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Effects of 2-mercaptoacetate in isolated liver mitochondria *in vitro*

Competitive inhibition of 3-hydroxybutyrate dehydrogenase and depression of the β-oxidation pathway

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The effects of 2-mercaptoacetate on the respiration rates induced by different substrates were studied *in vitro* in isolated liver mitochondria. With palmitoyl-L-carnitine or 2-oxoglutarate as the substrate, the ADP-stimulated respiration (State 3) was dose-dependently inhibited by 2-mercaptoacetate. With glutamate or succinate as the substrate, State-3 respiration was only slightly inhibited by 2-mercaptoacetate. In contrast, the oxidation rate of 3-hydroxybutyrate was competitively inhibited by 2-mercaptoacetate in both isolated mitochondria and submitochondrial particles. In uncoupled mitochondria and in mitochondria in which ATP- and GTP-dependent acyl-CoA biosynthesis was inhibited, the inhibitory effect of 2-mercaptoacetate on palmitoyl-L-carnitine oxidation was abolished; under the same conditions, however, inhibition of 3-hydroxybutyrate oxidation by 2-mercaptoacetate still persisted. These results led to the following conclusions: 2-mercaptoacetate itself enters the mitochondrial matrix, inhibits fatty acid oxidation through a mechanism requiring an energy-dependent activation of 2-mercaptoacetate and itself inhibits 3-hydroxybutyrate oxidation through a competitive inhibition of the membrane-bound 3-hydroxybutyrate dehydrogenase. This study also strongly suggests that the compound responsible for the inhibition of fatty acid oxidation is 2-mercaptoacetyl-CoA.

In studies from this laboratory (Sabourault *et al.*, 1979; Bauché *et al.*, 1981), we have investigated the mechanism through which the administration of 2-mercaptoacetate, a potent fatty-liver-inducing drug (Sabourault *et al.*, 1976), inhibits fatty acid oxidation in the liver (Sabourault *et al.*, 1979). Using liver mitochondria isolated from 2-mercaptoacetate-treated rats, we found that this inhibition was not due to impaired acyl-CoA transfer into the mitochondria, impaired respiratory-chain and citrate-cycle activities or impaired coupling of oxidation to phosphorylation, but was linked to an inhibition of long-chain fatty acyl-CoA dehydrogenase activity (Bauché *et al.*, 1981).

Since these results were obtained after treatment, it was decided to determine whether or not 2-mercaptoacetate itself is able to induce *in vitro* the same effects in isolated mitochondria from normal livers. This was the purpose of the present study, in which the effects of 2-mercaptoacetate on the respiration of normal rat liver mitochondria oxidizing CoA- and flavin-linked (palmitoyl-L-carnitine, 2-oxoglutarate), nicotinamide-nucleotide-linked (3-hydroxybutyrate, glutamate and malate) and flavin-linked (succinate) substrates were studied.

The results presented here clearly show that (1) 2-mercaptoacetate itself is able to enter the mitochondrial matrix, (2) exposure of mitochondria to 2-mercaptoacetate results in a marked inhibition of the oxidation of fatty acids and 3-hydroxybutyrate, (3) inhibition of fatty acid oxidation requires the activation of 2-mercaptoacetate through an energy-dependent process, (4) inhibition of 3-hydroxybutyrate oxidation is due to a competitive inhibition of 3-hydroxybutyrate dehydrogenase by 2-mercaptoacetate itself. This study also suggests that the most likely substrate responsible for the inhibitory effect of 2-mercaptoacetate on fatty acid oxidation is 2-mercaptoacetyl-CoA.

Materials and methods

Mitochondrial isolation

Overnight-starved female Wistar rats (200 ± 20 g) were killed by decapitation, and liver mitochondria were prepared by the method of Beattie (1968). The

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fluffy layer was discarded, and the mitochondrial pellet was washed three times by suspension and centrifugation in 0.25 M-sucrose at 4°C and was finally suspended in the same medium at 4°C at a protein concentration of 40–60 mg/ml. Protein was determined as described by Lowry *et al.* (1951).

Submitochondrial-particle preparation

This was done by the procedure described by Thayer & Rubin (1979). In this procedure, mitoplasts were first prepared from isolated mitochondria, after which the washed mitoplasts were suspended in medium A, which contained 10 mM-sodium Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.1), 1 mM-$MgCl_2$, 1 mM-sodium ATP, 5 mM-sodium succinate, 0.2 mM-NADH and bovine serum albumin (1 mg/ml) in a final volume of 2 ml. Additional 0.1 M-$MgCl_2$ was then added to give a final concentration of 12 mM. The suspension was sonicated for 4 × 15 s at 0°C with a Branson Sonifier (model 185) equipped with a microprobe operated at output setting no. 4. After sonication, the mixture was diluted to 8 ml with medium A and centrifuged at 20000 ***g*** for 10 min. The supernatant was removed and centrifuged at 150000 ***g*** for 1 h. The dark reddish-brown pellet was resuspended in 0.25 M-sucrose at a protein concentration of approx. 30 mg/ml. The yield was about 10 mg of submitochondrial-particle protein/100 mg of starting mitochondrial protein. Freshly prepared submitochondrial particles or particles stored at −20°C for 1 week were both used, since respiratory control, phosphorylation capacity and other energy-linked activities were maintained under these storage conditions.

Respiration

The rate of mitochondrial respiration was measured as previously described (Bauché *et al.*, 1981) with a Gilson oxygraph at 25°C by using a Clark oxygen electrode. The reaction medium (pH 7.4) consisted of 58 mM-KCl, 25 mM-NaCl, 6 mM-$MgCl_2$, 13 mM-K_2HPO_4, 3 mM-KH_2PO_4 and mitochondria equivalent to 2.5 mg of protein (final volume 1.6 ml). Unless otherwise stated, various concentrations of 2-mercaptoacetate were added 30 s before the addition of the respiratory substrates. Final concentrations of added respiratory substrates were: sodium DL-3-hydroxybutyrate, 14 mM; sodium succinate, 6 mM; sodium 2-oxoglutarate, 10 mM; sodium L-glutamate, 5 mM; sodium L-malate, 5 mM; palmitoyl-L-carnitine, 25 μM; octanoyl-DL-carnitine, 50 μM. In some experiments, NaF (12 mM) or 2,4-dinitrophenol (50 μM) was also added to the incubation medium. In the latter experiments, mitochondria were first preincubated for 3 min with 2,4-dinitrophenol, after which 2-mercaptoacetate was added to the incubation medium, and 30 s later respiration was initiated.

In all experiments, respiration was initiated by the addition of the substrates (State 4), after which 150 μM-ADP was added to induce an immediate increase in the rate of oxygen utilization (State 3; Chance & Williams, 1955). State-3 and -4 activities were determined by calculating the nmol of oxygen consumed/min per mg of mitochondrial protein added.

Measurement of 3-hydroxybutyrate dehydrogenase activity

This was done in both intact mitochondria and submitochondrial particles. In intact mitochondria, this activity was measured by determining the rate of respiration (State 3) induced by various concentrations of DL-3-hydroxybutyrate (1–25 mM) in the presence or absence of various concentrations of 2-mercaptoacetate (50–500 μM), as described above. In submitochondrial particles (0.5 mg of protein per assay), 3-hydroxybutyrate dehydrogenase activity was measured either polarographically or spectrophotometrically. In the former method, this enzyme activity was estimated by measuring the rate of respiration induced by 1 mM-NAD^+ and various concentrations of DL-3-hydroxybutyrate (2.5–14 mM), in the presence or absence of 100 μM-2,4-dinitrophenol. In these experiments, various concentrations of 2-mercaptoacetate (50–200 μM) were added 30 s before initiation of respiration. When 3-hydroxybutyrate dehydrogenase activity was measured spectrophotometrically, submitochondrial particles were incubated for 30 s at 25°C in a buffer (pH 7.4) consisting of 24 mM-glycylglycine, 8 mM-$MgCl_2$, 50 mM-KCl, 87 mM-sucrose, 2 μM-rotenone, with various concentrations of DL-3-hydroxybutyrate (1.25–10 mM) in the absence or presence of 25–100 μM-2-mercaptoacetate (final volume 2.0 ml). The reaction was started by addition of 1 mM-NAD^+, and the formation of NADH occurring during the first 30 s was determined by the increase in A_{340}. Specific activity of 3-hydroxybutyrate dehydrogenase is expressed as μmol of NAD^+ reduced/min per mg of submitochondrial protein (molar absorption coefficient 6.22 $mM^{-1} \cdot cm^{-1}$). The apparent K_i values were calculated from Dixon plots.

Statistical analysis

Statistical significance of the data was analysed by Student's *t* test.

Materials

The sources of the chemicals used in this study have been previously described (Bauché *et al.*, 1981).

Results

Table 1 shows the effects of 2-mercaptoacetate on the hepatic mitochondrial oxidation of palmitoyl-L-carnitine and 2-oxoglutarate. Irrespective of the substrate used, State-4 oxygen consumption was unaffected after 30s preincubation of mitochondria with 2-mercaptoacetate (50–1000μM). By contrast, the ADP-stimulated respiration (State 3) with palmitoyl-L-carnitine was dose-dependently inhibited by 2-mercaptoacetate (22, 31, 47 or 59% inhibition with 50, 100, 500 and 1000μM-2-mercaptoacetate respectively). With 2-oxoglutarate as the substrate, the State-3 activity was not significantly affected by 50 or 100μM-2-mercaptoacetate, whereas with 500 and 1000μM, 35 and 56% inhibition respectively were observed. These data indicate that 2-mercaptoacetate itself enters the mitochondrial matrix and inhibits *in vitro* the palmitoyl-L-carnitine oxidation, an effect that was previously observed *in vivo* (Bauché *et al.*, 1981) and which is very rapid in onset, since maximal inhibition was already obtained after 30s preincubation with 2-mercaptoacetate (results not shown). Moreover, comparison of the concentrations of 2-mercaptoacetate eliciting inhibition of β-oxidation *in vivo* (40μmol/100g body wt.) and *in vitro* (50–1000μM) suggests that the inhibition of acyl-CoA dehydrogenase found in washed mitochondria after administration of 2-mercaptoacetate (Bauché *et al.*, 1981) is probably irreversible.

The fact that 2-mercaptoacetate inhibits only the State-3 activity suggests that this effect could result from an inhibition of the adenine nucleotide translocase or the coupling of oxidation to phosphorylation. To test these hypotheses, we studied the effects of various concentrations of 2-mercaptoacetate on the hepatic mitochondrial oxidation of different substrates: malate plus glutamate; DL-3-hydroxybutyrate (nicotinamide-nucleotide-linked oxidations) and succinate (flavin-linked oxidation).

As with the substrates used in the experiments in Table 1, Table 2 shows that 2-mercaptoacetate failed to affect the State-4 activity when glutamate plus malate or DL-3-hydroxybutyrate were used as the substrates. However, in the presence of succinate, 2-mercaptoacetate produced a slight concentration-dependent stimulation of State-4 respiration. Concerning State-3 activity, distinct actions of 2-mercaptoacetate were found depending on the respiratory substrate used. For example, 500μM-2-mercaptoacetate produced only a slight inhibition of oxygen consumption when glutamate plus malate (14% inhibition) or succinate (19% inhibition) were used as oxidizable substrates. Contrasting with these data, 500μM-2-mercaptoacetate induced a more pronounced inhibition (47%) when DL-3-hydroxybutyrate, another nicotinamide-nucleotide-dependent substrate, was used. Because of the slight inhibition of glutamate respiration by 2-mercaptoacetate, these results led us to conclude that this compound has probably little or no effect on the activities of the respiratory chain, of the adenine nucleotide translocase, and of the coupling of oxidation to phosphorylation. In addition, the strong inhibition of DL-3-hydroxybutyrate-stimulated respiration by 2-mercaptoacetate suggests that this compound may inhibit either the activity of 3-hydroxybutyrate dehydrogenase or the transport of DL-3-hydroxybutyrate across the mitochondrial membrane.

Table 1. *Effects of 2-mercaptoacetate on the oxidation of various CoA-dependent substrates in coupled rat liver mitochondria*

The reaction chamber of the oxygen electrode contained 58mM-KCl, 25mM-NaCl, 6mM-$MgCl_2$, 13mM-K_2HPO_4, 3mM-KH_2PO_4 (pH 7.4) and mitochondria equivalent to 2.5mg of protein (final volume 1.6ml). Respiration was initiated with 25μM-palmitoyl-L-carnitine or 10mM-2-oxoglutarate. When present, 2-mercaptoacetate was added 30s before the addition of the respiratory substrates. Each value is the mean ± S.E.M. for the number of determinations in parentheses.

2-Mercaptoacetate (μM)	O_2 consumption (nmol/min per mg of protein)			
	Palmitoyl-L-carnitine		Sodium 2-oxoglutarate	
	State 4	State 3	State 4	State 3
0 (Control)	10.0 ± 1.1 (6)	27.9 ± 2.0 (6)	6.0 ± 1.0 (5)	25.4 ± 2.2 (5)
50	10.4 ± 1.5 (6)	21.7 ± 2.2 (6)	5.5 ± 0.2 (3)	23.4 ± 2.8 (3)
	$P > 0.05$	$0.001 < P < 0.01$	$P > 0.05$	$P > 0.05$
100	10.4 ± 1.7 (6)	19.4 ± 1.4 (6)	6.1 ± 0.6 (4)	22.5 ± 1.7 (4)
	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P > 0.05$
500	10.7 ± 2.2 (5)	14.7 ± 2.2 (5)	6.7 ± 1.0 (4)	16.4 ± 2.6 (4)
	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P < 0.001$
1000	10.0 ± 0.8 (6)	13.3 ± 2.6 (6)	7.1 ± 1.4 (5)	11.2 ± 1.7 (5)
	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P < 0.001$

Table 2. *Effects of 2-mercaptoacetate on the oxidation of various substrates in coupled rat liver mitochondria*
Experimental conditions and statistical analysis are as described in Table 1. Final concentrations of added respiratory substrates were: sodium L-glutamate, 5 mM; sodium L-malate, 5 mM; sodium DL-3-hydroxybutyrate, 14 mM; sodium succinate, 6 mM.

	O_2 consumption (nmol/min per mg of protein)					
	Sodium L-glutamate + sodium L-malate		Sodium DL-3-hydroxybutyrate		Sodium succinate	
2-Mercaptoacetate (μM)	State 4	State 3	State 4	State 3	State 4	State 3
0 (Control)	7.5 ± 1.2 (6)	44.7 ± 1.8 (6)	7.5 ± 0.7 (6)	23.7 ± 1.1 (6)	11.7 ± 1.1 (6)	57.0 ± 4.3 (6)
50	7.9 ± 1.3 (5)	43.8 ± 1.5 (5)	6.9 ± 0.3 (4)	20.0 ± 1.4 (4)	12.6 ± 1.4 (3)	55.5 ± 4.7 (3)
	$P > 0.05$	$P > 0.05$	$P > 0.05$	$0.001 < P < 0.01$	$P > 0.05$	$P > 0.05$
100	7.0 ± 0.9 (4)	41.5 ± 1.5 (4)	6.9 ± 0.4 (6)	18.4 ± 1.8 (6)	13.5 ± 1.0 (6)	56.8 ± 4.1 (6)
	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.001$	$0.02 < P < 0.05$	$P > 0.05$
500	9.0 ± 0.9 (5)	38.3 ± 2.3 (5)	7.3 ± 0.8 (6)	12.5 ± 1.3 (6)	14.8 ± 1.1 (6)	46.4 ± 2.7 (6)
	$P > 0.05$	$0.001 < P < 0.01$	$P > 0.05$	$P < 0.001$	$0.001 < P < 0.01$	$0.001 < P < 0.01$

Table 3. *Inhibition of DL-3-hydroxybutyrate oxidation by 2-mercaptoacetate in rat liver mitochondria and submitochondrial particles*
DL-3-Hydroxybutyrate oxidation was measured in the standard medium as described in the Materials and methods section in intact rat liver mitochondria (2.5 mg of protein) or in rat liver submitochondrial particles (0.5 mg of protein). The rate of respiration induced by various concentrations of DL-3-hydroxybutyrate (1–25 mM with mitochondria and 2.5–14 mM with submitochondrial particles) was determined in the absence or in the presence of 2-mercaptoacetate. The concentration ranges used were 50–500 μM- and 50–200 μM-2-mercaptoacetate with mitochondria and submitochondrial particles respectively. Each K_m and $V_{max.}$ value is the mean ± S.E.M. of the results from four separate experiments calculated by least-squares regression of the Michaelis–Menten equation. The K_i values were calculated from Dixon plots.

	Mitochondria		Submitochondrial particles	
	Control	+2-Mercaptoacetate	Control	+2-Mercaptoacetate
K_m (mM)	1.34 ± 0.35		1.41 ± 0.10	
K_i (mM)		0.029 ± 0.005		0.038 ± 0.007
$V_{max.}$ (nmol of O_2 consumed/min per mg of protein)	27.2 ± 2.3	27.6 ± 2.8 $P > 0.05$	132.2 ± 10.7	127.0 ± 21.7 $P > 0.05$

To test these hypotheses, we have first studied, in intact mitochondria, the inhibition of DL-3-hydroxybutyrate-stimulated respiration as a function of 2-mercaptoacetate concentration (50–500 μM). The data in Table 3, showing no significant difference between the $V_{max.}$ values obtained in the absence and in the presence of 2-mercaptoacetate, suggest that, under these conditions, 2-mercaptoacetate may be a potent competitive inhibitor of the membrane-bound enzymic oxidation of 3-hydroxybutyrate ($K_i = 0.029 \pm 0.005$ mM, $n = 4$). To eliminate the possible interference of 2-mercaptoacetate with the transport system of 3-hydroxybutyrate across the mitochondrial membrane, similar experiments were next repeated in submitochondrial particles. Here again, 2-mercaptoacetate induced a competitive inhibition of 3-hydroxybutyrate oxidation, and the K_i value calculated from these experiments (0.038 ± 0.007 mM, $n = 4$) was not statistically different from the K_i value found in intact mitochondria (Table 3).

To ensure that the results obtained in the above experiments could not be due to any interference of 2-mercaptoacetate with the respiratory-chain activity, we have more specifically investigated the influence of 2-mercaptoacetate on 3-hydroxybutyrate dehydrogenase activity in submitochondrial particles. These experiments, in which the enzymic activity was determined by measuring spectrophotometrically the kinetics of NAD^+ reduction, provided the evidence that 2-mercaptoacetate is indeed a competitive inhibitor of 3-hydroxybutyrate dehydrogenase with respect to 3-hydroxybutyrate ($K_i = 0.021 \pm 0.002$ mM, $n = 3$) (Fig. 1).

An important question is to determine whether 2-mercaptoacetate itself or one of its metabolites is responsible for the inhibition of fatty acid oxidation described above. By analogy with pent-4-enoic acid

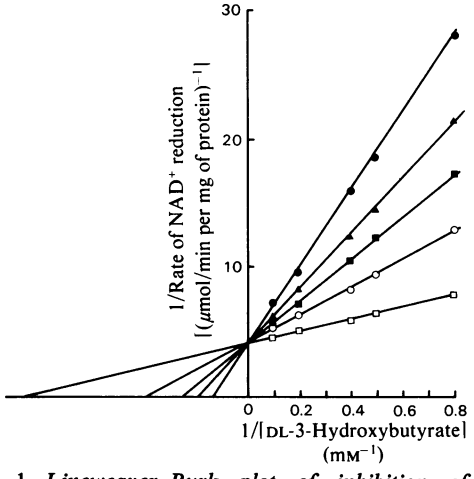

Fig. 1. *Lineweaver–Burk plot of inhibition of 3-hydroxybutyrate dehydrogenase by 2-mercaptoacetate in rat liver submitochondrial particles*

3-Hydroxybutyrate dehydrogenase activity was determined by NAD^+-linked spectrophotometric assays in submitochondrial particles (0.5 mg of protein per assay) as described in the Materials and methods section in the absence (□) and in the presence of 2-mercaptoacetate (final concns.: ○, 25 μM; ■, 50 μM; ▲, 75 μM; ●, 100 μM). The data shown are representative of one typical experiment. The K_m, K_i for 2-mercaptoacetate and $V_{max.}$ values (±S.E.M.) shown below were calculated from the data obtained by least-squares regression from three separate experiments, assuming competitive inhibition.

	Control	+2-Mercaptoacetate
K_m (mM)	1.16 ± 0.06	
K_i (mM)		0.021 ± 0.002
$V_{max.}$ (μmol of NAD^+ reduced/min per mg of protein)	0.24 ± 0.01	0.24 ± 0.02

(Holland & Sherratt, 1973), we first studied whether this inhibition was energy-dependent or not. Table 4 shows that, when mitochondria are depleted of ATP, 1 mM-2-mercaptoacetate no longer had an inhibitory effect on palmitoyl-L-carnitine oxidation, a finding which demonstrated that this action of 2-mercaptoacetate is energy-dependent. By contrast, Table 4 also indicates that, under the same conditions, the 2-mercaptoacetate inhibition of 3-hydroxybutyrate oxidation is still maintained (56% inhibition), a finding that gives an additional argument in favour of a direct effect of 2-mercaptoacetate on 3-hydroxybutyrate dehydrogenase. Since, as indicated above, the inhibition of fatty acid oxidation by 2-mercaptoacetate is energy-dependent, this raises the possibility that the active metabolite of 2-mercaptoacetate may be 2-mercaptoacetyl-CoA. To test this possibility, we next studied the ability of 2-mercaptoacetate to inhibit palmitoyl-L-carnitine oxidation in intact mitochondria incubated in the presence of 12 mM-fluoride and 16 mM-phosphate, conditions under which the ATP- and GTP-dependent acyl-CoA biosynthesis is prevented (Van Den Bergh, 1967). As shown in Table 4, the inhibitory effect of 1 mM-2-mercaptoacetate on palmitoyl-L-carnitine oxidation was markedly decreased under these conditions (only 24 ± 7% inhibition, $n = 5$, compared with the 52 ± 5% inhibition observed in the absence of fluoride, $n = 6$; see Table 1). On the contrary, the inhibitory effect of 2-mercaptoacetate on 3-hydroxybutyrate oxidation was of the same order of magnitude whether fluoride was present (45 ± 7% inhibition, $n = 4$) or not (50 ± 7% inhibition, $n = 5$) in the assay. As could be expected, Table 4 also shows that L-glutamate oxidation was unaltered by 2-mercaptoacetate, under these conditions.

Table 4. *Effects of 2-mercaptoacetate on the oxidation of various substrates in uncoupled and in fluoride-exposed isolated rat liver mitochondria*

Uncoupled mitochondria were prepared by incubating mitochondria (2.5 mg of protein) with 50 μM-2,4-dinitrophenol in the standard medium (final volume 1.6 ml); 3 min later 2-mercaptoacetate (1 mM) was added and, 30 s later, respiration was initiated. When respiration rates (State 3) were determined in the presence of fluoride, experimental conditions were as described in Tables 1 and 2, except that NaF (12 mM) was included in the standard medium. Final concentrations of respiratory substrates are as described in Tables 1 and 2.

	O_2 consumption (nmol/min per mg of protein)			
	Uncoupled mitochondria		Standard medium plus fluoride	
Substrate	Control	+2-Mercaptoacetate (1 mM)	Control	+2-Mercaptoacetate (1 mM)
Palmitoyl-L-carnitine	31.4 ± 0.7 (4)	29.2 ± 2.2 (4) $P > 0.05$	40.8 ± 1.9 (5)	31.0 ± 3.3 (5) $P < 0.001$
Sodium L-glutamate + sodium L-malate	39.1 ± 5.0 (4)	40.7 ± 5.5 (4) $P > 0.05$	68.5 ± 2.2 (5)	70.4 ± 5.1 (5) $P > 0.05$
Sodium DL-3-hydroxybutyrate	12.5 ± 0.8 (4)	5.5 ± 0.6 (4) $P < 0.001$	37.5 ± 3.5 (4)	20.7 ± 4.7 (4) $0.001 < P < 0.01$

Discussion

In a previous report (Bauché *et al.*, 1981), we showed that the administration of 2-mercaptoacetate to rats induced an almost complete inhibition of β-oxidation in the liver. Since it is not unusual to observe different influences of metabolically active agents on mitochondria when animals are treated *in vivo* and when the organelles are exposed to the compound *in vitro* (Higgins & Banks, 1971; Higgins & Friend, 1972; Higgins *et al.*, 1978), the aim of the present study was to determine whether 2-mercaptoacetate itself could induce the same inhibitory effects *in vitro* as it does *in vivo*.

Treatment of liver mitochondria with 2-mercaptoacetate gave some results similar to those obtained with rat liver mitochondria isolated 3h after the administration of this compound. In fact, under these two different experimental conditions, 2-mercaptoacetate was able to inhibit the palmitoyl-L-carnitine oxidation. The experiments performed *in vitro* extend our knowledge of the metabolic effects of 2-mercaptoacetate by indicating that this compound is able by itself to enter the mitochondrial matrix and that it needs an ATP-dependent activation process to inhibit the β-oxidation pathway. Furthermore, the experiments performed *in vitro* with inhibitors of the ATP- and GTP-dependent acyl-CoA synthetases suggest that the most likely metabolite responsible for the inhibition of palmitoyl-L-carnitine oxidation is 2-mercaptoacetyl-CoA. It seems therefore that there are some similarities between 2-mercaptoacetate and the four potent inhibitors of β-oxidation, pent-4-enoate (Holland & Sherratt, 1973), 2-bromo-octanoate (Raaka & Lowenstein, 1979*a*), 2-bromopalmitate (Chase & Tubbs, 1972) and hypoglycin A (Manchester, 1974; Kean, 1976; Sherratt & Osmundsen, 1976). In fact, it has been demonstrated that the activation into CoA esters of these inhibitors or of some of their metabolites was also required for eliciting their inhibitory effects on β-oxidation (Chase & Tubbs, 1972; Holland & Sherratt, 1973; Manchester, 1974; Kean, 1976; Sherratt & Osmundsen, 1976; Raaka & Lowenstein, 1979*b*; Wenz *et al.*, 1981). However, we have previously shown (Bauché *et al.*, 1981) that, like hypoglycin A and its metabolite, methylenecyclopropylacetyl-CoA (Kean, 1976; Wenz *et al.*, 1981), 2-mercaptoacetate inhibited β-oxidation *in vivo* through a specific inhibition of acyl-CoA dehydrogenase, a mechanism which is quite different from those involved in the inhibition of fatty acid oxidation by pent-4-enoate, 2-bromo-octanoate and 2-bromopalmitate (Holland *et al.*, 1973; Raaka & Lowenstein, 1979*b*; Chase & Tubbs, 1972). Therefore, besides hypoglycin A and its metabolite methylenecyclopropylacetyl-CoA (Wenz *et al.*, 1981), 2-mercaptoacetate appears to be a new and interesting inhibitory tool for studies on the β-oxidation pathway.

On the other hand, the present report provides evidence that 2-mercaptoacetate itself acts as a competitive inhibitor of 3-hydroxybutyrate dehydrogenase with respect to 3-hydroxybutyrate. This inhibitory effect could be due to its thiol group. In fact, it is possible that 2-mercaptoacetate may reduce the disulphide bond belonging to the active site of the enzyme (Phelps & Hatefi, 1981) or may establish disulphide bonds with the free thiol groups involved in the binding of the coenzyme to the apoenzyme (Latruffe & Gaudemer, 1974; Phelps & Hatefi, 1981). However, in experiments in which 2-mercaptoacetate was replaced by 2-mercaptoethanol (results not shown), we failed to observe any significant inhibition of 3-hydroxybutyrate dehydrogenase in submitochondrial particles. It seems thus that the mechanism suggested above cannot be evoked and that the most likely hypothesis is a competition between the carboxylic groups of 2-mercaptoacetate and 3-hydroxybutyrate.

Besides the inhibition of β-oxidation that was observed both *in vivo* and *in vitro*, there are, however, several differences between the metabolic effects of 2-mercaptoacetate in liver mitochondria observed *in vivo* (Bauché *et al.*, 1981) and those reported in the present paper. In fact, *in vivo* we previously noted an activation of State-4 and conversely an inhibition of State-3 activities with all the oxidizable substrates tested except with fatty acids (Bauché *et al.*, 1981). Occurrence of these two disturbances *in vivo* but not *in vitro* could well be explained by the intramitochondrial accumulation of long-chain acyl-CoA that was observed after 2-mercaptoacetate administration (Bauché *et al.*, 1981) and which probably resulted from the inhibition of fatty acid oxidation.

Another important metabolic disturbance also observed in liver of 2-mercaptoacetate-treated rats was a marked decrease in acetyl-CoA (Sabourault *et al.*, 1979). This decrease, which is also probably linked to the inhibition of β-oxidation, could alter by itself many other important mitochondrial metabolic processes. For instance, one of these disturbances could be to decrease pyruvate carboxylase activity (Utter & Keech, 1963), the occurrence of which is strongly suggested by the marked increase in pyruvate reported by us in the liver of 2-mercaptoacetate-treated rats (Sabourault *et al.*, 1979). Another possible metabolic disturbance that may also occur *in vivo* could be the inhibition of acetylglutamate synthesis and consequently the inhibition of ureogenesis. Further experiments are necessary to delineate in the liver the various metabolic effects of 2-mercaptoacetate, a potent mitochondrial poison as demonstrated in the present paper.

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