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.

mobilization of tissue calcium. In the present work we have measured the dose-response characteristic for vasopressin-stimulated cyclic AMP in the isolated toad (*Bufo marinus*) bladder, and have investigated, with rat kidney, the possibility that hormonal effects on tissue calcium may lead to an increased uptake of Ca^{2+} into mitochondria.

All toads were rapidly pitthed and the half bladders were excised and weighed. They were preincubated for 1 h in aerated frog Ringer solution and then for a further 20 min, as matched pairs, in the presence of a given dose of [8-arginine]-vasopressin or under control conditions. The tissue was homogenized and the cyclic AMP concentration determined using the technique described by Brooker, Thomas & Appelman (1968). The concentration of cyclic AMP was found to be about 2 pmol/mg wet wt. in control tissue and to be maximally stimulated by 260% at 100 munits of vasopressin/ml.

The Ca^{2+} concentration in rat kidney mitochondria, isolated from a 10% (w/v) homogenate (Rossi & Lehninger, 1963), was measured by atomic absorption spectroscopy in an EDTA-water system after washing of the mitochondria in iso-osmotic sucrose and extraction with 5% (w/v) trichloroacetic acid. The minced tissue was incubated for 20 min in the absence and in the presence of vasopressin (1–500 munits/ml). A dose response characteristic was obtained showing the Ca^{2+} concentration of rat kidney mitochondria to reach a minimum after vasopressin (2 munits/ml) treatment. At higher doses of hormone Ca^{2+} concentration gradually increases, reaching a value close to that of the control at 100 munits of vasopressin/ml.

It is concluded that the concentration of cyclic AMP in toad bladder may not be directly related to the hormonal stimulation of Na^{+} transport across the tissue. Our view of the possible role of cyclic AMP and Ca^{2+} in the mechanism of vasopressin action will be presented.

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Isolation of Glucagon-Like Immunoreactivity of Gut by Affinity Chromatography

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The gut of animals contains glucagon-like immunoreactivity (Moody *et al.* 1970; Valverde, Rigopoulou, Marco, Faloona & Unger, 1970). Antibodies coupled to Sepharose have been used for affinity chromatography of polypeptide hormones (Akanuma, Kuzuya, Hayashi, Ide & Kuzuya, 1970; Weintraub, 1970). We have used antibodies to pancreatic glucagon, that cross-react with glucagon-like immunoreactivity of gut, for affinity chromatography of the latter substance from pig ileum.

An antibody-Sepharose complex was prepared by coupling rabbit immunoglobulin G (260 mg) containing antibodies to crystalline pig glucagon (Novo Terapeutisk Laboratorium, Copenhagen, Denmark) to 100 g of Sepharose 4B (Cuatrecasas, 1970). ^{125}I -labelled glucagon was bound by the complex in 40 mM-phosphate buffer, pH 7.4, containing human serum albumin (5 g/l), thiomersalate (0.25 g/l) and Trasylol (aprotinin; 5000 units/ml) (FBA Pharmaceuticals, Haywards Heath, Sussex, U.K.). The most suitable solvent for the elution of ^{125}I -labelled glucagon from the antibody-Sepharose complex was 4.25 M-ethanol in 4 mM-hydrochloric acid, which can be removed subsequently by freeze-drying. When ^{125}I -labelled glucagon (15 ng) was applied to columns (4.5 cm $\times$ 0.6 cm) at pH 7.4, 8–10% of the radioactivity flowed through the columns. This is attributed to chemical changes in the hormone during iodination. After a wash with water, 65% of the ^{125}I -labelled glucagon was eluted as a single peak by 4.25 M-ethanol in 4 mM-hydrochloric acid. A further 3% was eluted by 6 M-guanidine, pH 2.5, so that more than 20% of the hormone remained tightly bound to the columns. This loss was prevented by pre-washing the columns with 4.25 M-ethanol in 4 mM-hydrochloric acid. Columns bound 30 μg of crystalline glucagon/g of antibody-Sepharose complex and retained their chromatographic properties after at least ten experiments.

When crystalline glucagon, glucagon (2000 ng/g of tissue) extracted (Kenny & Say, 1962) from pig pancreas or glucagon-like immunoreactivity (200 ng/g of tissue) from pig ileum were chromatographed, the elution profiles as determined by glucagon radioimmunoassay (Buchanan & McCarroll, 1971) were identical. Immunoreactivity was eluted in 5 ml as two close peaks. After affinity chromatography of pancreas extracts on larger columns (28 cm $\times$ 1.5 cm), *N*-terminal histidine was detected