3-MERCAPTOPICOLINIC ACID, A PREFERENTIAL INHIBITOR OF THE CYTOSOLIC PHOSPHOENOLPYRUVATE CARBOXYKINASE

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1. Introduction

3-Mercaptopicolinic acid has been shown to inhibit gluconeogenesis in both the rat and the guinea-pig [1] in a similar fashion to quinolinic acid [2,3] an inhibitor which is thought to block gluconeogenesis at the level of phosphoenolpyruvate carboxykinase (GTP: oxaloacetate carboxylase (trans-phosphorylating) EC 4.1.1.32). Quinolinic acid appears to be a more potent inhibitor of gluconeogenesis in the rat than in the guinea-pig [3,4], since phosphoenolpyruvate carboxykinase (PEPCK) in the latter animal has a bimodal distribution the mitochondrial activity being inaccessible to inhibition by the mitochondrial non-penetrant quinolinic acid. In this communication we attempt to define the site of inhibition of mercaptopicolinic acid and to assess its inhibitory potency relative to quinolinate.

2. Materials and methods

2.1. *Preparation of liver fractions*

Samples of liver taken at sacrifice were blotted, weighed and homogenized in 10 vol of ice-cold buffer containing 0.25 M sucrose, 5 mM Tris–HCl and 0.1 mM EGTA, pH 7.4. The homogenate was centrifuged at 600 *g* for 10 min to remove cell debris and the supernatant then centrifuged at 12 000 *g* for 10 min to give fractions containing the mitochondrial pellet, and microsomes plus the soluble components of the cells. These fractions were used for the assay of phosphoenolpyruvate carboxykinase activity by the method of Roobol and Alleyne [5], mitochondria being solubilized by the addition of Triton X100 (0.2%) prior to assay of enzyme activity.

2.2. *Gluconeogenesis in kidney cortex*

Kidney cortex obtained from 24-h starved rats or guinea-pigs were rinsed in cold 0.9% (w/v) NaCl and slices were cut with a Stadie-Riggs microtome. The slices were rinsed for 5 min in Krebs-Henseleit buffer, pH 7.4 [6], and incubated for one hour under $O_2 + CO_2$ (95:5) at 37°C with 10 mM lactate, pyruvate, or L-malate. Following incubation the slices were blotted and dried for dry weight determination and the medium deproteinised by addition of perchloric acid. Glucose in the medium was determined enzymically by the method of Slein [7]. In separate experiments we showed that the kidney cortex glycogen content of between 0.2 and 0.5 mg/g wet weight in both rats and guinea-pigs did not decrease significantly during the course of the incubation and therefore did not contribute glucose to the medium via glycogenolysis.

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3. Results

3.1. *Inhibition of gluconeogenesis*

Since it has been shown [1] that mercaptopicolinate inhibits gluconeogenesis from pyruvate but not glycerol, the effect of 0.1 mM mercaptopicolinate on

Table 1
Inhibition of gluconeogenesis in rat and guinea-pig kidney cortex slices by mercaptopicolinate and quinolinate

	Rate of glucose production nmol/h/mg dry wt					
	Pyruvate		Lactate		L-malate	
Mercaptopicolinate (100 μM)	–	+	–	+	–	+
Guinea-Pig	31.8 ± 1.8	10.7 ± 1.1	27.3 ± 2.5	16.3 ± 1.3	78.3 ± 13.1	18.5 ± 2.9
Rat	66.4 ± 8.6	21.2 ± 5.3	36.0 ± 4.9	16.9 ± 1.6	98.7 ± 10.2	20.5 ± 4.1
Quinolinate (5 mM)	–	+	–	+	–	+
Guinea-Pig	35.4 ± 27.0	27.5 ± 4.1	22.2 ± 2.9	19.4 ± 2.1	72.4 ± 10.2	17.3 ± 2.4
Rat	87.3 ± 18.6	25.6 ± 2.7	40.7 ± 1.6	18.0 ± 1.4	103.5 ± 8.9	22.0 ± 3.8

Values expressed are the means of six observations ± S.E.M. The experiments were carried out as described in methods section at the inhibitor concentrations shown.

gluconeogenesis from pyruvate and L-malate in kidney cortex was tested. In both guinea-pig and rat inhibition of glucose production was evident from both pyruvate, lactate and L-malate. When 5 mM quinolinate was added to rat kidney cortex slices the inhibition of gluconeogenesis observed with lactate and pyruvate as substrates was of a similar order to that observed with 100 μM mercaptopicolinate. However, with guinea-pig kidney cortex slices inhibition by quinolinate was not nearly as marked as with mercaptopicolinate. At concentrations lower than 0.1 mM, mercaptopicolinate was inhibitory to gluconeogenesis from pyruvate in both guinea-pig and the rat to roughly equal extents (table 2).

3.2. *Inhibition of phosphoenolpyruvate carboxykinase (PEPCK)*

Since gluconeogenesis in kidney cortex was inhibited by mercaptopicolinate and quinolinate at a level which suggested a block at PEPCK and since quinolinate is a known inhibitor of PEPCK [2] a comparison of the kinetics of PEPCK inhibition was made for these two compounds. At different concentrations of oxaloacetate the effect of increasing concentrations of quinolinate were tested on the activity of PEPCK both in the supernatant fraction and the mitochondrial fraction of guinea-pig liver. Dixon plots of the activities obtained indicated that quinolinate inhibited non-competitively with respect

Table 2
Inhibition of gluconeogenesis by mercaptopicolinate from pyruvate in guinea-pigs and rat kidney cortex slices

	Inhibitor μM			
	0	10	25	100
Glucose Production		nmol/h/mg dry wt		
Guinea Pig	30.8 ± 4.5	23.5 ± 3.7 (24%)	12.3 ± 2.0 (59.8)	6.6 ± 0.6 (78.6)
Rat	75.2 ± 5.8	47.6 ± 4.2 (38%)	31.9 ± 4.2 (57.7)	21.4 ± 4.9 (71.5)

Values are the means of six observations ± S.E.M. The experiment was carried out as described in the methods section at a pyruvate concentration of 10 mM. The percentage inhibition is given in brackets for each concentration of inhibitor.

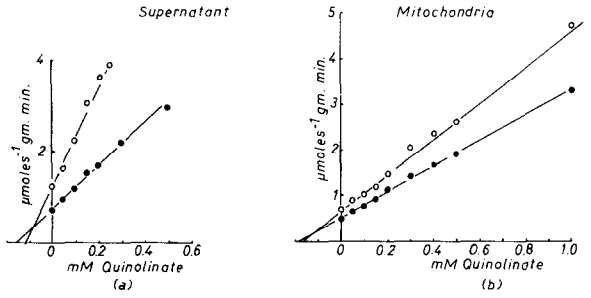

Fig.1. Reciprocal velocity against inhibitor concentration plots for quinolinate inhibition of (a) supernatant and (b) mitochondrial PEPCK. Experiments were conducted using increasing concentrations of quinolinate at oxaloacetate concentrations of (a) 17.6 μM (● – ●) and 9.6 μM (○---- ○), and (b) 19.2 μM (● – ●) and 10.7 μM (○—○). ITP was present at 5 mM and Mn^{++} at 1 mM temperature of 37°C. The velocity of the PEPCK reaction was measured in the direction of PEP synthesis as described in the methods section.

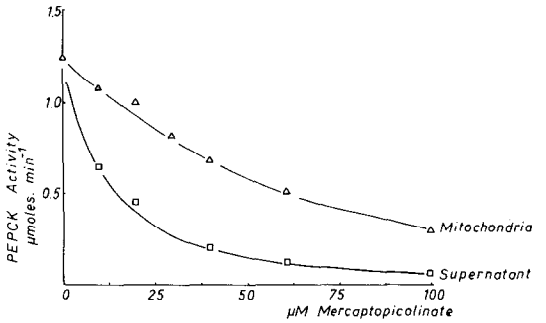

Fig.3. The velocity of PEPCK activity as a function of mercaptopicolinate concentration for mitochondrial (△ – △) and supernatant (□ – □) fractions of guinea-pig liver adjusted to the same initial velocity of reaction in the absence of inhibitor. The oxaloacetate concentration was 19.2 μM, ITP 5 mM, and Mn^{++} was 1 mM at a temperature of 37°C. The velocity of the PEPCK reaction was measured as described in the methods section.

to oxaloacetate in both fractions, the K_i 0.13 mM for the supernatant fraction and 0.17 mM for the mitochondrial fraction (fig.1).

When these activities were monitored as a function of mercaptopicolinate concentration, plots again indicated non-competitive inhibition of PEPCK with respect to oxaloacetate with both mitochondrial and supernatant enzymes (fig.2). However, the K_i values obtained were much lower than with quinolinate and markedly different in supernatant and mitochondrial fractions being 5 μM for the former and 56 μM for the latter activity. The activities of the mitochondrial and supernatant PEPCK activities were adjusted to the same level in the absence of inhibitor at the same oxaloacetate concentration and the activity monitored as a function of increasing mercaptopicolinate concentration (fig.3). Much greater inhibition was obtained at any one concentration of added inhibitor with the supernatant enzyme. Since mitochondria were solubilised with Triton X100 before assay of PEPCK, Triton X100 (0.2%) was added to the supernatant enzyme to rule out any possibility of an effect of Triton on the observed K_i. The rates of reaction in the presence of the Triton X100 were identical with those in the absence of detergent and the K_i was again 5 μM. When the activity of PEPCK was monitored at two different concentrations of Mn^{++} as a function of mercaptopicolinate concentration, Dixon plots again indicated non-competitive inhibition, the ten-fold difference in K_i again being evident between mitochondrial and supernatant enzymes. Increasing the concentration Mn^{++} did not appear to 'protect' against mercaptopicolinate inhibition as was observed with quinolinate inhibiton [2]. Non-competitive kinetics were also obtained with respect to ITP.

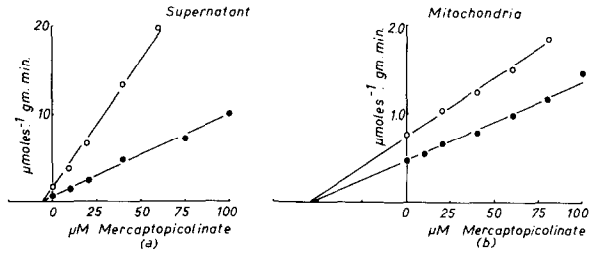

Fig.2. Reciprocal velocity against inhibitor concentration plots for 3-mercaptopicolinate inhibition of (a) supernatant and (b) mitochondrial PEPCK. Experiments were conducted using increasing concentrations of mercaptopicolinate at oxaloacetate concentration of (a) 17.6 μM (● – ●) and 9.6 μM (○ – ○) and (b) 19.2 μM (● – ●) and 10.7 μM (○ – ○). ITP was present at 5 mM and Mn^{++} at 1 mM at a temperature of 37°C. The velocity of the PEPCK reaction was measured in the direction of PEP synthesis as described in the methods section.

3.3. *Permeability of mitochondria to 3-mercaptopicolinate*

When guinea-pig liver or kidney mitochondria were suspended in a solution of 150 mM ammonium mercaptopicolinate pH 7.4 at 30°C and optical density changes at 620 nm monitored [8], they were observed to swell rapidly at a rate equivalent to one third of that observed with 100 mM ammonium phosphate and twice that observed with 150 mM ammonium thiocynate. The swelling of the mitochondria in mercaptopicolinate, unlike that in phosphate, was not inhibited by mersalyl. Since both phosphate and thiocyanate are mitochondrial penetrants, on this basis we classify mercaptopicolinate as a mitochondrial penetrant.

4. Discussion

Mercaptopicolinate is an inhibitor of PEPCK at low concentrations and seems to be more selective for the cytosolic enzyme than the mitochondrial enzyme. It resembles quinolinate in its inhibitory behaviour except that significant inhibition occurs at much lower concentrations. The effect of this inhibition on gluconeogenesis, however, differs from quinolinate in that quinolinate is a poor inhibitor of gluconeogenesis in guinea-pig where PEPCK distribution is bimodal [4,9] compared with the rat where PEPCK is almost entirely cytosolic. This lack of inhibition of gluconeogenesis in the guinea-pig which has been described previously [3,4] was attributed to the fact that quinolinate as a mitochondrial nonpenetrant would be unable to inhibit mitochondrial PEPCK and thus allow mitochondrial generation of PEP for the gluconeogenic pathway. Mercaptopicolinate, being a monocarboxylate anion and thus a mitochondrial penetrant can inhibit both mitochondrial and cytosolic PEPCK and thus block gluconeogenesis in the guinea-pig very effectively. However, the difference in the K_i for mercaptopicolinate between mitochondrial and supernatant enzymes would suggest that at low concentrations of the inhibitor, the mitochondrial enzyme would still be able to provide PEP for gluconeogenesis in conditions where the cytosolic enzyme was inhibited. We were unable to demonstrate any such effect in vitro (table 3) by testing low concentration of mercaptopicolinite on gluconeogenesis in rat and guinea-pig kidney cortex. Despite the fact that the PEPCK in the two cellular compartments are different proteins immunologically [10], the kinetic behaviour of the two species is very similar [11] with respect to all substrates, except for perhaps Mg^{++}. It is interesting therefore that mercaptopicolinate is able to show such specificity with respect to the cytosolic enzyme. Since in vitro the inhibitor appears to be more specific for the cytosolic enzyme, it is surprising that at low concentrations gluconeogenesis in the rat is inhibited not much more than in the guinea-pig since the enzyme in rat is predominantly cytosolic.

Acknowledgements

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