Plasma Adenosine 3′,5′-Monophosphate in Antiinsulin-Treated Rats

Adenosine 3′, 5′-monophosphate (cAMP) an intracellular mediator of the actions of a number of hormones[1] may escape from its cells of origin into the extracellular fluids, including plasma[2,3], urine[2] and cerebrospinal fluid[1].

Since cyclic AMP in plasma is in a dynamic equilibrium with intracellular pools of the nucleotides[3], cyclic nucleotide efflux from the cells may provide a measure of cyclic nucleotide metabolism. As plasma levels of cyclic AMP and cyclic guanosine 3′, 5′-monophosphate (cGMP) do not change in fasting[4] and the plasma glucose and free fatty acid concentration are affected in diabetes and fasting, we became interested in possible changes in cyclic AMP in plasma after antiinsulin treatment.

Materials and methods. Male rats (260–300 g) of Sprague-Dawley strain were used. Cyclic 3′, 5′AMP was purchased from Boehringer Co., U.K. Adenosine-8-^{3}H-3′, 5′monophosphate was obtained from Amersham, Bucks., U.K.

Equal amounts of antiinsulin serum and normal serum were injected into the femoral vein of ether anaesthetized rats. Samples of blood were collected from tail vein at different time intervals. The blood was treated with equal volume of 0.25 *M* $ZnSO_4$ and 0.25 *M* $Ba(OH)_2$ immediately to precipitate the protein and then centrifuged. Supernatant was used for cyclic AMP and blood glucose determinations. Antiinsulin serum was prepared as described by BARLING and BELOFF-CHAIN[5]. Blood glucose was estimated by the method of MARKS and LLOYD[6].

An aliquot of the above supernatant was evaporated to dryness and redissolved in 50 m*M* *Tris*-HCl buffer, pH 7.4 (containing 8 m*M* theophylline and 6 m*M* 2-mercaptoethanol). Cyclic AMP was then assayed by the method described by DAS[7] at two dilutions and accompanied by standard control. It was observed in this study that the technique of BROWN et al.[8] of absorbing unreacted radioactive AMP with charcoal could be conveniently modified to the filtration method for separation. The sample was then added to a reaction mixture containing radioactive cyclic AMP, binding protein and *Tris* buffer and incubated for 1 h 30 min at 4 °C. After incubation 1 ml of 20 m*M* KH_2PO_4 buffer, containing 6 m*M* 2-mercaptoethanol was added and the solution immediately passed through a Millipore filter (0.45 μm, 25 mm diameter). The tube and paper were carefully washed free of radioactive cyclic AMP with KH_2PO_4 buffer containing 2-mercaptoethanol. Addition of mercaptoethanol to the washing buffer stabilized the protein complex and yielded a very consistent and repeatable result compared to the charcoal absorption method. In this study cyclic AMP was assayed by both methods and the results were found to be very similar.

Effect of antiinsulin serum on cyclic AMP levels in rat plasma

Time after antiinsulin injection (min)	Blood glucose (mg/100 ml ± S.E.)	Blood cyclic AMP (*p*mole/ml ± S.E.)
0	95 ± 4.5	19 ± 0.9
15	150 ± 7.8	20 ± 1.1
30	250 ± 20.4	22 ± 2.5
45	260 ± 15.9	18 ± 1.8
60	310 ± 25.7	22 ± 3.0
90	280 ± 10.8	21 ± 0.8
120	200 ± 8.2	20 ± 1.0

Results are for 6 rats.

Results and discussion. It has been shown that glucagon substantially increased the cyclic AMP levels[2,9,10], while insulin lowered the release of cyclic AMP in perfused rat livers. The release of cyclic nucleotides from heart or adipose tissue in response to glucagon or insulin stimulation was not systematically studied. The levels of insulin and glucagon in human plasma is thought to be responsible for the adaptation of the body to the metabolic changes[11]. Antiinsulin injection must severely displace the insulin/glucagon ratio, so that plasma glucagon levels are increased, although reliable data on plasma glucagon concentrations are not yet available.

The above results (Table) show that when there is a steady increase in blood glucose levels probably due to a decrease in insulin/glucagon ratio, there is no significant change in blood cyclic AMP concentrations. These findings are in agreement with those of TURINSKY[4] who demonstrated the absence of the effect of fasting on plasma cyclic AMP levels and concluded that this was a result of a rapid balance between efflux and uptake of cyclic AMP in different tissues. Plasma adenosine 3′, 5′-monophosphate was assayed at different time intervals after an i.v. injection of antiinsulin serum. No significant change in adenosine 3′, 5′-monophosphate level was observed within 2 h after injection. A rapid dynamic equilibrium of influx and efflux between different tissue and plasma adenosine3′, 5′-monophosphate is postulated.

Résumé. On a dosé le 3′, 5′-monophosphate d'adénosine du plasma, à différents intervalles, après une injection i.v. de sérum antinsuline. On n'enregistra aucun changement important du taux de 3′, 5′-monophosphate d'adénosine pendant les 2 h suivantes. On en déduit qu'un équilibre dynamique rapide de l'influx et de l'efflux du 3′-5′-monophosphate d'adénosine s'établit entre les différents tissus et le plasma.

I. DAS[12,13]

Department of Biochemistry,
Imperial College of Science and Technology,
London, S.W.7 (England),
11 March 1974.

[1] G.A. ROBISON, R.W. BUTCHER and E.W. SUTHERLAND, in *Cyclic AMP* (Academic Press, New York and London 1971), p. 17.
[2] A.E. BROADUS, N.I. KAMINSKY, R.C. NORTHCUTT, J.G. HARDMAN, E.W. SUTHERLAND and G.W. LIDDLE, J. clin. Invest. *49*, 2237 (1970).
[3] A.E. BROADUS, N.I. KAMINSKY, J.G. HARDMAN, E.W. SUTHERLAND and G.W. LIDDLE, J. clin. Invest. *49*, 2222 (1970).
[4] J. TURINSKY, J. Proc. Soc. exp. Biol. Med. *142*, 1189 (1973).
[5] P. BARLING and A. BELOFF-CHAIN, Horm. Metab. Res. *5*, 154 (1973).
[6] V. MARKS and K. LLOYD, Proc. Ass. clin. Biochem. *2*, 176 (1963).
[7] I. DAS, Horm. Metab. Res. *5*, 330 (1973).
[8] B.L. BROWN, J.D.M. ALBANO, R.P. EKINS, A.M. SGHERZI and W. TAMPION, Biochem. J. *121*, 561 (1971).
[9] J.H. EXTON, S.B. LEWIS, R.J. HO, G.A. ROBISON and C.R. PARK, Ann. N.Y. Acad. Sci. *185*, 85 (1971).
[10] J. KUSTER, J. ZAPF and A. JAKOB, FEBS Lett. *32*, 73 (1973).
[11] R.H. UNGER, Diabetes *20*, 834 (1971).
[12] The author is most grateful for the support and suggestion of Prof. Sir ERNST CHAIN. The skilled technical assistance of HELGA WILLE is gratefully acknowledged.
[13] Present address: Department of Experimental Pathology, Section of Biochemistry, Cardiothoracic Institute, Brompton London, SW3 6 HP (England).