Mechanism of the Acute cAMP-Induced Decrease in P-450$_{17\alpha}$ in Cultured Mouse Leydig Cells[a]

LOUISE M. PERKINS AND ANITA H. PAYNE[b]

Departments of Biological Chemistry and Obstetrics and Gynecology and the Reproductive Endocrinology Program University of Michigan Ann Arbor, Michigan 48109

Recent studies from our laboratory have shown that treatment of primary cultures of mouse Leydig cells with a high dose of cAMP results in an oxygen-mediated, steroid product-enhanced decrease in the microsomal 17α-hydroxylase (P-450$_{17\alpha}$) enzyme activity.[1,2] These observations are consistent with the hypothesis proposed by Hornsby[3] that these decreases in P-450 enzyme activity occur when steroid products, acting as pseudosubstrates, bind to the P-450 protein and enhance the generation of damaging oxygen free-radicals, which either directly or indirectly inactivate the enzyme.[3]

The present study was designed to investigate the mechanism by which the oxygen-dependent, product-induced decrease in P-450$_{17\alpha}$ activity occurs. The amount of P-450$_{17\alpha}$ was determined in lysates of cultured mouse Leydig cells treated for 48 h with 8-Br-cAMP (cAMP) or steroids by immunoblotting to determine if decreases in P-450$_{17\alpha}$ enzyme activity are due to damage to, and concomitant loss of, the enzyme protein or due to an inactivation of the catalytic activity of the enzyme with no change in the amount of the enzyme protein. In addition, the amount of mitochondrial cholesterol side-chain cleavage enzyme (P-450$_{scc}$) was determined in the same preparations to evaluate if the two enzymes are regulated in the same manner or are regulated differentially.

Adult mouse Leydig cells were purified and allowed to attach 3 h prior to the initiation of treatment with cAMP or steroids. Following the 48 h treatment period, P-450$_{17\alpha}$ enzyme activity was determined by the conversion of [^{3}H]progesterone to [^{3}H]products, and cultured cells were lysed with a sodium dodecyl sulfate (SDS)-containing buffer. Aliquots of the lysates were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting with antibody specific for P-450$_{17\alpha}$. In some cases, immunoblots were treated with antibody to P-450$_{scc}$ following immunoblotting with anti-P-450$_{17\alpha}$. Bound antibody was detected with [^{125}I]protein A and autoradiography.

FIGURE 1 shows that 1.0 mM cAMP or 2 μM testosterone (T) causes a decrease in the amount of P-450$_{17\alpha}$ similar to the decrease in activity of the enzyme. In the presence of aminoglutethimide (AG), which inhibits T synthesis, the

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[b] Address correspondence to Anita H. Payne, Steroid Research Unit, L1221-0278 Women's Hospital, The University of Michigan, Ann Arbor, MI 48109-0278.

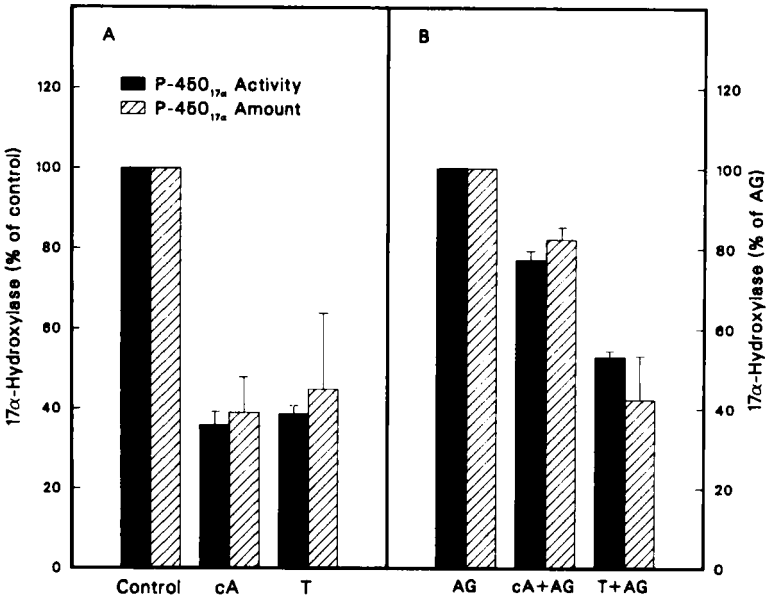

FIGURE 1. Comparison of changes in P-450$_{17\alpha}$ activity and amount relative to control in the absence or presence of aminoglutethimide. Leydig cell cultures were maintained for 48 h at 19% oxygen and treated with medium only (Control), 1.0 mM 8-Br-cAMP (cA), 2 μM testosterone (T), 0.5 mM aminoglutethimide (AG), cA + AG, or T + AG. Cell lysates were prepared and immunoblotted for P-450$_{17\alpha}$. The relative amount of P-450$_{17\alpha}$ in a given experiment was determined by densitometry and expressed as percent of control. P-450$_{17\alpha}$ enzyme activity was measured in duplicate dishes in the same experiment. Values represent the mean and the range of two experiments.

cAMP-induced decrease but not the T-induced decrease is inhibited. Furthermore, the changes in the amount of the enzyme accompany the changes in the enzyme activity in every case. The cAMP-induced decrease in the amount of P-450$_{17\alpha}$ can be prevented by maintaining the cultures at reduced oxygen tension (1% O_2) during the treatment period, although the total T synthesis is unchanged under these conditions. The product-induced loss is not a steroid receptor-mediated event, because neither cortisol, estradiol, nor the androgen agonist, methyltrienolone, were able to cause the decrease. Decreased *de novo* synthesis of the P-450 is not involved in the process inasmuch as cycloheximide does not affect the loss of P-450$_{17\alpha}$ due to T. FIGURE 2 demonstrates that cAMP and T alter the amount of P-450$_{17\alpha}$ specifically, because the amount of P-450$_{scc}$ did not change following the same treatment.

The results presented in this study suggest that the mechanism by which acute cAMP treatment enhances loss of P-450$_{17\alpha}$ activity is to increase damage to the enzyme. The damaged protein resulting from this process is more prone to degradation than the intact enzyme. The data are consistent with the proposal that steroid products cause oxygen-mediated decreases in P-450$_{17\alpha}$ activity by acting as so-called pseudosubstrates, which bind to the enzyme and enhance the genera-

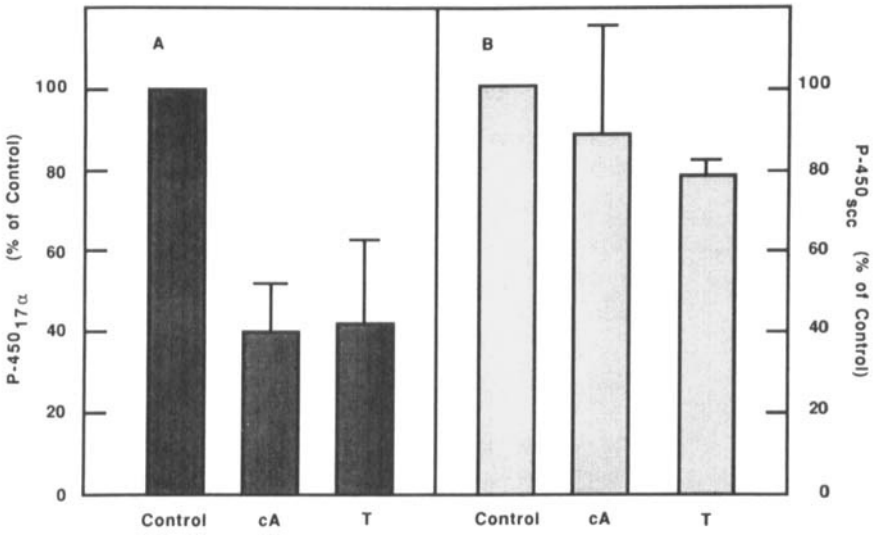

FIGURE 2. Comparison of changes in the amount of P-450$_{17\alpha}$ (dark bars) and P-450$_{scc}$ (light bars) after treatment with cAMP or testosterone. Leydig cell cultures were maintained for 48 h at 19% oxygen and treated with medium only (Control), 1.0 mM 8-Br-cAMP (cA), or 2 μM testosterone (T). Cell lysates were prepared and immunoblotted for P-450$_{17\alpha}$ followed by immunoblotting of the same nitrocellulose for P-450$_{scc}$. The relative amount of each P-450 was determined by densitometry and is expressed as percent of control for that protein. Values represent the mean and the range of two experiments.

tion of damaging oxygen free radicals. This process is specific for P-450$_{17\alpha}$, and thus under these conditions, the P-450$_{17\alpha}$ and P-450$_{scc}$ are not coordinately controlled.

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