

cyclase activity decreased, even though Mg^{2+} was maintained in excess and sodium fluoride was present at optimum concentration.

Differential-centrifugation studies have shown that all particulate fractions of rat corpus-luteum homogenate contain adenylate cyclase and 'adenosine triphosphatase'. The cyclases of 5000g and 54000g pellets show a linear increase in the production of cyclic AMP with increasing tissue concentration in contrast with that of the 600g pellet.

Sonication increases adenylate cyclase and 'adenosine triphosphatase' activities in the 54000g pellet and high-speed supernatant at the expense of those in the 600g and 5000g pellets, but the solubilization was small and non-selective.

Triton X-100 (1%) increases the amount of adenylate cyclase in 54000g supernatant, but this is at the expense of a large overall inactivation of the enzyme. Lubrol-PX (1%) was somewhat less effective, and treatment of homogenate with deoxycholate (1%) resulted in elimination of adenylate cyclase activity.

Detection of adenylate cyclase activity in soluble fractions of luteal homogenate is complicated by the presence of 3':5'-cyclic nucleotide phospho-

diesterases, but, even after removal of cytosol before detergent treatment of the particulate fractions, the amount of adenylate cyclase solubilized was still small compared with the overall loss of activity.

Jungas, R. L. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 757.

Marsh, J. M. (1969). In *Progress in Endocrinology*, p. 83. Ed. by Gual, C. Amsterdam: North-Holland Publishing Co.

Parlow, A. F. (1958). *Fedn Proc. Fedn Am. Soc. exp. Biol.* **17**, 402.

Rall, T. W. & Sutherland, E. W. (1958). *J. biol. Chem.* **232**, 1965.

Reiner, J. M., Tsuboi, K. K. & Hudson, P. B. (1955). *Archs Biochem. Biophys.* **56**, 165.

Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1968). *A. Rev. Biochem.* **37**, 149.

Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1970). In *Fundamental Concepts in Drug-Receptor Interactions*, p. 59. Ed. by Danielli, J. F., Moran, J. F. & Triggle, D. J. New York: Academic Press Inc.

Stansfield, D. A. & Robinson, J. W. (1965). *Endocrinology*, **76**, 390.

Sutherland, E. W., Hardman, J. G., Butcher, R. W. & Broadus, A. E. (1969). In *Progress in Endocrinology*, p. 26. Ed. by Gual, C. Amsterdam: North-Holland Publishing Co.

COMMUNICATIONS

A Modified Enzymic Assay for Adenosine 3':5'-Cyclic Monophosphate

By M. JOHNSON and H. S. A. SHERRATT. (*Department of Pharmacology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.*)

An understanding of the physiological role of cyclic AMP (adenosine 3':5'-cyclic monophosphate) depends on its successful assay. Here we outline a method based on the conversion of phosphorylase *b* into phosphorylase *a*, which indirectly requires cyclic AMP. This conversion is catalysed by phosphorylase *b* kinase, which is activated by phosphorylase *b* kinase kinase, the latter reaction having an absolute requirement for cyclic AMP (Walsh, Perkins & Krebs, 1968). Earlier assay methods that used phosphorylase *b* were only successful owing to variable amounts of phosphorylase *b* kinase found in inactive preparations of phosphorylase *b* kinase. In this method the optimum amount of purified phosphorylase *b* kinase kinase (Walsh *et al.* 1968) is added.

Tissues are rapidly frozen in isopentane or with tongs, cooled to $-196^{\circ}C$. Samples (10–25mg) are weighted at $-30^{\circ}C$ and powdered while frozen. The powder is homogenized in 5 vol. of 10% (w/v) trichloroacetic acid at $0^{\circ}C$ and after centrifugation

the supernatant is extracted four times with 5 vol. of water-saturated ether. Interfering nucleotides are removed by precipitation with zinc sulphate and barium hydroxide and centrifugation (G. Krishna, see Rodbell, 1967). The final supernatants are applied to cellulose plates with micro-syringes and chromatographed with propan-2-ol-water-35M-ammonia (14:3:3, by vol.) (Goldberg, Lerner, Sasko & O'Toole, 1968) with cyclic AMP markers. After the chromatogram has been dried, the areas containing cyclic AMP (R_f 0.45) are eluted with 0.5ml of water. This process gives recoveries of cyclic AMP of 90–95%.

The eluate (100 μ l) containing cyclic AMP is added to 260 μ l of a medium in a cuvette, containing 30mM-tris, 30mM-sodium glycerol 2-phosphate, 2.5mM-EDTA, 25mM-2-hydroxyethanethiol, 0.075mM-ATP, 0.25mM- Mg^{2+} , 2000 units of Norit A-treated crystalline rabbit muscle phosphorylase *b* (Fischer & Krebs, 1962), 12.5 μ g of non-activated phosphorylase *b* kinase (Krebs *et al.* 1964) and 1.9 μ g of phosphorylase *b* kinase kinase at pH 6.8 at $35^{\circ}C$. After 1 min the formation of phosphorylase *a* is terminated by the addition of 1.0ml of ice-cold 20mM-phosphate containing 0.5mM-NADP⁺ and 0.1% of bovine serum albumin at pH 6.8. Phosphoglucomutase (1 U) and glucose 6-phosphate dehydro-

genase (0.3U) were then added in a volume of 200 μ l. The increase in fluorescence due to NADPH formed in the coupled assay of phosphorylase *a* at 35°C is continuously recorded after the reaction has been started by the addition of 100 μ l of 1% purified shell-fish glycogen. The rates after 5 min are compared with those obtained with cyclic AMP standards and an extraction blank, and are directly proportional to the logarithm of the cyclic AMP concentrations in the range 1–100 pmol/assay mixture.

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Fischer, E. H. & Krebs, E. G. (1962). In *Methods in Enzymology*, vol. 5, p. 369. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Goldberg, N. D., Lerner, J., Sasko, H. & O'Toole, A. G. (1968). *Analyt. Biochem.* **28**, 523.

Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L. & Fischer, E. H. (1964). *Biochemistry, Easton*, **3**, 1022.

Rodbell, M. (1967). *J. biol. Chem.* **242**, 5744.

Walsh, D. A., Perkins, J. P. & Krebs, E. G. (1968). *J. biol. Chem.* **243**, 3763.

The Assay of Adenosine 3':5'-Cyclic Monophosphate by Saturation Analysis

By B. L. BROWN, R. P. EKINS and W. TAMPION. (*Institute of Nuclear Medicine, The Middlesex Hospital Medical School, London W1N 7RL, and Department of Biochemistry, Royal Free Hospital Medical School, London W.C.1, U.K.*)

The principle of saturation analysis, first applied to the measurement of thyroxine (Ekens, 1960) and vitamin B₁₂ (Barakat & Ekens, 1961), has been used to develop a simple and sensitive assay for cyclic AMP (adenosine 3':5'-cyclic monophosphate). The assay depends upon the specific cyclic AMP-binding protein in adrenal tissue described by Gill & Garren (1969), the specificity and reaction-energy characteristics of which suggested its possible use as a specific reagent for assay purposes (Brown & Ekens, 1970).

Bovine adrenal cortices were homogenized in a medium comprising 0.25 M-sucrose, 50 mM-tris, pH 7.4, 25 mM-potassium chloride and 5 mM-magnesium chloride. After centrifugation at 4°C at 2000g for 5 min, the supernatant was respun at 5000g for 15 min. The resultant supernatant was stored at -20°C in 0.5 ml portions. Dilutions of this preparation in 50 mM-tris buffer, pH 7.4 (containing 8 mM-theophylline and 6 mM-2-mercaptoethanol), were used as the binding agent.

Standard response curves were obtained by

incubating serial dilutions of cyclic AMP (0–15 pmol) with cyclic [³H]AMP and a dilution of the binding agent at 4°C. After 1.5 h a suspension of 10 mg of charcoal (Norit GSX) in tris buffer containing bovine serum albumin (2%, w/v) was added to each tube to separate the free and bound moieties. The radioactivity of portions of the supernatant obtained after centrifugation (i.e. the bound fraction) was counted in a liquid-scintillation spectrometer and the radioactivity observed was related to the total initial ³H radioactivity present. In the determination of cyclic AMP in biological material cyclic [³H]AMP was added to the sample before extraction and the radioactivity served in the determination both of extraction recovery and the distribution ratio. The appropriate controls, blanks and standards were included in each run.

Investigations of the specificity characteristics of the binding protein have indicated that, of all the nucleotides and nucleosides studied, only cyclic GMP (guanosine 3':5'-cyclic monophosphate) demonstrated measurable cross-reaction (0.3% under normal assay conditions). The lower limit of detection of cyclic AMP in this system is approx. 0.2 pmol/incubation tube.

The application of this assay system to biological extracts will be discussed.

Barakat, R. M. & Ekens, R. P. (1961). *Lancet*, ii, 25.

Brown, B. L. & Ekens, R. P. (1970). *Clin. Sci.* **38**, 27 F.

Ekens, R. P. (1960). *Clinica chim. Acta*, **5**, 453.

Gill, G. N. & Garren, L. D. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 512.

The Effects of Secretin, Pancreozymin and Acetylcholine on the Concentration of Adenosine 3':5'-Cyclic Monophosphate in Cat Pancreas

By M. JOHNSON, H. S. A. SHERRATT, R. M. CASE and T. SCRATCHERD. (*Departments of Pharmacology and Physiology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.*)

The effect of secretin, but not that of pancreozymin, can be mimicked by the dibutyryl derivative of cyclic AMP (adenosine 3':5'-cyclic monophosphate) in the isolated cat pancreas (Case, Laundry & Scratcherd, 1969). By contrast cyclic AMP and its dibutyryl derivative have been reported to increase enzyme extrusion by isolated mouse and rabbit pancreas (Kulka & Sternlicht, 1968; Ridderstap & Bonting, 1969; Knodell, Toskes, Heber & Brooks, 1970). The present study was designed to assess more accurately the role of cyclic AMP in the action of secretin and pancreozymin.

Young lean cats, starved for 18 h, were anaesthetized with Nembutal and their pancreatic ducts were