Effect of 3-Mercaptopicolinic Acid on Gluconeogenesis and Gluconeogenic Metabolite Concentrations in the Isolated Perfused Rat Liver

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3-Mercaptopicolinic acid inhibited gluconeogenesis from lactate and alanine, but not dihydroxyacetone, in the perfused rat liver. Hepatic metabolite concentrations suggested that gluconeogenesis was inhibited at phosphoenolpyruvate carboxykinase. The compound is very effective at low concentrations, and seems an ideal agent for use in studying metabolic regulation involving gluconeogenesis and anaplerotic mechanisms.

Previous studies (DiTullio *et al.*, 1974) have demonstrated that 3-mercaptopicolinic acid (SKF-34288) produces hypoglycaemia in starving rats, mice and guinea pigs, as well as in alloxan-diabetic rats. In addition, it inhibited gluconeogenesis from lactate in rat kidney-cortex and liver slices, and from lactate, pyruvate and alanine, but not fructose, in perfused rat livers. The purpose of the present investigation was to determine the site(s) of interaction of 3-mercaptopicolinic acid on hepatic gluconeogenesis. The isolated perfused rat liver was used as a model, and hepatic gluconeogenic and tricarboxylic acid-cycle intermediates were measured.

Materials and methods

Animals, perfusion technique and determinations. Male Sprague–Dawley rats (180–220 g; Charles River Breeding Laboratory, Wilmington, Mass., U.S.A.) starved for 18 h were used for all experiments. Surgical isolation of the liver, the perfusion apparatus and technique, $^{14}\text{CO}_2$ collection, and metabolite determinations on perfusate and frozen liver samples have been described in detail elsewhere (Toews *et al.*, 1970; Goodman *et al.*, 1973). All substrates, cofactors and enzymes were products of Boehringer Mannheim Corp. (New York, N.Y., U.S.A.) or Sigma Chemical Co. (St. Louis, Mo., U.S.A.). [$\text{U-}^{14}\text{C}$]Alanine was from New England Nuclear (Boston, Mass., U.S.A.).

Substrate and 3-mercaptopicolinic acid additions. L-Alanine, lactate or dihydroxyacetone were used as substrates at a concentration of 10 mM. When alanine was used, a 1 M-alanine solution was infused at a rate of $10.3 \mu\text{mol/min}$. This rate of infusion was found to maintain the alanine concentration at $10 \text{ mM} \pm 10\%$. 3-Mercaptopicolinic acid (supplied by Dr. H. L. Saunders of the Smith, Kline and French Laboratories, Philadelphia, Pa., U.S.A.) was dissolved in 0.1 M-NaOH and added directly to the perfusate, initially or at 15 min to give the desired concentration (see the Results section). Controls received the same volume of the drug vehicle.

Results

Effect of 3-mercaptopicolinic acid on gluconeogenesis. At 1.0 mM, 3-mercaptopicolinic acid inhibited gluconeogenesis from lactate by 93% (control, 47; +drug, $4 \mu\text{mol/h}$ per g fresh wt.; $P < 0.01$), irrespective of whether the compound was added to the perfusion medium together with, or 15 min before or after, the addition of the substrate. 3-Mercaptopicolinic acid (added initially) at 0.1 and 1.0 mM inhibited gluconeogenesis from 10 mM-alanine by 61 and 84% respectively (control, 18; +0.1 mM-drug, 7; +1.0 mM-drug, $3 \mu\text{mol/h}$ per g fresh wt.; $P < 0.01$ versus control). This effect was not due to an inhibition of alanine utilization, since the concentration of alanine in the perfusate remained essentially constant in both the control and the drug group after the beginning of an alanine infusion. Glycogen content (before and after perfusion) in each liver did not significantly change. In the presence of 10 mM-dihydroxyacetone, 1.0 mM-3-mercaptopicolinic acid did not inhibit gluconeogenesis, suggesting that the compound does not inhibit gluconeogenesis by a non-specific toxic effect on the liver, and that its site(s) of action is prior to dihydroxyacetone phosphate.

Effect of 3-mercaptopicolinic acid on hepatic metabolite concentrations. To determine more specifically the site of inhibition of gluconeogenesis, hepatic metabolites were measured in the presence of alanine and 0.1 mM-mercaptopicolinic acid. The results are presented in Table 1. The concentrations of lactate, pyruvate, oxaloacetate and malate were elevated, whereas the concentrations of phosphoenolpyruvate, 2-phosphoglycerate, 3-phosphoglycerate, glycerophosphate, fructose 1,6-diphosphate and glucose 6-phosphate were decreased from control values. The slight increase in lactate and the decrease in glucose 6-phosphate, however, were not significant compared with the control group. This metabolite pattern suggests inhibition of gluconeogenesis at phosphoenolpyruvate carboxykinase (EC 4.1.1.32). In addi-

Table 1. *Effect of 3-mercaptopycolinic acid on rat liver metabolite concentrations*

Frozen-tissue biopsies were obtained after 60 min of perfusion in the presence of 10 mM-alanine and 10 mM-alanine plus 0.1 mM-3-mercaptopycolinic acid added at zero time. Each value represents the mean $\pm$ S.E.M. of six to nine determinations, and are expressed as nmol/g fresh wt. * and ** indicate significantly different from control at $P < 0.05$ and 0.01 respectively.

Metabolite	Metabolite concentration (nmol/g fresh wt. of tissue)	
	Control	+3-Mercaptopycolinic acid
Glucose 6-phosphate	41.5 $\pm$ 6.3	25.5 $\pm$ 4.2
Fructose 1,6-diphosphate	11.4 $\pm$ 1.2	4.5 $\pm$ 0.8**
3-Phosphoglycerate	809 $\pm$ 190	231 $\pm$ 16*
2-Phosphoglycerate	182 $\pm$ 18	121 $\pm$ 9*
Phosphoenolpyruvate	197 $\pm$ 37	58 $\pm$ 13**
Pyruvate	69 $\pm$ 20	146 $\pm$ 18*
Lactate	447 $\pm$ 42	483 $\pm$ 28
Acetyl-CoA	32.6 $\pm$ 3.7	13.2 $\pm$ 1.7**
Citrate	167 $\pm$ 36	362 $\pm$ 62*
2-Oxoglutarate	168 $\pm$ 19	270 $\pm$ 55
Malate	95 $\pm$ 12	176 $\pm$ 13**
Oxaloacetate	2.9 $\pm$ 0.1	10.3 $\pm$ 1.6**
3-Hydroxybutyrate	65 $\pm$ 13	80 $\pm$ 7
Acetoacetate	392 $\pm$ 10	344 $\pm$ 11
Alanine	15700 $\pm$ 750	15000 $\pm$ 995
Glutamate	6300 $\pm$ 345	6700 $\pm$ 234
Aspartate	786 $\pm$ 107	1608 $\pm$ 12**
Glycerol phosphate	250 $\pm$ 28	114 $\pm$ 9**
ATP	1903 $\pm$ 329	2293 $\pm$ 173
ADP	611 $\pm$ 70	570 $\pm$ 21
AMP	162 $\pm$ 6	184 $\pm$ 15

tion, 3-mercaptopycolinic acid increased citrate, 2-oxoglutarate and aspartate, and decreased acetyl-CoA. The compound did not alter hepatic O_2 consumption, adenine nucleotide concentrations or inhibit fatty acid oxidation, as indicated by rates of ketogenesis in the presence of 1.0 mM-linoleate (results not shown). Further, in the presence of linoleate, the compound caused a more reduced state of the mitochondrial compartment (increased β -hydroxybutyrate/acetoacetate ratio) than in the control plus linoleate; however, the mechanism by which this occurs requires further investigation.

Stoichiometry of alanine utilization. To determine the disposition of the carbon moieties of alanine into the products formed, the carbon compounds known to be derived from alanine were tabulated. It is assumed that alanine utilization was approximately equal to its rate of infusion in these studies, since alanine utilization was not affected by the compound, and that its concentration remained fairly constant throughout the perfusion period. This would result in a utilization rate of about 600 μ mol/h. In the control group, the amounts of alanine converted into glucose, lactate, pyruvate and $^{14}CO_2$ were 324, 108, 28 and 104 μ mol/h respectively, accounting for 94% of the alanine utilized. In the presence of 0.1 mM-3-mercaptopycolinic acid, alanine flux into glucose,

lactate, pyruvate and $^{14}CO_2$ was 140, 92, 103 and 126 μ mol/h respectively, accounting for only 77% of the alanine utilized. The fate of the remaining 23% is not known.

Discussion

The data from the present investigations indicate that 3-mercaptopycolinic acid markedly inhibits gluconeogenesis from lactate and alanine. This effect could be produced immediately when 1.0 mM-3-mercaptopycolinic acid was present, suggesting a rapid accumulation of the compound by the hepatocyte to its site of inhibition. When metabolite concentrations were measured in the presence of alanine and 3-mercaptopycolinic acid, a crossover point was established between oxaloacetate and phosphoenolpyruvate, suggesting that the site of inhibition of gluconeogenesis is located at phosphoenolpyruvate carboxykinase. Studies have also been conducted *in vitro* on partially purified preparations of phosphoenolpyruvate carboxykinase from rat liver, and it was demonstrated that 3-mercaptopycolinic acid inhibited both the conversion of phosphoenolpyruvate into oxaloacetate and the conversion of oxaloacetate into phosphoenolpyruvate (H. L. Saunders, personal communication). It is not known, however, how the compound interacts with the enzyme. In any event,

these studies strengthen the contention that 3-mercaptopycolinic acid is an inhibitor of phosphoenolpyruvate carboxykinase.

It is noteworthy that the structure of 3-mercaptopycolinic acid is somewhat similar to quinolinic acid, a metabolite of tryptophan. H. A. Lardy and his colleagues (Ray *et al.*, 1966; Veneziale *et al.*, 1967) have studied the effects of tryptophan and its metabolites on hepatic metabolism, and have found inhibition of gluconeogenesis from lactate and alanine. In their studies, the metabolite pattern in perfused rat liver was very similar to that reported in the present communication. Their studies have not been able to demonstrate a decrease in the activity of phosphoenolpyruvate carboxykinase when assayed in a high-speed supernatant fraction of rat liver after the treatment with tryptophan or one of its metabolites. However, Williamson *et al.* (1971) have demonstrated that quinolinic acid administration to starved rats inhibits hepatic phosphoenolpyruvate carboxykinase activity in the direction of oxaloacetate synthesis.

The question may be raised as to whether the modes of action of 3-mercaptopycolinic acid and quinolinic acid on gluconeogenesis are similar. 3-Mercaptopycolinic acid seems to be a more effective and potent inhibitor of gluconeogenesis than quinolinic acid. DiTullio *et al.* (1974) reported that, in the perfused rat liver, 2.3 mM-quinolinic acid inhibited gluconeogenesis from lactate by only 27%, whereas 0.1 mM-3-mercaptopycolinic acid inhibited gluconeogenesis from lactate by 60%. In addition, heterocyclic acids such as quinolinic acid and 3-mercaptopycolinic acid, having a carboxyl group in the α -position to the ring N atom, can form complexes with transition-metal ions (Bailar, 1956). It has been reported that certain bivalent transition-metal ions such as Mn^{2+} and Fe^{2+} activate phosphoenolpyruvate carboxykinase (Foster *et al.*, 1967). Veneziale *et al.* (1967) postulated that the inhibition of gluconeogenesis by quinolinic acid could be due to the compound chelating the metal ion necessary for the activity of phosphoenolpyruvate carboxykinase. Indeed, in their studies on the perfused liver, when stoichiometric amounts of

$MnCl_2$ were added to the perfusion fluid containing quinolinic acid, they reported that the inhibition of gluconeogenesis was overcome. However, in the present studies, 0.5 mM- $MnCl_2$ was unable to overcome the block imposed by 3-mercaptopycolinic acid on gluconeogenesis (results not shown).

Although the inhibition of phosphoenolpyruvate carboxykinase by 3-mercaptopycolinic acid seems to be primary and not secondary to other events, it does not preclude the possible involvement of other sites being affected either directly or after the block at phosphoenolpyruvate carboxykinase is established. In order to determine this, as well as the mode of action of 3-mercaptopycolinic acid, additional studies are warranted.

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