Effect of Methylmalonic Acid on Gluconeogenesis in Isolated Rat and Guinea-Pig Hepatocytes

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Gluconeogenesis from pyruvate, alanine, lactate and propionate was inhibited by methylmalonate in both rat and guinea-pig hepatocytes. The effect was dose-dependent. Gluconeogenesis from glycerol and xylitol was not affected.

Methylmalonic acid accumulates in tissues and blood of patients with methylmalonyl-CoA mutase deficiency and vitamin B₁₂ deficiency (for reviews see Rosenberg, 1972; Rosenberg & Sriver, 1974). Metabolic ketoacidosis and hypoglycaemia are usual features of the disease methylmalonic aciduria, which results from the inability to metabolize methylmalonyl-CoA further (Rosenberg, 1972). Because methylmalonyl-CoA inhibits pyruvate carboxylase, a key enzyme in the gluconeogenic pathway, it has been suggested that the hypoglycaemia might result from a derangement in gluconeogenesis (Utter, 1970). In a study of propionate metabolism in vitamin B₁₂-deficient rats, Weidemann *et al.* (1970) found that methylmalonate inhibited gluconeogenesis from propionate. The effect cannot be attributed to inhibition of pyruvate carboxylase, since the conversion of propionate to glucose does not involve this enzyme. Studies with isolated rat liver mitochondria have shown that methylmalonic acid is a potent inhibitor of the dicarboxylate carrier system of the inner mitochondrial membrane (Halperin *et al.*, 1971). It also inhibits tricarboxylic acid exchange on the tricarboxylate transporter as well as the malate/phosphate exchange. On the basis of these studies, Halperin *et al.* (1971) hypothesized that methylmalonic acid might inhibit gluconeogenesis by interfering with the transport of metabolites across the mitochondrial membrane. The present study was undertaken to determine the effect of methylmalonate on glucose synthesis from a variety of substrates in isolated hepatocytes.

Materials and Methods

Chemicals and animals

Methylmalonic acid was purchased from Calbiochem., Los Angeles, CA, U.S.A. The sources of all other chemicals were given previously (Arinze & Rowley, 1975). Male guinea pigs (200–250 g) of the Hartley strain were purchased from the Williams Kentucky Cavies, Fern Creek, KY, U.S.A. Male Sprague-Dawley rats were purchased from Harland Industries, Indianapolis, IN, U.S.A. The

animals were fed on Purina laboratory guinea-pig or rat chow *ad libitum* and were given free access to water at all times. Food was withdrawn for 24 h (rats) or 46–50 h (guinea pigs) before all experiments.

Isolation of hepatocytes and incubation

The preparation of hepatocytes and incubation conditions were as described previously (Arinze & Rowley, 1975). Methylmalonic acid was dissolved in Krebs-Ringer bicarbonate buffer (Umbreit *et al.*, 1964), and the solution was adjusted to pH 7.4 before addition to the incubation medium. Glucose was measured enzymically by the method of Slein (1965).

Results and Discussion

Table 1 shows the effect of methylmalonate on gluconeogenesis from a variety of substrates in guinea-pig hepatocytes. At a concentration of 5 mM, methylmalonate significantly inhibited gluconeogenesis from lactate and pyruvate. At higher concentrations of methylmalonate, glucose synthesis from alanine and propionate was also inhibited. In general, gluconeogenesis from all four substrates was inhibited by about 30 and 45–60% at methylmalonate concentrations of 10 and 20 mM respectively. Gluconeogenesis from glycerol and xylitol was not affected at all concentrations of methylmalonate tested.

Table 2 shows that gluconeogenesis from pyruvate, lactate and alanine was also inhibited in rat hepatocytes by methylmalonate. The extent of inhibition was approximately the same in the two species, except that, with lactate as substrate, the effect was somewhat greater in rat than in guinea-pig hepatocytes. The difference, although small, may be related to the location of phosphoenolpyruvate carboxykinase (Hanson & Garber, 1972) in the liver of the two species. Unlike in rat liver, gluconeogenesis from lactate in guinea-pig liver involves the malate/aspartate shuttle, as well as direct transfer of phosphoenolpyruvate out of the mitochondria

Table 1. *Effect of methylmalonate on gluconeogenesis in guinea-pig hepatocytes*

Incubations were carried out for 50 min in duplicate. The concentration of the various substrates was 2 mM and methylmalonate was added to give the final concentrations indicated. Appropriate controls were included for all additions, and glucose formation in the absence of substrate was subtracted. Values are means $\pm$ S.E.M. for the numbers of experiments indicated in parentheses. Values that are statistically significant (Student's *t* test) compared with controls are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. Abbreviation: n.d., not determined.

Addition	Gluconeogenesis (nmol/min per g of cells) from:					
	Pyruvate (10)	Lactate (7)	Alanine (9)	Propionate (6)	Glycerol (6)	Xylitol (4)
None	425 $\pm$ 24	387 $\pm$ 24	219 $\pm$ 24	571 $\pm$ 45	427 $\pm$ 42	518 $\pm$ 49
Methylmalonate (2.5 mM)	367 $\pm$ 58	n.d.	170 $\pm$ 34	n.d.	n.d.	528 $\pm$ 56
Methylmalonate (5 mM)	346 $\pm$ 15**	309 $\pm$ 25**	177 $\pm$ 26	465 $\pm$ 55	435 $\pm$ 46	460 $\pm$ 60
Methylmalonate (10 mM)	305 $\pm$ 36**	263 $\pm$ 50**	149 $\pm$ 31	404 $\pm$ 49*	420 $\pm$ 36	676 $\pm$ 79
Methylmalonate (20 mM)	186 $\pm$ 32***	172 $\pm$ 49***	89 $\pm$ 23*	318 $\pm$ 38***	384 $\pm$ 39	n.d.

Table 2. *Effect of methylmalonate on gluconeogenesis in rat hepatocytes*

Incubations were carried out as in Table 1. Values are means $\pm$ S.E.M. for the numbers of experiments indicated in parentheses. Values that are statistically significant (Student's *t* test) compared with controls are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001

Addition	Gluconeogenesis (nmol/min per g of cells) from:			
	Pyruvate (6)	Lactate (4)	Alanine (5)	Glycerol (6)
None	417 $\pm$ 21	378 $\pm$ 10	232 $\pm$ 28	819 $\pm$ 72
Methylmalonate (10 mM)	295 $\pm$ 26**	207 $\pm$ 23***	143 $\pm$ 24*	886 $\pm$ 79
Methylmalonate (20 mM)	236 $\pm$ 27***	129 $\pm$ 16***	104 $\pm$ 19*	779 $\pm$ 81

(Arinze *et al.*, 1973). In hepatocytes prepared from both species glucose production from glycerol was not affected by additions of methylmalonate.

Since glucose production from glycerol and xylitol, which enter the gluconeogenic pathway above the level of triose phosphates, was not affected, the data in Tables 1 and 2 suggest that the site of inhibition in the gluconeogenic pathway lies below the level of the trioses. The effect observed is clearly dose-dependent. Halperin *et al.* (1971) have shown that methylmalonate interferes with di- and tri-carboxylate exchange systems across the mitochondrial membrane, as well as with the malate/phosphate exchange. The latter effect is similar to the inhibition of malate transport in mitochondria by *n*-butylmalonate (Robinson & Chappell, 1967), which is known to inhibit gluconeogenesis from pyruvate (Williamson *et al.*, 1970). By analogy, pentylmalonate, another malonate derivative that affects transport across the mitochondrial membrane (Robinson *et al.*, 1971), may also be expected to inhibit gluconeogenesis. Taken together these results suggest that methylmalonate inhibits gluconeogenesis by interfering with metabolite transport across the mitochondrial membrane.

The concentration at which methylmalonate inhibited gluconeogenesis in our experiments is

similar to that which was shown to inhibit malate and 2-oxoglutarate transport in isolated rat liver mitochondria (Halperin *et al.*, 1971). The lowest concentration (2.5 mM) of methylmalonate tested inhibited gluconeogenesis from pyruvate and alanine in guinea-pig liver cells by about 20%, but had a much greater effect on metabolite transport in mitochondria. In the studies of Weidemann *et al.* (1970) with rat kidney-cortex slices gluconeogenesis from propionate was inhibited by about 25% by 5 mM-methylmalonate. The concentration of methylmalonate (5–10 mM) that significantly inhibits gluconeogenesis in isolated hepatocytes or kidney-cortex slices is higher than the observed plasma concentrations (up to 3 mM) of methylmalonate in cases of methylmalonic aciduria (Oberholzer *et al.*, 1967; Morrow *et al.*, 1969; Rosenberg, 1972). However, in the present study and that of Weidemann *et al.* (1970) the uptake of methylmalonate from the incubation medium was not determined. There are only a few studies (see Morrow *et al.*, 1969) that directly assess the hepatic or renal concentrations of methylmalonate in this disease. Such studies are necessary to establish whether the hypoglycaemia associated with the disease is causally related to a possible inhibition of gluconeogenesis *in vivo* by methylmalonate.

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