubation chamber was used and the gas was a certified mixture of 1% O2, 5% CO2, 94% N2 (Mathesons Gas Products). When required, Me2SO was added to a final concentration of 100 μM. The number of cells added to each culture dish was measured by counting aliquots of the cell suspension in a Coulter counter. To determine the percentage of Leydig cells, aliquots of the cell suspension were added to glass slides, dried, and subjected to histochemical staining for 3β-hydroxysteroid dehydrogenase as previously described (27) and examined for stained cells under light microscope. The percentage of Leydig cells was 70–80% and all results are expressed per 105 Leydig cells.

Measurement of Steroidogenic Enzyme Activities

The initial enzyme activities were determined following a 3-h attachment period. Subsequently, enzyme activities were determined at 24-h intervals. Fresh medium was added and the cultures were incubated for a 1-h wash period to deplete endogenous substrate prior to the determination of enzyme activity. Enzyme activities were determined, following the 1-h wash period, by incubating replicate culture dishes with a saturating concentration of the appropriate 3H-substrate (10 mM, 1 μCi) dissolved in 100 mM Me2SO in 1 ml of culture medium. Both the wash procedure and the determination of the enzyme activity were done at 37 °C in a humidified atmosphere of 95% air, 5% CO2. The enzyme reactions were stopped by the addition of 9.1 ml of 1 N NaOH. 50 μg of 13C-steroids (600 cpm) corresponding to the products of the reaction were added and the media was removed to an extraction tube. The culture dish was washed with 1 ml of basified medium which was combined with the original medium and extracted with 5 volumes of toluene. The toluene was evaporated and the steroids were taken up in 2 X 150 μl of methanol applied to the following thin-layer chromatography (TLC) plates (Gelman Sciences, Inc.), and separated in chloroform/ethanol (7:1). Steroids were visualized under UV light or by exposure to iodine vapor and the appropriate spots were cut from the sheet and the radioactivity associated with the product(s) was determined by liquid scintillation spectroscopy. Preliminary experiments established that 10 μM substrate with a saturating concentration for all the enzyme activities reported here and that each of the enzyme assays was linear with respect to time and with respect to the number of Leydig cells.

The absence of artifacts due to dilution of the specific activity of the substrate by endogenous substrate was demonstrated as follows. When cells were incubated with 10 μM substrate or 1 μM 8-Br-CAMP during the initial 3 h of culture, no decrease in conversion of 3H-substrate to 3H-products was observed. This indicates that any increase in the size of the substrate pool resulting from the stimulation of steroidogenesis is effectively removed during the 1-h wash period and does not artifically reduce the conversion of 3H-substrate to 3H-products during the assay of enzyme activity.

Δ-3β-HydroxySteroid Dehydrogenase-isomerase Activity—Δ-3β-Hydroxysteroid dehydrogenase-isomerase activity was determined by measuring the conversion of [3H]pregnenolone to [3H]progesterone, [3H]17α-hydroxyprogesterone, [3H]androstenedione, and [3H]Testosterone, as described above. The area corresponding to androstenedione was eluted with methanol and chromatographed to ensure complete separation of androstenedione and pregnenolone.

17α-Hydroxylase Activity—17α-Hydroxylase activity was determined by measuring the conversion of [3H]progesterone to [3H]17α-hydroxyprogesterone, [3H]androstenedione, and [3H]testosterone, as described above. The area corresponding to androstenedione was eluted with methanol and chromatographed to ensure complete separation of androstenedione and progesterone.

17α,20 Lyase Activity—17α,20 Lyase activity was determined by measuring the conversion of [3H]17β-hydroxyprogesterone to [3H]androstenedione and [3H]testosterone, as described above. Since 17α-hydroxypregesterone and testosterone co-migrate in the solvent system used, the area corresponding to these steroids was eluted with methanol and acetylated with pyridine/acetic anhydride (2:1) for 18 h. The testosterone acetate was separated from 17α-hydroxypregesterone by chromatography in chloroform/ethanol (7:1).

Testosterone Production in Response to 8-Br-CAMP

Following the daily medium change, replicate culture dishes were incubated for 3 h in the presence of 1 μM 8-Br-CAMP. At the end of the incubation, the medium was collected and centrifuged at 300 X g for 20 min to remove denatured protein. The supernatant media was then transferred to a clean tube and stored at -20 °C until determination of testosterone by radioimmunoassay, as previously described (28).
To determine the effects of desensitization on these activities, the control/desensitized as the factors. To determine the effects of the repeated for the following pairs of groups

conditions and time as the factors. In order to distinguish between the effects of oxygen tension and those of MeS0, the analysis was repeated for the following pairs of groups: (a) 19% O₂ versus 19% O₂ + MeS0 (b) 19% O₂ versus 1% O₂ + MeS0 versus 1% O₂ (d) 19% O₂ + MeS0 versus 1% O₂ + MeS0, and (e) 1% O₂ versus 1% O₂ + MeS0. All analyses were done using the computer program BMDP2V (29).

RESULTS

Effects of Oxygen Tension and MeS0 on 24-h Testosterone Production—Media from control and desensitized cultures were collected every 24 h for 4 days and analyzed for testosterone (Fig. 1). Testosterone production of control Leydig cells tended to increase slightly during the culture period, but it was not affected by the reduction of oxygen tension or the inclusion of MeS0 in the medium. Leydig cell cultures were desensitized by treatment with 1 mM 8-Br-cAMP during the first 24 h of culture only. This treatment caused a 15-fold increase in testosterone production over basal levels on day 1 of culture. Testosterone production by desensitized cultures remained elevated on day 2 of culture and then declined to values similar to those of controls on days 3 and 4 of culture. It is clear from these data that neither reduction of oxygen tension nor the inclusion of MeS0 in the culture medium diminished the capacity of cultured Leydig cells to increase their testosterone production in response to stimulation with 8-Br-cAMP. It can be inferred from this that the number of turnovers of the hydroxylase and lyase, which are involved in testosterone production, were equivalent under these conditions. Therefore, decreased metabolism of substrate at 1% O₂ cannot account for the differences discussed below in the P-450 activities of desensitized Leydig cell cultures maintained at high and low oxygen tension.

Effects of Oxygen Tension and MeS0 on 17α-Hydroxylase and C17-20 Lyase Activities—The effects of reduction of oxygen tension or the addition of MeS0 to the culture medium on 17α-hydroxylase and C17-20 lyase activities in cultures of control Leydig cells are shown in Fig. 2. In all cases, the enzyme activities were determined as described under "Materials and Methods" after a 3-h attachment period on day 0 and then at 24-h intervals. Cells were maintained at 19% O₂ (C), 19% O₂ + 100 mM MeS0 (E), 1% O₂ (F), and 1% O₂ + 100 mM MeS0 (B). Determinations were done in duplicate. Values are the means; bars indicate the range of two experiments.

**Fig. 2.** Effects of oxygen tension and MeS0 on P-450 activities of control Leydig cells in primary culture. Enzyme activities were determined as described under "Materials and Methods" after a 3-h attachment period on day 0 and then at 24-h intervals. Cells were maintained at 19% O₂ (C), 19% O₂ + 100 mM MeS0 (E), 1% O₂ (F), and 1% O₂ + 100 mM MeS0 (B). Determinations were done in duplicate. Values are the means; bars indicate the range of two experiments.

**Fig. 1.** Daily testosterone production of control and desensitized Leydig cells in primary culture. Leydig cells were desensitized by incubation with 1 mM 8-Br-cAMP during the initial 24 h of culture only. Culture medium was replaced at 24-h intervals and analyzed for testosterone by radioimmunoassay. Cells were maintained under the following conditions: 19% O₂ (C), 19% O₂ + 100 mM MeS0 (E), 1% O₂ (F), and 1% O₂ + 100 mM MeS0 (B). Values are means of two experiments, each of which was done in duplicate.

---

2 P. G. Quinn and A. H. Payne, unpublished data.
Desensitization and P-450 Damage in Cultured Leydig Cells

FIG. 3. Effects of oxygen tension and Me_{2}SO on 17α-hydroxylase activity of control and desensitized Leydig cells in primary culture. 17α-Hydroxylase activity was determined as described under "Materials and Methods" after a 3-h attachment period on day 0 of culture and then at 24 and 48 h in control (□) and desensitized (■) Leydig cell cultures. Desensitized cultures were treated with 1 mM 8-Br-cAMP during the initial 24 h of culture. Determinations were done in duplicate. Values are the means; bars indicate the range of two experiments.

different (p > 0.05) than controls during the initial 48 h of culture.

Δ^α-3β-Hydroxysteroid Dehydrogenase-isomerase Activity of Control and Densensitized Leydig Cells Maintained under Different Culture Conditions—The activity of 3β-hydroxysteroid dehydrogenase-isomerase, a microsomal enzyme which is not a P-450 enzyme, was determined after 3 h and again on day 2 of culture in control and desensitized Leydig cells maintained under various conditions. It is clear from the data in Fig. 5 that desensitization did not decrease this enzyme activity under any culture conditions tested and that the level of this activity remained similar to control values during the initial 48 h of culture. This is in marked contrast to the effects of desensitization and culture conditions on microsomal P-450 activities.

Acute 8-Br-cAMP-stimulated Testosterone Production—The maximum steroidogenic capacity of control and desensitized Leydig cells was assessed by incubating replicate cultures with a maximally stimulatory dose of 8-Br-cAMP (1 mM) for 3 h and determining the amount of testosterone produced. Preliminary data (not shown) indicated 8-Br-cAMP-stimulated testosterone production was linear over the time period employed. The maximum rate of testosterone production of

FIG. 5. Δ^α-3β-hydroxysteroid dehydrogenase-isomerase activity of cultured Leydig cells. Enzyme activity was determined as described under "Materials and Methods" after 3 h in culture and at 48 h in control (□) and desensitized (■) Leydig cells. Determinations were done in duplicate. Values are the means; bars indicate range of two experiments.

FIG. 6. Maximal testosterone production in response to 8-Br-cAMP of cultured Leydig cells. Cultured Leydig cells were incubated in fresh medium containing 1 mM 8-Br-cAMP for a 3-h period at 0, 24, and 48 h. Control cells (□) had not been previously exposed to 8-Br-cAMP, whereas desensitized cells (■) had been treated with 1 mM 8-Br-cAMP during the initial 24 h of culture. Determinations were done in duplicate. Values are the means; bars indicate the range of two experiments.
Leydig cells during the first 3 h in culture and the maximum rate of testosterone production of both control and desensitized Leydig cells at 24 and 48 h is shown in Fig. 6. The data are expressed as the rate of testosterone production (nanomoles of testosterone per mg protein, on the basis of co-purification of these activities from normal Leydig cells maintained under standard culture conditions, and is the mechanism by which these P-450 activities are rapidly reduced in desensitized Leydig cells. The data also demonstrate that the reduction of microsomal P-450 activities is not the factor that limits the steroidogenic capacity of desensitized Leydig cells.

Recent studies have suggested that both 17α-hydroxylase and 17α,20β-lase activities in the same P-450 protein, on the basis of co-purification of these activities from neonatal pig testis microsomes (39). The P-450 activities of the mouse Leydig cell resemble those of the neonatal pig in that the lase activity is double the hydroxylase activity but differ in that pregnenolone is preferentially metabolized via the Δ5 rather than the Δ4 pathway. In the present study, the 17α-hydroxylase and 17α,20β-lase activities responded in a parallel fashion to changes in culture conditions and treatment with cAMP. This pattern of change in P-450 activities is obviously consistent with the hypothesis that one enzyme catalyzes both reactions. It is also consistent with the existence of two independent, but closely related, P-450 enzymes which catalyze the hydroxylation and cleavage of 17α-hydroxyprogesterone, both of which are sensitive to oxygen-mediated damage.

The current study indicates that microsomal P-450 activities, 17α-hydroxylase and 17α,20β-lase, of cultured Leydig cells are very sensitive to oxygen-mediated damage. The lag period of 24 h in the decline of these P-450's is probably due to the utilization of endogenous antioxidants, and once these are depleted the P-450 activities decline rapidly. The observation that Me3SO, a potent scavenger of hydroxyl radicals, was partially effective in preventing this decline, as was the reduction of oxygen tension, together with the observation that Me3SO and low O2 act synergistically to prevent this decline in P-450 activities, suggests that more than one form of active oxygen is responsible for the decline in P-450 activities. Reactive oxygen species (superoxide and H2O2) are generated during catalysis by P-450 and by autoxidation of P-450 (21-24, 31). The production of O2 and H2O2 is often increased by pseudosubstrates (24, 32-34). In the presence of ferrous iron and ADP, H2O2 is converted to hydroxyl radicals via the Fenton reaction (24). H2O2 and other organic peroxides can react with the heme of P-450 and inactivate the enzyme (21, 24, 35). In addition, both hydroxyl radicals and superoxide anions can initiate lipid peroxidation (24). Lipoyl acid hydroperoxide has been shown to destroy the heme of purified cytochrome P-450 enzymes (38). In studies of microsomal preparations of P-450, the extent of lipid peroxidation correlated well with the extent of inactivation of the P-450 (25). Thus, the cytochrome P-450 enzyme and/or its associated reductase can generate active oxygen species which destroy the heme group directly or indirectly, by initiating peroxidation of the membrane lipids in which it is embedded.

The increased rate of loss of P-450 activities in desensitized Leydig cells was not diminished by Me3SO but was entirely prevented by reduction of the oxygen tension. This finding suggests that hydroxyl radicals are not involved in this process or that they are produced in the vicinity of the P-450 and react with the heme without being released into the bulk solution where they can react with Me3SO. The large and rapid reduction of hydroxylase and lase activities of desensitized Leydig cells at 19% O2 is consistent with the model described by Hornby (18) for inactivation of 11β-hydroxylase by cortisol in adrenocortical cell cultures. In that study, rapid inactivation of the 11β-hydroxylase was promoted by a variety of steroids which were either substrates for, or the 11β-hydroxylated products of, the enzyme; 11α-hydroxylated steroids and 11-ketosteroids were without effect. Hornby and Hotchkiss (18) proposed that interaction of products or pseudosubstrates with the cytochrome P-450 led to release of damaging oxygen free-radicals from the P-450-pseudosubstrate complex due to the inability of the steroid to be hydroxylated. In this regard, it should be noted that in the process of desensitization, Leydig cells are exposed to high concentrations (~2 μM) of the product, testosterone, in the initial period of culture when dramatic reductions in the cytochrome P-450 activities take place. Preliminary results indicate that treatment of Leydig cells with 2 μM androstenedione with Me3SO for 24 h at 19% O2 results in losses of 17α-hydroxylase and C17,20-lase activities similar to those observed in desensitized Leydig cells. Treatment with 2 μM cortisol or 112-hydroxyandrostenedione or 17α-methyltestosterone did not reduce the P-450 activities and the decreases induced by treatment with androstenedione and testosterone were prevented when cultures were maintained at 1% O2 instead of 19% O2. Further studies are underway to define more precisely the chemical nature of the steroid(s) which induce this loss of microsomal P-450 activities.

8-Br-cAMP was used in this study, rather than luteinizing hormone or human chorionic gonadotropin, to induce steroidogenic desensitization independently of luteinizing hormone receptor down regulation. Studies from this and other laboratories have demonstrated that desensitization of steroidogenesis is independent of receptor down regulation (37-39). In addition, preliminary experiments showed that luteinizing hormone and 8-Br-cAMP produced identical enzyme losses and decreases in testosterone production when used to induce steroidogenic desensitization. The results of this study cannot, at the present time, be resolved with the studies indicating that increased production of estradiol is responsible for the decrease in the 17α-hydroxylase and C17,20-lase activities of desensitized Leydig cells (3, 7, 8). Since the hydroxylase and lase activities are not diminished at low oxygen tension, it seems unlikely that aromatase, which is a microsomal P-450 enzyme that catalyzes the conversion of testosterone to estradiol, would be impaired under these conditions. Regardless of any role which estradiol may play in the desensitization process, it is clear from the present studies that reductions in hydroxylase and lase activities do not cause depressed testosterone synthesis in desensitized Leydig cells, at least during the first 48 h. Reductions of up to 75% of these activities did...
Desensitization and P-450 Damage in Cultured Leydig Cells

not decrease the testosterone biosynthetic capacity of control Leydig cells and preservation of the activities at control levels did not prevent the decline in testosterone biosynthetic capacity. These observations indicate that the decline of the activities of these P-450 activities to less than 10% of the original values, as observed on day 5 of culture at 19% O2, would make these activities rate-limiting in testosterone synthesis and would therefore decrease steroidogenic capacity as observed by Murphy and Moger (17).

The absence of a correlation between reductions in P-450 activities and steroidogenic capacity is not an entirely unexpected result in light of other metabolic defects which have been shown to exist in desensitized Leydig cells. A previous study from this laboratory provided evidence that depletion of cholesterol stores, due to the prolonged stimulation of steriodogenesis resulting from gonadotropin treatment in vivo was the major limiting factor in testosterone biosynthetic capacity of desensitized rat Leydig cells (14). In agreement with this study, Freeman and Ascoli (15) found that addition of low density lipoprotein cholesterol to cultured murine Leydig tumor cells could overcome gonadotropin-induced steriodogenic desensitization. In addition, Charreau et al. (16) found that exogenous cholesterol partially restored the pregnenolone production of desensitized Leydig cells and that the activity of hydroxymethylglutaryl CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, was significantly depressed at 24 and 72 h following treatment with gonadotropin. Recently, Luketich et al. (17) provided evidence that the mitochondrial cholesterol side chain cleavage enzyme, also a P-450-dependent activity, was reduced by 47% 24 h after administration of a desensitizing dose of human choric gonadotropin to mice. Since cholesterol side chain cleavage is probably the rate-limiting step in testicular steroidogenesis, as it is in the adrenal, reduction of this activity would be expected to have an important influence on testosterone biosynthetic capacity.

Taken together, these various studies indicate that several defects exist in the testosterone biosynthetic pathway of desensitized Leydig cells. Cholesterol stores are depleted both by decreased synthesis and by increased metabolism, and both mitochondrial and microsomal cytochrome P-450 activities are reduced. In addition, there is evidence that the relative importance of these defects is influenced by the dose of gonadotropin used to induce desensitization (1, 3, 8). The present study demonstrates that the defect in mitochondrial P-450 activities, in the absence of substrate depletion, as occurs in control Leydig cells at 19% O2, does not reduce the steroidogenic capacity of cultured Leydig cells.

The Leydig cell culture system described in this report provides an excellent model for study of the desensitization process at the molecular level. The direct effects of gonadotropin and cAMP treatment on Leydig cell function can be determined in the absence of complications arising from feedback effects of the hypothalamic pituitary axis. In addition, factors contributing to the various defects can be independently and readily manipulated.

Acknowledgments—We wish to thank Dr. Morton Brown of the Department of Biostatistics for valuable advice on the statistical analyses. We gratefully acknowledge the technical assistance of Tammi DeShong and Kathy Krisch and the assistance of Sharon King in the typing of the manuscript.

REFERENCES

Oxygen-mediated damage of microsomal cytochrome P-450 enzymes in cultured Leydig cells. Role in steroidogenic desensitization.

P. G. Quinn and A. H. Payne


Access the most updated version of this article at http://www.jbc.org/content/259/7/4130

Alerts:
  - When this article is cited
  - When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/7/4130.full.html#ref-list-1