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Adenosine 3':5'-Cyclic Monophosphate as the Intracellular Mediator of the Action of Adrenocorticotrophin on the Adrenal Cortex

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Adrenocorticotrophin *in vitro* (Haynes, 1958) and *in vivo* (Grahame-Smith, Butcher, Ney & Sutherland, 1967) causes the accumulation of cyclic AMP (adenosine 3':5'-cyclic monophosphate) in the adrenal cortex. Cyclic AMP *in vitro* (Haynes, Koritz & Peron, 1959) and *in vivo* (Imura, Matsukara, Matsuyama, Sitsuda & Miyake, 1965) stimulates adrenocortical steroidogenesis. Grahame-Smith *et al.* (1967) showed that the increases in adrenal cyclic AMP induced by adrenocorticotrophin occurred before increases in the rate of adrenal steroidogenesis, that increasing doses of adrenocorticotrophin produced increasing concentration of adrenal cyclic AMP as steroidogenesis was progressively stimulated and that adrenal concentrations of cyclic AMP remained elevated while the rate of steroidogenesis was maintained. In addition, the potency of analogues of adrenocorticotrophin in producing stimulation of adrenal steroidogenesis was reflected in their potency in producing increases in adrenal cyclic AMP concentrations. Adrenocorticotrophin produces increases in adrenal cyclic AMP by stimulating adenylate cyclase activity (Grahame-Smith *et al.* 1967; Taunton, Roth & Pastan, 1967; Lefkowitz, Roth, Pricer & Pastan, 1970). Lefkowitz *et al.* (1970) have shown that cell-free adrenal extracts containing small fragments of cell membrane bind ^{125}I -labelled adrenocorticotrophin and that this binding is associated with stimulation of adenylate cyclase activity. The degree of binding of derivatives of ^{125}I -labelled adrenocorticotrophin was paralleled by their capacity to stimulate adenylate cyclase activity. The location of adenylate cyclase in other cells (Davoren & Sutherland, 1963; Sutherland, Oye & Butcher, 1965) suggests that adrenocorticotrophin acts on the plasma membrane of the adrenocortical cell to stimulate adenylate cyclase activity and to increase the intracellular concentration of cyclic AMP, which then acts as the intracellular mediator (second messenger) of adrenocorticotrophin.

The sequence of events through which cyclic AMP acts to stimulate steroidogenesis is not yet fully understood, but some clues are available. Inhibitors of protein synthesis block the stimulation

of adrenal steroidogenesis produced *in vitro* by adrenocorticotrophin and cyclic AMP (Ferguson, 1963), though inhibition of protein synthesis does not prevent the adrenocorticotrophin stimulation of adenylate cyclase (Grahame-Smith *et al.* 1967). Garren, Ney & Davis (1965) showed that in the rat *in vivo* cycloheximide administered after adrenocorticotrophin dramatically decreased corticosterone secretion to base-line values within 20 min. Garren and his colleagues (see Garren, 1968) have reached the conclusion that the cyclic AMP-mediated action of adrenocorticotrophin to stimulate steroidogenesis is dependent on the activation of a specific regulator protein, with a rapid turnover rate, which in turn increases in some way the conversion of cholesterol into pregnenolone, the rate-limiting step in steroid biosynthesis (Stone & Hechter, 1954; Karaboyas & Koritz, 1956). This step takes place in the mitochondrion (Halkerston, Eichhorn & Hechter, 1961) and is catalysed by an organized enzyme complex (Tchen, 1968) that converts cholesterol into 20α -hydroxycholesterol, then into $20\alpha,22\xi$ -dihydroxycholesterol and finally into pregnenolone (for references see Garren, 1968). The precise source of the initial substrate cholesterol is not clear. Adrenocorticotrophin increases both the rate of adrenal synthesis (Dexter, Fishman, Ney & Liddle, 1967) and the mobilization of cholesterol from cholesterol esters, though the latter is not blocked by inhibitors of protein synthesis (Davis & Garren, 1966) whereas steroidogenesis is, suggesting that the supply of cholesterol is not regulated by the protein-mediated action of adrenocorticotrophin.

Gill & Garren (1969) have found a protein associated with the microsomal fraction of adrenal cortex that shows some specificity in binding cyclic AMP. They suggest that cyclic AMP might act on this binding protein to alter its function by modifying its structure. They hypothesize that this protein then activates the synthesis of the protein regulating steroidogenesis. Farese (1967) has isolated from adrenal slices treated with adrenocorticotrophin a protein-like factor that appears to stimulate steroidogenesis in a cell-free system. This might be the hypothetical protein regulator of steroidogenesis. Koritz & Kumar (1970) have presented evidence to suggest that cyclic AMP-mediated adrenocorticotrophin stimulation of pregnenolone synthesis does not involve an increase in the amount of an enzyme that participates in pregnenolone synthesis but does affect the activity of such an enzyme. They point out that pregnenolone feeds back to inhibit its own synthesis, and they interpret their experimental results as indicating that the rate of efflux of newly synthesized pregnenolone from the mitochondrion is the factor controlled by the cyclic AMP-mediated action of adrenocorticotrophin. The postulated regulatory protein would then act to

increase the rate of efflux of pregnenolone from the mitochondrion.

Besides acutely stimulating steroidogenesis adrenocorticotrophin also causes adrenocortical growth. Grahame-Smith *et al.* (1967) showed in the rat that at a time after hypophysectomy when adrenal weight had fallen and the acute steroidogenic response to adrenocorticotrophin had disappeared the sensitivity of the adrenal cyclic AMP response to adrenocorticotrophin was undiminished. R. L. Ney (1969) has since shown that cyclic AMP is capable of maintaining adrenal weight in hypophysectomized rats. These experiments indicate that cyclic AMP may also be the intracellular mediator of this action of adrenocorticotrophin.

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The Role of 3':5'-Cyclic Nucleotide Phosphodiesterase in Regulating Tissue Concentrations of Adenosine 3':5'-Cyclic Monophosphate

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Specificity studies on 3':5'-cyclic nucleotide phosphodiesterases of various tissues (Nair, 1962; Butcher & Sutherland, 1962; Drummond & Perrott-Yee, 1961) suggest that their sole function is to regulate concentrations of 3':5'-cyclic nucleotides. Since cyclic GMP (guanosine 3':5'-cyclic monophosphate) has been found to occur widely (Goldberg, Dietz & O'Toole, 1969; Ishikawa, Ishikawa, Davis & Sutherland, 1969), however, it cannot be assumed that they regulate the concentration of cyclic AMP (adenosine 3':5'-cyclic monophosphate) exclusively. No phosphodiesterase has been shown to be absolutely specific to a single cyclic nucleotide, though enzymes with distinct preferences for either purine nucleotides or pyrimidine nucleotides are known (Hardman & Sutherland, 1965).

Although the subject has not been systematically studied it appears that the phosphodiesterases of various tissues are not identical. Since the relative activity of ox heart phosphodiesterase towards cyclic UMP (uridine 3':5'-cyclic monophosphate) gradually changes during purification (Hardman & Sutherland, 1965), we may conclude that the enzyme multiplicity extends to individual organs. It is not clear whether in this instance the enzymes are derived from different cells within the organ. Certainly in epididymal adipose tissue only about one-quarter of the phosphodiesterase activity resides in the fat-cells (Hepp, Menahan, Wieland & Williams, 1969), the remainder being found in the stromal-vascular fraction (J. I. Davies, unpublished work).

The possibility still remains that individual cells contain more than one enzyme species. Alternatively, differences in its physical environment, as reflected by its soluble or particulate distribution, may affect its properties.

The problems posed by possible heterogeneity in enzyme preparations are illustrated by the existing confusion over the K_m values of various phosphodiesterases. Using an enzyme preparation from rat brain and concentrations of cyclic AMP within the range 0.1–1.0 mM, Brooker, Thomas & Appleman (1968), like many others, obtained a K_m value of the order of 10^{-4} M. With lower concentrations of cyclic AMP, however, the same enzyme preparation yielded a K_m value of 10^{-6} M. This anomaly can be interpreted in several ways. First, the preparation may contain two distinct enzymes or, alternatively, a single enzyme in different