EFFECTS OF ACTINOMYCIN D ON HORMONE-INDUCED STEROIDOGENESIS BY SUPERFUSED RAT ADRENAL GLANDS*

M. K. Mostafapour and T. T. Tchen
Department of Chemistry
Wayne State University
Detroit, Michigan 48202

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SUMMARY - Under in vitro superfusion conditions, actinomycin-D potentiates the steroidogenic effect of ACTH and delays the onset of the refractory state in the rat adrenal gland. Actinomycin-D also reverses the inhibitory effect of cyanoketone which blocks the conversion of pregnenolone to progesterone. It is postulated that the delayed refractoriness may be due to a slower nuclear action of the hormone resulting in the synthesis of an inhibitory protein.

INTRODUCTION

It is well established that ACTH (or dibutyryl-c-AMP)-induced synthesis and secretion of glucocorticoids is abolished by inhibitors of protein synthesis but not by actinomycin D (1-5). Careful examination of these reports, however, shows that in most instances, actinomycin D had a slight stimulatory effect. More recently, Shin and Sato (6) reported that this stimulatory effect of actinomycin D is variable and dependent upon the concentration of actinomycin D. We wish to report here that the stimulatory effect of actinomycin D on hormone-induced glucocorticoid secretion by superfused rat adrenal glands is two fold. There is both an increase in the rate of steroid output and a prolongation of the duration of active steroid output. Furthermore, actinomycin specifically reversed the inhibition of steroidogenesis by cyanoketone (2α-cyano-

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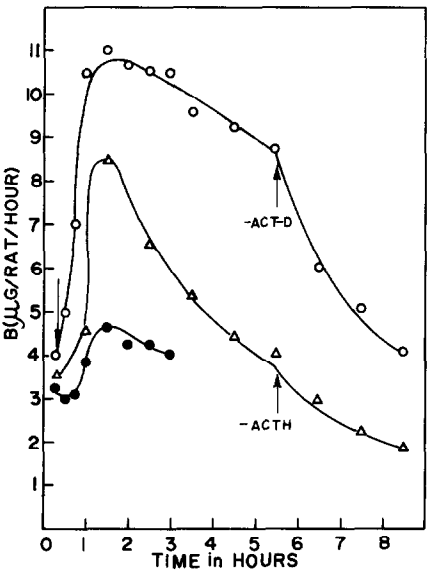

Figure 1 Effect of actinomycin-D on ACTH stimulated corticosterone output rate by superfused rat adrenals. The superfusion conditions are as described earlier (8).

o—o: corticosterone output in the presence of both ACTH (0.1 U/ml) and actinomycin-D (20 μg/ml). (-Act-D) denotes removal of Act-D but continued superfusion with ACTH. Δ—Δ : corticosterone output in the presence of ACTH. (-ACTH) denotes removal of ACTH from superfusion fluid. ●—●: with Act-D but without ACTH. The time of addition of ACTH and/or actinomycin D (Act-D) are indicated by arrow.

4,4,17α-trimethyl-androst-5-en-17β-ol-3-one) which blocks the conversion of pregnenolone to progesterone (7) a process which involves the translocation of pregnenolone from mitochondria into the cytoplasm.

MATERIALS AND METHODS

Cyanoketone was a gift from Dow Chemical Co. Actinomycin-D was obtained through Sigma. Dissolution of cyanoketone and actinomycin-D in the buffer was aided by a small quantity of ethanol. Other materials and methods were as previously described (8).

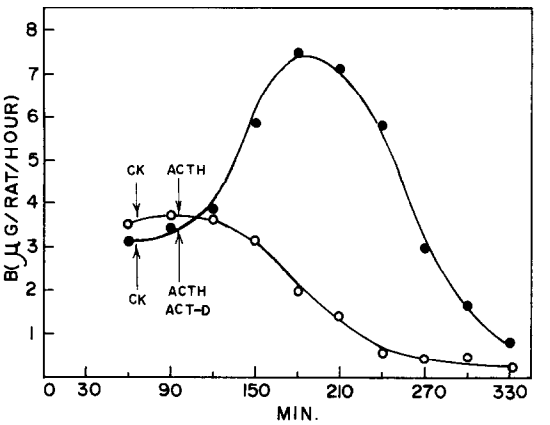

Figure 2 Reversal of cyanoketone inhibition of ACTH stimulated corticosterone (B) output by actinomycin D (Act-D). The conditions are the same as in Figure 1.

o—o: corticosterone output in the presence of cyanoketone (10^{-4}M) and ACTH (0.1 U/ml). •—•: corticosterone output in the presence of cyanoketone, ACTH and actinomycin D. The time of addition of cyanoketone (CK), ACTH and actinomycin D (Act-D) are indicated by arrows. It is clear that cyanoketone inhibits the effect of ACTH and that the presence of actinomycin D reverses this inhibition and yields the typical output profile obtained with ACTH alone (compare with Figure 1).

RESULTS

The stimulation of hormone-induced steroidogenesis by actinomycin D is shown in Figure 1. Presence of actinomycin D (20 μg/ml) and ACTH together yielded higher rate of steroid output and delayed the onset of "refractory" state (decline of steroid output). This latter effect of actinomycin D is readily reversed when actinomycin D is removed from the superfusion fluid. In the absence of ACTH, the addition of actinomycin D to the superfusing fluid resulted only in a slightly higher steroid output. (Fig. 1)

Actinomycin D also specifically reverses the inhibition of

hormone-induced steroidogenesis by cyanoketone which blocks the transformation of pregnenolone to progesterone and leads to accumulation of pregnenolone in the mitochondria (7). As shown in Figure 2, superfusion of adrenals by ACTH and cyanoketone (1×10^{-4}M) did not produce enhanced steroidogenesis whereas the further addition of actinomycin D gave the typical hormone-induced increase in glucocorticoid output. The inhibitory effect of aminoglutethimide (inhibitor of sidechain cleavage) or of metopyrone (inhibitor of P_{450}) is not reversed by actinomycin D. (Mostafapour and Tchen, unpublished)

DISCUSSION

The stimulation of steroidogenesis by ACTH is still poorly understood. Although there is general consensus that it involves the synthesis of a labile protein from a pre-formed mRNA, the identity and function of this labile protein is still uncertain (9). It is also well known that prolonged treatment of adrenals by ACTH or dibutyryl-c-AMP leads to a "refractory" state where steroidogenesis is no longer stimulated by either the hormone or its second messenger (10-14). It is tempting to speculate that the adrenals may have a built-in guard against over-production of glucocorticoids during prolonged stress (prolonged ACTH stimulation). If such were the case, stimulatory effect of actinomycin D may be explained in the following way. If c-AMP causes a rapid synthesis of a stimulatory protein from existing mRNA and a slower synthesis of a mRNA which eventually leads to the formation of an inhibitory protein, one would expect ACTH-treated adrenals to enter rapidly into an active state and then gradually shift into a refractory state. In the presence of actinomycin D, one would find only the synthesis

of the stimulatory protein but not the inhibitory-mRNA-protein. The resulting kinetics of steroidogenesis would then resemble that seen experimentally in Figure 1. This hypothesis is, however, admittedly highly speculative and is offered only for the want of a better explanation of the effect of actinomycin D.

The reversal of the inhibitory effect of cyanoketone by actinomycin D is even more difficult to explain. Presumably, actinomycin D somehow facilitates the transport of pregnenolone out of the mitochondria and the subsequent oxidation to progesterone. It is not known whether this is due to a direct or an indirect effect on the mitochondria. Further work is in progress in the hope of clarifying this effect of actinomycin D.

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