COMMUNICATIONS

 β 1-24-Adrenocorticotrophin Diazotized to Polyacrylamide: Effects on Isolated Adrenal Cells

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The retention of biological activity by several polypeptide hormones when covalently attached to large polymers has suggested that these hormones are acting at the cell surface. We now report effects of β 1-24-ACTH* coupled to polyacrylamide on an adrenal-cell suspension. Schimmer, Ueda & Sato (1968) have reported effects of a similar preparation on monolayer cultures of adrenal tissue.

Suspensions of cells were prepared from decapsulated rat adrenals (Richardson & Schulster, 1970). Full dissociation of the adrenal tissue after digestion with collagenase (0.5 mg/ml) was achieved by the use of a homogenizer fitted with a silicone rubber pestle (clearance 0.3 mm) (Jacob & Bhargava, 1962). After incubations of the cell suspension, corticosterone was estimated in cells plus medium by radioimmunoassay (precision at 1 ng, 70 pg; sensitivity, 30 pg). β 1-24-ACTH (Synacthen; CIBA, Basle, Switzerland) was coupled to a *p*-aminophenyl derivative of polyacrylamide (Enzacryl AA; Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.) by diazotization (Barker, Somers, Epton & McLaren, 1970). Subsequent to coupling the resin was washed extensively with 0.1M-phosphate buffer, pH 7.4, and then with 0.5M-sodium chloride in the same buffer. The beads were finally suspended and stored in 0.9% sodium chloride.

β 1-24-ACTH-polyacrylamide added to a suspension of adrenal cells produced a stimulation of steroidogenesis (unincubated suspensions contained 0.029 μ g of corticosterone/gland; control suspensions incubated for 1 h contained 0.029 μ g of corticosterone/gland; suspensions incubated for 1 h with 5 mg of β 1-24-ACTH-polyacrylamide contained 1.02 μ g of corticosterone/gland; suspensions incubated with 10 munits of ACTH contained 1.38 μ g of corticosterone/gland).

Experiments have shown that the activity shown by β 1-24-ACTH-polyacrylamide is not due to proteolytic cleavage of biologically active peptides from the complex. First, when cells were incubated with β 1-24-ACTH-polyacrylamide, the medium obtained after centrifugation was found to be inactive when tested for its ability to stimulate

steroidogenesis in a further batch of cells. Secondly, incubations were carried out in which β 1-24-ACTH-polyacrylamide and cells were separated by a sintered-glass disc (pore size 5–15 μ m) that would allow free passage of medium. Under these conditions β 1-24-ACTH-polyacrylamide was completely ineffective in stimulating steroidogenesis (control suspension incubated for 1 h contained 0.050 μ g of corticosterone/gland; suspension incubated with 5 mg of β 1-24-ACTH-polyacrylamide contained 1.04 μ g of corticosterone/gland; suspension separated from β 1-24-ACTH-polyacrylamide by sintered-glass disc contained 0.049 μ g of corticosterone/gland).

Our results suggest that ACTH can stimulate steroidogenesis without entering the adrenal cell, functioning by an interaction with cell-surface receptors.

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Effect of Vasopressin on the Uptake of Calcium Ions by Kidney Mitochondria and on the Concentration of Adenosine 3':5'-Cyclic Monophosphate in Toad Bladder

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Effects of [8-arginine]-vasopressin on Na⁺ and water transport across the isolated toad bladder have been considered in terms of a direct permeability effect of the hormone and an effect mediated by cyclic AMP (adenosine 3':5'-cyclic phosphate) (Snart, 1970). The nature of the direct permeability effect of the hormone is believed to be associated with the adsorption of the hormone on membrane receptor sites (Snart & Sanyal, 1968), a process that may involve the release of membrane-bound Ca²⁺ (Kafka & Pak, 1969). Such an interaction may be responsible for the hormonal activation of the adenylate cyclase system, which has been associated by Rasmussen (1970) with the

* Abbreviation: ACTH, adrenocorticotrophin.