CONTROL OF GLUCONEOGENESIS BY ACETYL COA IN RATS TREATED WITH GLUCAGON AND ANTI-INSULIN SERUM *

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Glucagon has recently been shown to have a direct lipolytic action on liver (Bewsher and Ashmore, 1966). Increased hepatic lipolysis, plus enhanced availability of plasma fatty acids, causes an elevation of acetyl CoA and fatty acyl CoA levels (Williamson, Herczeg, Coles and Danish, 1966). Such elevated levels of acetyl CoA presumably account for the increased rate of ketogenesis observed with glucagon.

Acute effects of insulin deficiency, induced by administration of guinea pig anti-insulin serum (AIS) to rats, are similar to effects produced by glucagon: elevation of plasma glucose, free fatty acid, and ketone levels, plus an increased rate of gluconeogenesis (Wagle and Ashmore, 1963; Wagle and Ashmore, 1964; Tarrant, Mahler, and Ashmore, 1964). These findings have led to the suggestion that the early metabolic changes in experimental insulin deficiency may be induced by hormones with actions normally counterbalanced by insulin (Wright, 1965). Such hormones are glucagon, epinephrine, ACTH and corticosteroids (Mahler, Stafford, Tarrant and Ashmore, 1964; Jungas and Ball, 1963).

Data presented in this paper supports the concept that the lipolytic, ketogenic, and gluconeogenic effects of glucagon are revealed in the insulin-deficient rat. It is suggested that stimulation of gluconeogenesis, both with glucagon and with AIS, is secondary to enhanced lipolysis and is mediated through facilitation of pyruvic carboxylase by elevated hepatic levels of acetyl CoA.

Methods. Male, fed Holtzman rats weighing 200-220 g. were used. Acute

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insulin insufficiency was produced by injecting 2 ml. (equivalent to 6 units of beef-insulin) AIS into the tail vein (Wright, 1965). Crystalline glucagon (lot no. 258-234B-167-1, courtesy of Lilly Research Laboratories) was injected (1.25 mg./kg. body weight) at the same time as the AIS. Rats were anesthetized by a subcutaneous injection of sodium pentobarbital (60 mg./kg. body weight) which was given 30 min. before sacrifice. The livers were rapidly frozen *in situ* with tongs precooled in liquid N_2. The serum from each rat was assayed for unneutralized insulin antibody at the time of death to insure that the AIS was given in sufficient amount to neutralize the available insulin. Metabolic intermediates were measured as described elsewhere (Williamson, 1965; Williamson, *et al.*, 1966). Blood ketones were determined by the method of Mellanby and Williamson (1963), and free fatty acids by the method of Novák (1965).

Results. AIS produced a marked increase in plasma levels of glucose, FFA, and ketones over a 2-hour period. Glucagon added with AIS for 30 min. enhanced all changes, particularly free fatty acids and β-hydroxybutyrate (Table 1).

Table 1

PLASMA GLUCOSE, FFA AND KETONE LEVELS IN RATS TREATED WITH ANTI-INSULIN SERUM AND GLUCAGON

Results are the mean ± standard error of the mean, with 12 rats in the control group and six rats in each of the other groups.

Intermediate	Control	AIS 30 min.	AIS 2 hrs.	AIS plus Glucagon 30 min.
Glucose (mg. %)	155 ± 4	227 ± 16	274 ± 16	423 ± 11
FFA (mμEq./ml.)	285 ± 30	829 ± 115	821 ± 58	1363 ± 123
Acetoacetate (μM)	86 ± 11	116 ± 17	260 ± 27	279 ± 46
β-Hydroxybutyrate (μM)	221 ± 26	413 ± 60	506 ± 83	1176 ± 144

Elevated plasma levels of free fatty acids and ketones, produced by either AIS or AIS plus glucagon, were associated with diminished levels of

CoA and elevated levels of acetyl CoA and fatty acyl CoA (Fig. 1). Although glucagon produced no further increase of acetyl CoA above that found with AIS after 30 min., the levels of fatty acyl CoA were increased significantly.

Gluconeogenesis is facilitated by AIS (Wagle, et al., 1963; Exton, et al., 1966) and by glucagon (Struck, Ashmore, and Wieland, 1965; Garcia, et al., 1966; Exton and Park, 1966), and confirmed in our experiments by measuring the incorporation of ^{14}C-bicarbonate into plasma glucose. These studies showed a significant increase in the incorporation of counts into glucose after treatment of rats with AIS, which was further increased by glucagon.

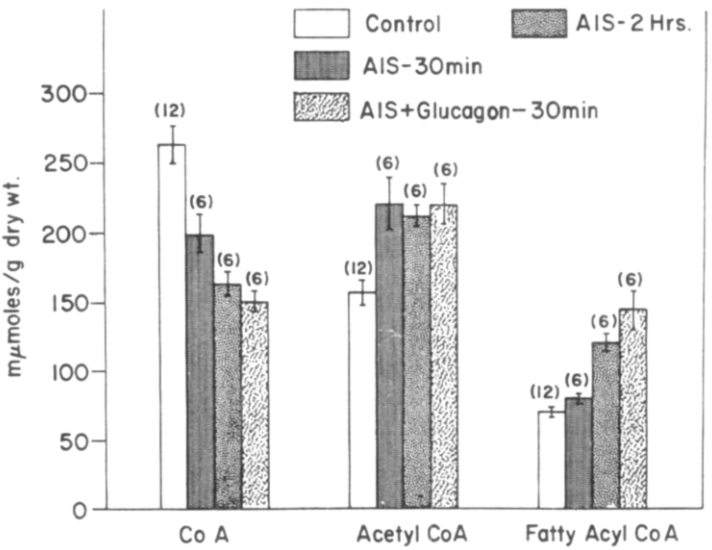

Fig. 1. Effects of anti-insulin serum (AIS) and AIS plus glucagon on the levels of CoA, acetyl CoA and acyl CoA in rat liver in vivo. Numbers in parentheses refer to the number of animals in each group. The vertical bars represent two standard errors of the mean.

To find out if possible activation of pyruvic carboxylase by acetyl CoA (Utter and Keech, 1963) could be detected in vivo, tissue levels of intermediates in the sequence from lactate to phosphoenolpyruvate (PEP) were measured in different groups of rat-livers (Table 2). Malate was included in the sequence since it is in equilibrium with oxalacetate, due to the high activity of malate dehydrogenase. The tissue levels of lactate, pyruvate, oxalacetate and PEP decreased after 30 min. treatment with AIS, while malate levels increased. After 2 hrs. treatment with AIS, levels of lactate and

pyruvate remained depressed relative to the control values, but malate levels showed a further increase and oxalacetate and PEP levels rose slightly above the control. When glucagon was given with AIS, levels of lactate, pyruvate and oxalacetate decreased relative to controls while levels of malate and PEP increased.

Table 2

EFFECTS OF AIS AND GLUCAGON ON METABOLIC INTERMEDIATES IN RAT LIVER IN VIVO

Results are the mean ± standard error of the mean, with 12 rats in the control group and 6 rats in each of the other groups.

Intermediate mμmoles/g. dry wt.	Control	AIS 30 min.	AIS 2 hrs.	AIS plus Glucagon 30 min.
Lactate	5860 ± 470	2427 ± 149*	3920 ± 670*	2830 ± 258*
Pyruvate	363 ± 30	98 ± 7*	175 ± 32 *	110 ± 12*
Oxalacetate	15.2 ± 0.9	11.2 ± 0.7*	15.6 ± 1.0	12.5 ± 1.0
Malate	1238 ± 81	1690 ± 145*	1948 ± 145*	2224 ± 217*
P-Enolpyruvate	320 ± 14	272 ± 12*	343 ± 41	504 ± 86*

*$P < 0.05$ relative to controls.

Discussion. Elevated levels of acetyl CoA have been found in livers of alloxan-diabetic rats (Wieland and Weiss, 1963), starved rats (Bortz and Lynen, 1963; Tubbs and Garland, 1964) and fed rats treated with glucagon (Williamson, et al., 1966). The present results extend these observations to the acutely insulin-deficient animal, in which maximum increases of acetyl CoA were obtained after 30 min. These altered hormonal and nutritional states are each associated with enhanced rates of ketogenesis and gluconeogenesis.

Increased hepatic levels of acetyl CoA and fatty acyl CoA are undoubtedly caused by an enhanced availability of fatty acids, due primarily to increased lipolysis in adipose tissue, as shown by the elevated plasma FFA levels. The sharp rise of fatty acyl CoA in the livers of rats treated with AIS and glucagon compared with AIS treatment alone suggests that it may be due to the direct action of glucagon on hepatic triglyceride lipase (Bewsher and Ashmore, 1966).

In studies with perfused rat livers supplied with 20 mM lactate as substrate, Exton, et al. (1966) observed changes in levels of intermediates of the gluconeogenic pathway with glucagon and cyclic 3',5'-AMP which were similar to those observed here, i.e., a decrease of lactate and pyruvate and an increase of malate and PEP. Therefore, Exton and collaborators inferred that an enzyme step (or steps) between pyruvate and PEP was stimulated by cyclic 3',5'-AMP.

Although the present results do not obviate the conclusions of Exton et al. (1966), we prefer a different interpretation. Rather than cyclic 3',5'-AMP affecting enzyme steps of the gluconeogenic sequence directly (for which there is no experimental evidence), we suggest that the effect is mediated through the facilitation of pyruvic carboxylase by the elevated levels of acetyl CoA (Utter and Keech, 1963).

One difficulty with this interpretation is that the tissue level of oxalacetate fails to rise as would be expected if its rate of formation increased. Malate levels, however, increased in each of the experimental groups. Contrary to the assumption made by Exton and Park (1966) that the redox potential of the NAD system remains constant when gluconeogenesis is stimulated by glucagon, the results of Table 2 show an increase in the ratios of both lactate/pyruvate and malate/oxalacetate with AIS and AIS plus glucagon treatments, indicating a more reduced state of the NAD system. This is brought about by enhanced oxidation of fatty acid in mitochondria (Williamson, Kreisberg and Felts, 1966). The observed fall of oxalacetate is, therefore, explained by the attempt of malate dehydrogenase to maintain equilibrium at a more negative pyridine nucleotide redox potential (see Hohorst and Reim, 1961). This results in an immediate fall of oxalacetate levels, despite a more rapid rate of formation, until malate rises sufficiently to support an elevated oxalacetate level at the new redox state of the NAD system. This effect is well illustrated by the increase of oxalacetate levels with duration of AIS treatment after an initial drop (Table 2).

Possible control at the PEP-carboxykinase step in neither confirmed nor disproved by the present observations. The finding of greatly-elevated PEP levels after AIS plus glucagon treatment may represent a facilitation of PEP formation from oxalacetate, but the observation can be explained equally well by an inhibition of pyruvic kinase. The latter explanation may represent the more likely possibility, since octanoic acid has been found to inhibit pyruvic kinase directly (G. Weber, personal communication).

REFERENCES

Bewsher, P. D. and Ashmore, J. Biochem. Biophys. Res. Communs. In Press.

Bortz, W. M. and Lynen, F. Biochem. Z. 339, 77 (1963).

Exton, J. H., Jefferson, L. S., Butcher, R. W. and Park, C. R. Amer. J. Med. 40, 709 (1966).

Exton, J. H. and Park, C. R. Pharmacol. Revs. 18, 181 (1966).

Garcia, A., Williamson, J. R. and Cahill, G. F. Diabetes 15, 188 (1966).

Hohorst, H. J. and Reim, M. Biochem. Biophys. Res. Communs. 4, 159 (1961).

Jungas, R. L. and Ball, E. G. Biochem. 2, 383 (1963).

Mahler, R., Stafford, W. S., Tarrant, M. E. and Ashmore, J. Diabetes 13, 297 (1964).

Mellanby, J. and Williamson, D. H. In H. Bergmeyer (editor). Methods of Enzymatic Analysis, New York: Academic Press, 1963, p. 454.

Novák, M. J. Lipid Res. 6, 431 (1965).

Struck, E., Ashmore, J. and Wieland, O. Biochem. Z. 343, 107 (1965).

Tarrant, M. E., Mahler, R. and Ashmore, J. J. biol. Chem. 239, 1714 (1964).

Tubbs, P. K. and Garland, P. B. Biochem. J. 93, 550 (1964).

Utter, M. F. and Keech, D. B. J. biol. Chem. 238, 2603 (1963).

Wagle, S. R. and Ashmore, J. J. biol. Chem. 238, 17 (1963).

Wagle, S. R. and Ashmore, J. J. biol. Chem. 239, 1289 (1964).

Wieland, O. and Weiss, L. Biochem. Biophys. Res. Communs. 10, 1333 (1963).

Williamson, J. R. J. biol. Chem. 240, 2308 (1965).

Williamson, J. R., Herczeg, B., Coles, H. and Danish, R. Biochem. Biophys. Res. Communs. In Press.

Wright, P. H. In B. S. Leibel and G. A. Wrenshall (editors). On the Nature and Treatment of Diabetes, New York: Excerpta Medica Foundation, 1965, p. 354.