The effect of insulin on adenosine 3':5'-monophosphate and guanosine 3':5'-monophosphate concentrations in human plasma

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Summary

1. The action of insulin on plasma cyclic nucleotide concentrations in normal human subjects has been studied after intravenous injection, alone and in combination with glucagon.

2. After injection of insulin alone there was an initial small, though not significant, decrease in plasma cyclic AMP at 15 min followed by an increase to more than twice the initial concentration at 30 min. The increase was absent when hypoglycaemia was lessened by infusion of glucose after insulin injection.

3. Injection of insulin caused no significant change in plasma cyclic GMP concentration, whether or not glucose was infused after the hormone.

4. Glucagon (3–300 nmol, 10–1000 µg), caused a dose-dependent increase in plasma cyclic AMP concentration. The rise in plasma cyclic AMP produced by 3 or 30 nmol of glucagon was not significantly modified by simultaneous injection of insulin (44 nmol; 6 units).

Key words: cyclic AMP, cyclic GMP, glucagon, insulin, radioimmunoassay.

Introduction

Adenosine 3':5'-monophosphate (cyclic AMP) is widely accepted as the intracellular mediator of the hepatic effects of glucagon (Robison, Butcher & Sutherland, 1971). The intravenous administration of glucagon causes a rapid increase in the plasma concentration of cyclic AMP, which is thought to be caused by hepatic production of the nucleotide (Broadus, Kaminsky, Northcutt, Hardman, Sutherland & Liddle, 1970; Liljenquist, Bomboy, Lewis, Sinclair-Smith, Felts, Lacy, Crofford & Liddle, 1974; Strange & Mjos, 1975). Glucagon does not appear directly to influence the plasma concentration of guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Broadus et al., 1970).

Insulin antagonizes the effect of glucagon on the formation and release of cyclic AMP in perfused liver of animals in vitro (Park, Lewis & Exton, 1972; Kuster, Zapf & Jakob, 1973). It has been reported that insulin increases the content of cyclic GMP in various tissues in vitro (Illiano, Tell, Siegel & Cuatrecasas, 1973; Walaas, Walaas & Gronnerod, 1974). We have investigated whether such actions of insulin in vitro are reflected in changes in the concentration of cyclic nucleotides in human plasma after intravenous injection of insulin alone or in combination with glucagon.

Patients and methods

Subjects studied were from a group of fourteen healthy, non-obese, male volunteers, aged between 19 and 32 years. Basal blood samples were taken after an overnight fast. Insulin (44 nmol; 6 units) and/or glucagon (3, 30 or 300 nmol; 10, 100 or 1000 µg) were injected into an antecubital vein, and further samples were taken at 5, 15, 30 and 45 min. Paired tests, for
example, comparing the effect of glucagon with that of glucagon plus insulin, were carried out on the same subjects on consecutive days.

Blood samples for cyclic nucleotides were taken in sequestrene tubes and kept on ice before separation. The plasma was deproteinized with an equal volume of trichloroacetic acid (100 g/l), and the acid was removed by shaking with four portions (5 vol.) of water-saturated ether. Samples were stored at -20°C until assay. Cyclic AMP was measured directly by a highly specific radioimmunoassay (Siddle, Kane-Maguire & Campbell, 1973), after addition of CaCl₂ in excess of sequestrene present. Samples for cyclic GMP were absorbed on to 0.4 cm x 2 cm columns of AG-1 X2 resin, 200-400 mesh, chloride form (Bio-Rad Laboratories, Richmond, Calif., U.S.A.). The column was washed with 5 ml of water and 2.5 ml of HCl (50 mmol/l) and then cyclic GMP was eluted with 2.5 ml of HCl (500 mmol/l). The eluate was freeze-dried and redissolved in assay buffer to give fivefold concentration relative to the original plasma. Recovery of cyclic GMP in this procedure was always greater than 95%. Cyclic GMP was measured by radioimmunoassay, by use of antibody prepared by the methods of Steiner, Parker & Kipnis (1972). The assay incubations contained 0.4 pmol of cyclic [³H]GMP, 15-20 Ci/mmol (The Radiochemical Centre, Amersham, Bucks., U.K.), sufficient antibody to bind approximately 50% of the tracer, and standard or sample, in a final volume of 60 μl of potassium phosphate (100 mmol/l, pH 7.0). In other respects the assay was carried out as for cyclic AMP (Siddle et al., 1973). Each of two antibodies used showed high specificity for cyclic GMP (relative concentrations for 50% displacement of 0.4 pmol of tracer in assay were: cyclic GMP 1; cyclic AMP 20 or 4000; 5'-GMP and 5'-AMP > 10⁶), and in any case cyclic GMP was separated from cyclic AMP and other nucleotides by the column elution.

Blood glucose was measured by a standard glucose oxidase Auto-analyzer method (Boehringer Corp., London). Statistical significance of differences was assessed by Student's t-test or paired t-test, as appropriate.

**Results**

Mean fasting concentrations from all tests in the current study were: blood glucose 4.0±0.1 mmol/l (mean ± SEM, forty-four tests); plasma cyclic AMP 28±2 nmol/l (forty-four tests); plasma cyclic GMP 3.6±0.3 mmol/l (thirteen tests). Results throughout have been expressed as mean percentage changes relative to the appropriate fasting concentration in order to emphasize consistent patterns of change even where there were variations in initial concentrations.

The concentrations of both cyclic AMP and cyclic GMP in plasma were measured after the injection of insulin or after a control injection of sodium chloride solution (150 mmol/l; saline). Cyclic AMP concentration (Fig. 1) fell by 17% over 45 min in the control test (P<0.01). The reason for this fall is unknown. After insulin, cyclic AMP concentration fell by 16% at 15 min and although this was a greater fall than that observed 15 min after the saline infusion, the difference was not statistically significant (P>0.05). At 30 and 45 min after insulin injection, there was a highly significant rise (P<0.001) in plasma cyclic AMP concentration to more than twice the initial value. Cyclic GMP concentration (Fig. 1) was slightly elevated at all time points after insulin, but at no time was the difference statistically significant
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(P > 0.05) from the values observed after injection of saline.

In a separate test on three of the subjects (results not shown), injection of insulin was followed by an infusion of glucose [100 ml of 0.55 mol/l (10%) solution over 30 min], which lessened the hypoglycaemic effect of insulin and prevented hypoglycaemic symptoms. No effect of insulin on plasma cyclic nucleotide concentrations was observed under these conditions, and the rise in cyclic AMP at 30 and 45 min after insulin was prevented.

Injection of glucagon caused a dose-dependent rise in the concentration of plasma cyclic AMP. Glucagon doses used in the present work (3 or 30 nmol, Fig. 2) were submaximal relative to the dose of 300 nmol (1 mg) used in a previous study (Elkeles, Lazarus, Siddle & Campbell, 1975a), which produced a twenty- to forty-fold increase in plasma cyclic AMP. The increase in blood glucose produced after 300 nmol of glucagon was greater than that seen after 3 nmol, but not significantly different from that after 30 nmol of glucagon.

The effect of insulin on the glucagon-induced rise in plasma cyclic AMP was tested in subjects who received glucagon alone or glucagon and insulin simultaneously, on successive days. Insulin (44 nmol) had no significant effect on the concentration of cyclic AMP at 5 and 15 min produced by 3 or 30 nmol of glucagon (Fig. 2). The mean values (±SEM) for ratios of plasma cyclic AMP concentrations with insulin/without insulin, calculated from the paired values for each subject, were: 30 nmol of glucagon: 5 min, 0.91 ± 0.08; 15 min, 1.11 ± 0.22; 3 nmol of glucagon: 5 min, 0.94 ± 0.08; 15 min, 0.93 ± 0.04.

With 44 nmol of insulin plus 3 nmol of glucagon, there was a second rise in cyclic AMP at 30 min (Fig. 2) (P < 0.02 relative to glucagon alone at 30 min and relative to glucagon plus insulin at 15 min) similar to that occurring at this time with insulin alone (Fig. 1).

After injection of insulin with glucagon, there was a sharp fall in blood glucose concentration, with maximal hypoglycaemia at 30 min. However, this was preceded in every subject by a small rise in blood glucose at 5 min (Fig. 2).

**Discussion**

Measurements of changes in extracellular cyclic nucleotides have been used to investigate tissue responses to hormones both in vitro (Park et al., 1972; Kuster et al., 1973) and in vivo (Murad, 1973; Liljenquist et al., 1974; Elkeles et al., 1975a). The function of cyclic nucleotide release from tissues is unknown and the precise relationship to tissue nucleotide content uncertain. The present study was designed to investigate whether measurement of plasma cyclic nucleotides might provide a convenient index of tissue responsiveness to insulin in man.

Insulin has been reported to lower cyclic AMP content (Robison et al., 1971; Park et al., 1972) and raise cyclic GMP content (Illiano et al., 1973; Walaas et al., 1974) of various tissues in vitro. However, insulin had no significant effect on plasma cyclic AMP or cyclic GMP concentrations in the first 15 min after injection in man (Fig. 1). An increase in cyclic AMP which occurred 30 min after injection (Fig. 1) was prevented by glucose infusion and was probably caused by an increase in the secretion of glucagon (Ohneda, Aguilar-Parada, Eisentraut & Unger, 1969; Persson, Gyntelberg, Heding & Boss-Nielsen, 1971) or catecholamines (Young, Landsberg & Knopp, 1974; Christensen, Alberti & Brandsborg,
in response to the severe hypoglycaemia. A rise in plasma cyclic AMP during insulin-induced hypoglycaemia has also been demonstrated by Hamet, Lowder, Hardman & Liddle (1975) who presented evidence that this was secondary to β-adrenergic receptor stimulation. Broadus et al. (1970) found no significant changes in the urinary excretion of cyclic AMP and cyclic GMP after elevation of endogenous insulin secretion during glucose-tolerance tests, and reported a twofold increase in cyclic AMP and cyclic GMP after elevation of cyclic AMP and cyclic GMP after elevation of insulin/glucagon ratios used to demonstrate hepatic effects of insulin. We are grateful to Professor C. N. Hales and Professor R. F. Mahler for their encouragement. This work was supported by a grant to Professor Hales from the British Diabetic Association.

References


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