Microsomal Cytochrome P-450 Enzyme Damage in Cultured Leydig Cells: Relation to Steroidogenic Desensitization[a]

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It is well established that treatment of animals with a single high dose of LH or hCG results in steroidogenic desensitization of Leydig cells.[1,2] Desensitized Leydig cells exhibit a decreased capacity to produce testosterone in response to subsequent stimulation with gonadotropins or cAMP analogues, and have decreased activities of the microsomal P-450 enzymes, 17α-hydroxylase, and C_{17-20} lyase. The present study provides evidence that the decreases in microsomal P-450 activities are caused by oxygen-derived, free-radical damage of the enzymes, and, furthermore, that the decrease in microsomal P-450 activities are not the primary cause of the decrease in testosterone-producing capacity observed in desensitized Leydig cells.

Primary cultures of purified, adult mouse Leydig cells were maintained in serum-free medium at 32°C, in a humidified atmosphere of 19% O_2 (95% air/5% CO_2) or 1% O_2, in the presence and absence of the antioxidant dimethyl sulfoxide. The culture medium was changed daily and the activities of microsomal enzymes were determined by quantifying the conversion of ^{3}H-substrate to ^{3}H-products during a 1 hr incubation at 37°C, in an atmosphere of 19% O_2. The maximal capacity to produce testosterone was determined by incubating replicate cultures with 1 mM 8-Br-cAMP for a 3 hr period and measuring testosterone in the medium by radioimmunoassay.

1 mM 8-Br-cAMP was added to half of the Leydig cells, during the initial 24 hr of culture only, to induce steroidogenic desensitization. The other half served as controls. Testosterone production by desensitized Leydig cells was 15-fold and threefold greater than that of controls on days one and two of culture, respectively. The addition of dimethyl sulfoxide to the culture medium and/or reduction of the oxygen tension had no effect on testosterone production by control or desensitized Leydig cells.

The data presented in TABLE 1 demonstrate that, when control Leydig cell cultures are incubated in 19% O_2, the microsomal P-450 activities, 17 α-hydroxylase and C_{17-20} lyase, are stable during the first 24 hr but decrease markedly by 48

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TABLE 1. Microsomal Enzyme Activities and Maximal Testosterone Production of Cultured Leydig Cells

Treatment	nmol products · h^{-1} · 10^5 Leydig cells^{-1}			
	17α-Hydroxylase Activity	C_{17-20} Lyase Activity	Δ^5-3β-Hydroxysteroid Dehydrogenase Activity	Maximal Testosterone Production
I. 19% O_2				
Day 0	2.062 ± 0.200[a]	3.750 ± 0.950	2.310 ± 0.206	0.147 ± 0.008
Day 1 control	1.753 ± 0.284	3.650 ± 0.248	N.D.[b]	0.160 ± 0.006
Day 1 desensitized	0.971 ± 0.054	2.272 ± 0.157	N.D.	0.079 ± 0.003
Day 2 control	0.473 ± 0.043	1.234 ± 0.161	2.333 ± 0.139	0.150 ± 0.021
Day 2 desensitized	0.163 ± 0.027	0.327 ± 0.147	2.269 ± 0.020	0.037 ± 0.008
II. 19% O_2 + Dimethyl sulfoxide				
Day 0	2.033 ± 0.137	3.481 ± 0.109	2.259 ± 0.103	0.176 ± 0.035
Day 1 control	1.945 ± 0.056	3.601 ± 0.083	N.D.	0.185 ± 0.005
Day 1 desensitized	1.184 ± 0.185	2.557 ± 0.241	N.D.	0.079 ± 0.006
Day 2 control	1.041 ± 0.217	2.171 ± 0.137	2.205 ± 0.014	0.193 ± 0.005
Day 2 desensitized	0.348 ± 0.105	0.967 ± 0.112	1.987 ± 0.030	0.039 ± 0.001
III. 1% 0_2				
Day 0	2.139 ± 0.226	3.221 ± 0.323	2.342 ± 0.160	0.161 ± 0.010
Day 1 control	2.452 ± 0.342	4.170 ± 0.560	N.D.	0.237 ± 0.011
Day 1 desensitized	2.287 ± 0.470	3.838 ± 0.811	N.D.	0.107 ± 0.021
Day 2 control	1.560 ± 0.085	3.418 ± 0.370	2.145 ± 0.047	0.239 ± 0.014
Day 2 desensitized	1.436 ± 0.054	2.952 ± 0.483	1.954 ± 0.090	0.085 ± 0.012
IV. 1% O_2 + Dimethyl sulfoxide				
Day 0	3.050 ± 0.110	4.132 ± 0.198	2.866 ± 0.475	0.175 ± 0.023
Day 1 control	3.198 ± 0.777	5.522 ± 0.288	N.D.	0.229 ± 0.009
Day 1 desensitized	2.691 ± 0.623	5.047 ± 0.278	N.D.	0.106 ± 0.020
Day 2 control	2.851 ± 0.641	5.317 ± 0.739	2.131 ± 0.455	0.289 ± 0.007
Day 2 desensitized	2.085 ± 0.371	4.075 ± 0.644	1.721 ± 0.202	0.083 ± 0.008

[a] $\bar{X}$ ± S.D. [b] Not determined.

hr. This decline in P-450 activities was partially prevented by addition of the antioxidant dimethyl sulfoxide to the medium or by reduction of the oxygen tension. The combined effects of these treatments were synergistic in preserving the P-450 activities of control Leydig cells. In contrast, the P-450 activities of desensitized Leydig cells maintained at 19% O_2 were reduced to 50 and 33% of control values at 24 and 48 hr, respectively. However, when Leydig cells were cultured in an atmosphere of 1% O_2, the cAMP-induced decrease in the P-450 activities was essentially prevented. The activity of Δ^5-3β-hydroxysteroid dehydrogenase-isomerase, a microsomal enzyme that is not P-450–dependent, was stable in both control and desensitized Leydig cells during the 48 hr culture period, indicating that the decrease in microsomal P-450 activities was specific and not due to damage of the cells and/or the smooth endoplasmic reticulum. These data are consistent with the hypothesis that oxygen-mediated damage is responsible for the time-dependent decrease in 17α-hydroxylase and C_{17-20} lyase activities of control Leydig cells, and is the mechanism by which these activities are further decreased in desensitized Leydig cells. The large and rapid reduction of hydroxylase and lyase activities of desensitized Leydig cells at 19% O_2 is consistent with the model described by Hornsby for inactivation of the adrenal P-450 enzyme, 11β-hydroxylase, by the product, cortisol.[3] In this model, interaction of products (pseudosubstrates) with the enzyme leads to release of damaging free radicals from the P-450·pseudosubstrate complex, due to the inability of the steroid product to be hydroxylated. In this regard, it should be noted that during the desensitization process, Leydig cells are exposed to high concentrations (~ 2 μM) of the product, testosterone. Recent results, which indicate that a 24 hr incubation of Leydig cells with high (2 μM) but not low (0.2 μM) concentrations of testosterone causes the same extent of decrease in P-450 activities as incubation with cAMP, and that this decrease is prevented by reduction of the oxygen tension, support the hypothesis that decreases in microsomal P-450 activities of desensitized Leydig cells are caused by steroid pseudosubstrate-induced, oxygen-derived, free-radical damage of these P-450 enzymes.

The capacity of control Leydig cells to produce testosterone in response to cAMP was not diminished at any time or under any culture conditions tested, even though P-450 activities of cultures maintained at 19% O_2 had decreased by 75% after 48 hr. In contrast, the capacity of desensitized Leydig cells had decreased by 50% at 24 hr under all culture conditions, in spite of the fact that Leydig cells maintained at 1% O_2 did not exhibit decreased P-450 activity at this time. Thus, the decrease in maximal capacity for testosterone production of desensitized Leydig cells cannot be attributed to the decrease in 17α-hydroxylase and C_{17-20} lyase activities and must be caused by other factors, such as a decrease in cholesterol side-chain cleavage activity[4] or depletion of cholesterol stores.[5,6]

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