Regulation of *de Novo* Synthesis of Cytochrome P-450$_{17\alpha}$ in Mouse Leydig Cell Cultures[a]

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Studies from this laboratory have shown that chronic (long term) treatment of mouse Leydig cell cultures with LH, or its intracellular second messenger, cAMP, causes a time-dependent increase in 17α-hydroxylase activity.[1] This cAMP-induced increase in P-450$_{17\alpha}$ enzyme activity is enhanced by aminoglutethimide (AG), an inhibitor of cholesterol metabolism. The AG enhancement of cAMP-induced 17α-hydroxylase activity can be reversed by supplying exogenous testosterone (T) to the cAMP + AG-treated cultures.[2] This finding suggests that testosterone produced during the cAMP induction of P-450$_{17\alpha}$ activity negatively regulates the extent of this induction.

The present study was designed to examine whether the cAMP-induced increase and testosterone-mediated decrease in P-450$_{17\alpha}$ activity are due to changes in the total amount of specific enzyme protein, changes in the rate of *de novo* synthesis of P-450$_{17\alpha}$, or activation or inactivation of preexisting enzyme protein. Purified Leydig cells were maintained in culture for 7 days prior to the initiation of treatment with 0.05 mM 8-Br-cAMP, 0.5 mM AG, cAMP + AG, or cAMP + AG + 5 μM T. To determine *de novo* synthesis of P-450$_{17\alpha}$, cultures were incubated for 3 h in medium containing [^{35}S]methionine. Immunoprecipitable P-450$_{17\alpha}$ was separated by SDS-gel electrophoresis and visualized by fluorography. [^{35}S]methionine P-450$_{17\alpha}$ was quantitated by laser densitometry. The total amount of P-450$_{17\alpha}$ was determined by immunoblotting (Western blotting); SDS-gel resolved Leydig cell proteins were transferred to nitrocellulose paper, incubated with anti-P-450$_{17\alpha}$ antibody, and bound antibody was detected with iodinated protein A. The total amount of P-450$_{17\alpha}$ was quantitated by laser densitometry. 17α-Hydroxylase activity was determined after a 1 h wash, by measuring the conversion of [^{3}H]progesterone to [^{3}H]steroid products during a 1 h incubation.

To determine whether the 8-Br-cAMP-stimulated induction of 17α-hydroxylase activity was mediated by an increase in the rate of *de novo* synthesis of P-450$_{17\alpha}$, the rate of incorporation of [^{35}S]methionine into newly synthesized enzyme protein was measured over a 4-day period. The results shown in FIGURE 1 demonstrate that treatment of Leydig cells with 8-Br-cAMP resulted in the induction of *de novo* synthesis of P-450$_{17\alpha}$. The rate of synthesis approximately doubled when cells were treated with 8-Br-cAMP + AG. To test if the AG-enhancement of

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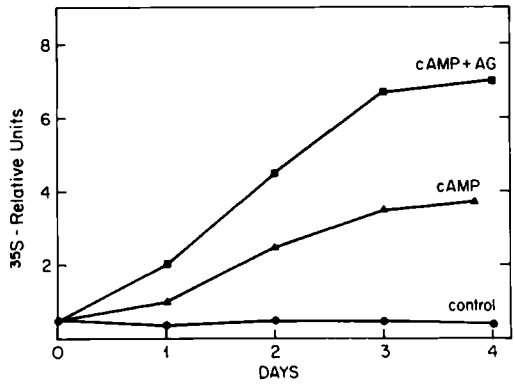

FIGURE 1. Effect of aminoglutethimide (AG) on the rate of *de novo* synthesis of P-450$_{17\alpha}$. Leydig cell cultures were treated with control media, media containing 8-Br-cAMP, or 8-Br-cAMP + AG. At the indicated time, *de novo* synthesis was determined as described in the text. Each point on the curve represents immunoprecipitated P-450$_{17\alpha}$ isolated from an equal number of trichloroacetic acid precipitable counts.

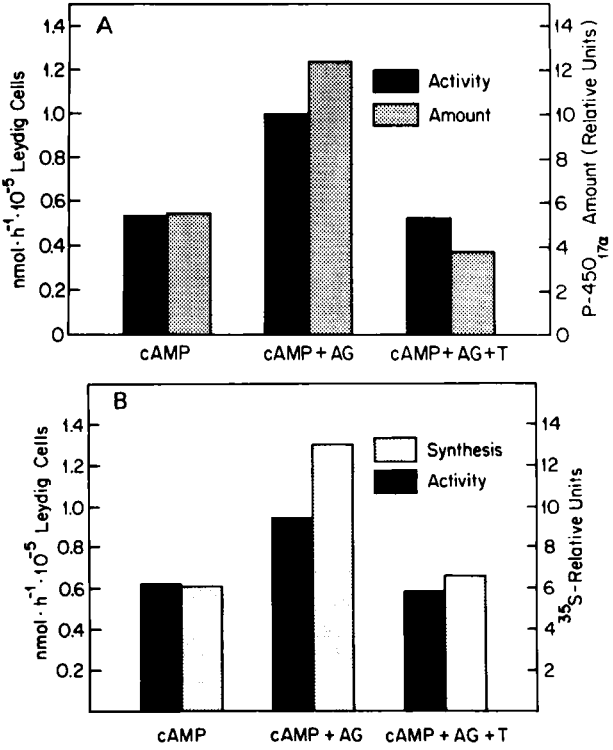

FIGURE 2. Effect of cAMP, AG, and testosterone on 17α-hydroxylase activity, total amount, and *de novo* synthesis of P-450$_{17\alpha}$. Leydig cell cultures were treated for 4 days as indicated and assayed as described in the text. **A:** 17α-hydroxylase activity ( ); total immunoreactive P-450$_{17\alpha}$ ( ); **B:** 17a-hydroxylase activity ( ) and *de novo* synthesis ( ).

8-Br-cAMP-stimulated induction was due to the inhibition of testosterone production, exogenous testosterone was supplied to 8-Br-cAMP + AG-treated cultures. 17α-Hydroxylase activity was determined in parallel with total amount (FIG. 2A) or with *de novo* synthesis of P-450$_{17\alpha}$ (FIG. 2B). The results, shown in FIGURE 2, demonstrate that changes in enzyme activity are correlated to changes in both the total amount of P-450$_{17\alpha}$ and its rate of synthesis.

The results demonstrate the following. cAMP-induced increases in activity are due to increased accumulation of P-450$_{17\alpha}$ enzyme protein resulting from increased synthesis of P-450$_{17\alpha}$ and are not due to the activation of a pool of preexisting enzyme protein. The AG enhancement of cAMP induction of *de novo* synthesis of P-450$_{17\alpha}$ can be reversed by the addition of exogenously added testosterone. These data indicate that testosterone produced during cAMP induction of P-450$_{17\alpha}$ negatively regulates the activity of this cytochrome P-450 enzyme by decreasing its *de novo* synthesis.

REFERENCES

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