INHIBITORY EFFECT OF 2-MERCAPTOACETATE ON FATTY ACID OXIDATION IN THE LIVER

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1. Introduction

We have shown that 2-mercaptoethanol administration to the rat is followed by the induction of a fatty liver [1,2]. Among the different possible mechanisms responsible for this induction, increased peripheral fatty acid mobilization seems to play an important role [3,4], but is probably not the unique alteration involved in the pathogenesis of this type of fatty liver. In fact, ketogenesis from octanoate was found markedly reduced in liver homogenates from 2-mercaptoethanol treated rats, suggesting that, in addition to increased fatty acid input into the liver, inhibition of hepatic fatty oxidation may also occur in these animals [5].

In [3,4] we have found that 2-mercaptoacetate administration also increased the rate of fatty acid mobilization and induced a fatty liver. Moreover, administration of pyrazole, an inhibitor of alcohol dehydrogenase [6], was shown to prevent the 2-mercaptoethanol -(but not the 2-mercaptoacetate-) induced fatty liver, suggesting that the toxic effects of 2-mercaptoethanol could be due to 2-mercaptoacetate rather than to 2-mercaptoethanol per se [4].

The aim of this study was to determine whether 2-mercaptoacetate may inhibit ketogenesis and, if so, whether such an inhibition could or not be related to a decreased rate of fatty acid oxidation in the liver. The present results show that 2-mercaptoacetate administration does, in fact, inhibit ketogenesis, an effect which is indeed related to β-oxidation inhibition. Besides, 2-mercaptoacetate administration induces a dramatic enhancement of hepatic pyruvate level (> 15-fold), the mechanism of the latter effect is discussed in relation to β-oxidation inhibition, hepatic CoA-SH decrease or inhibition of pyruvate transport across the mitochondrial membrane.

2. Materials and methods

Female Wistar rats (200 ± 20 g) were fasted overnight preceding the experiments. 2-Mercaptoacetate (40 μmol/100 g body wt) was administered intraperitoneally as in [3]; control rats received an equal volume of saline. Animals were sacrificed by decapitation 3 h later. For ketogenesis studies, part of the liver was immediately excised and homogenized in ice-cold potassium phosphate buffer (0.1 M, pH 7.0). The final volume of homogenate (ml) was adjusted to 3-times the weight of liver (g) then subjected to 1 cycle of freeze thawing [7] before being used for the determination of the ketogenesis rate.

Ketone body synthesis with octanoate or with acetyl-CoA as substrate was studied according to [7] with a slight modification (presence of NAD^+ in the assay when ketogenesis from acetyl-CoA was studied). After a preincubation period of 2 min at 37°C the homogenates were added to the reaction mixtures containing the ketogenic substrate. When octanoate was the substrate, the final incubation mixture (3 ml) consisted of 0.1 M Tris–HCl buffer (pH 8.5), 5 mM

Table 1
Ketone-body synthesis by liver homogenates from 2-mercaptoacetate treated rats

Animals	Ketogenesis (acetoacetate + 3-hydroxybutyrate) (μ mol . g liver wet wt^{-1} . h^{-1}) from:		
	Endogenous substrates	Octanoate	Acetyl-CoA
Controls	78.2 ± 5.9 (19)	24.5 ± 7.0 (7)	57.0 ± 13.5 (7)
2-Mercaptoacetate treated	38.6 + 5.5 (16)	6.9 + 3.2 (8)	65.4 ± 10.7 (6)
	$p < 0.001$	$p < 0.001$	$p > 0.5$

Experimental conditions are described in section 2. Each value is the mean ± SE, with no. determinations in parentheses

$MgCl_2$, 5 mM ATP, 0.3 mM CoA-SH, 0.66 mM NAD^+, 0.5 ml homogenate and 3.3 mM octanoate. When acetyl-CoA was the substrate, the incubation mixture contained a generating system for acetyl-CoA consisting of 0.1 M Tris–HCl buffer (pH 8.5), 5 mM $MgCl_2$, 5 mM ATP, 1.5 mM CoA-SH, 30 units phosphotransacetylase, 0.66 mM NAD^+, 0.5 ml homogenate and 27 mM acetylphosphate (final vol. 3 ml). Incubations were performed in parallel but in the absence of ketogenic substrates to determine the rate of 'endogenous ketogenesis'. After 4 min, the incubation mixtures were poured into centrifuge tubes containing 0.3 ml ice-cold $HClO_4$ (70%, w/v). After neutralization, acetoacetate and 3-hydroxybutyrate were determined enzymatically according to [8].

In parallel with these determinations, liver CoA-SH [9] acetyl-CoA [10], acyl-CoA [11] and pyruvate [12] levels were determined according to the enzymatic methods cited. Blood glucose was also determined according to [13]. Enzymes, coenzymes and substrates were from Sigma and 2-mercaptoacetate from Merck.

3. Results

As shown in table 1, ketogenesis from endogenous substrates was 50% reduced in liver homogenates from 2-mercaptoacetate-treated rats. This treatment also markedly altered the rate of ketone body synthesis from octanoate as substrate (75% inhibition). In contrast, with acetyl-CoA (acetylphosphate) as substrate, the net production of acetoacetate and 3-hydroxybutyrate was not significantly modified by 2-mercaptoacetate administration. These discrepant effects of 2-mercaptoacetate on ketogenesis from octanoate and acetylphosphate could be linked to the different concentrations of CoA-SH present in each of these experiments. However when ketogenesis from octanoate was determined under the same conditions as those used for the determination of ketogenesis from acetylphosphate (1.5 mM CoA-SH instead of 0.3 mM), the magnitude of the ketogenesis inhibition was not altered (results not shown).

As shown in table 2, 3 h after 2-mercaptoacetate

Table 2
Effects of 2-mercaptoacetate administration on liver CoA-SH, acetyl-CoA, acyl-CoA and pyruvate levels and on blood glucose in the rat

Animals	Liver concentrations (nmol/g wet wt) of:					Blood glucose (mmol/l)
	CoA-SH	Acetyl-CoA	Acyl-CoA	CoA-SH + acetyl CoA + acyl-CoA	Pyruvate	
Controls (6)	44 ± 10	123 ± 12	59 ± 5	226 ± 13	96 ± 12	5.1 ± 0.5
2-Mercaptoacetate treated (6)	9 ± 4	27 ± 6	130 ± 10	166 ± 10	1481 ± 18	3.0 ± 0.5
	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$0.001 < p < 0.01$

Experimental conditions are described in section 2. Each value is the mean ± SE., with no. determinations in parentheses

administration, the hepatic levels of free CoA-SH and acetyl-CoA were markedly decreased, falling to ~20% of the control values. At the same time, on the contrary, the hepatic acyl-CoA level was increased (+ 120%), an effect which did not completely balance, however, the reduction in free CoA-SH and acetyl-CoA concentrations. Moreover, 2-mercaptoacetate treatment induced both a dramatic increase (> 15-fold) of the hepatic pyruvate level and a significant reduction (−40%) of the blood glucose level (table 2).

4. Discussion

We show that 2-mercaptoacetate administration induces an increase in liver acyl-CoA, a decrease in liver acetyl-CoA and a reduction of the rate of ketogenesis from octanoate, but not from acetyl-CoA. It can thus be assumed that these effects are all related to an inhibition of the β-oxidation pathway. This can be due either to a decrease in the availability of acyl-CoA in the mitochondria or, as shown in the case of 4-pentenoate administration [14–16], to a selective and direct inhibition of one or more of the enzymes involved in this pathway (acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase or 3-ketoacyl-CoA thiolase, but not acetoacetyl-CoA thiolase since this enzyme is also involved in ketogenesis from acetyl-CoA, a pathway which is unaltered by 2-mercaptoacetate). Although the intramitochondrial acyl-CoA level was not specifically assessed in these experiments, the marked increase in hepatic acyl-CoA level found in the 2-mercaptoacetate treated rats would suggest that the acyl-CoA availability for β-oxidation is not reduced in the liver of these animals. Therefore, the most probable mechanism concerned in the 2-mercaptoacetate-induced β-oxidation inhibition appears to be the inhibition of one or more of the enzymes involved in this pathway.

From these experiments it is clear that hepatic disturbances other than those affecting β-oxidation (but which could be related at least in part to inhibition of this pathway) also occur after 2-mercaptoacetate administration. In fact, it has been shown that, in these fasted rats, the hepatic pyruvate level was considerably increased, an effect which could result from a direct and/or an indirect inhibition of the mitochondrial utilization of pyruvate by 2-mercaptoacetate.

(i) The latter compound may indeed directly impede the mitochondrial metabolism of pyruvate by combining with the thiol group which belongs to the carrier involved in pyruvate transport across the mitochondrial membrane [17].

(ii) As 2-mercaptoacetate inhibits β-oxidation, this compound may also indirectly interfere with the mitochondrial utilization of pyruvate: in fact, pyruvate carboxylase activity, which is elevated in these animals because of their fasting status [18], should be reduced as a consequence of the drop in acetyl-CoA level [19]. Besides, evidence in favour of the inhibition of this neoglucogenic enzyme is provided by the hypoglycaemia observed in the treated animals.

(iii) The marked decrease in free CoA-SH availability observed in the liver of 2-mercaptoacetate treated rats would also contribute to reduce the rate of pyruvate utilization by the mitochondria as it is a factor known to induce deinhibition of pyruvate dehydrogenase kinase [20].

Considering now the results in table 2, it is obvious that the fall in the free CoA-SH + acetyl-CoA levels found in the liver after 2-mercaptoacetate treatment is only partly balanced by the concomitant increase in the acyl-CoA concentration due to β-oxidation inhibition. This indicates that, in the treated-animals, part of the free CoA-SH (~ 60 nmol/g wet wt) has become unavailable for acylation reactions. Among the different mechanisms which could be evoked to explain such a gap, it is tempting to speculate upon a possible competition of 2-mercaptoacetate with acetate, a competition which should lead to mercaptoacetyl-CoA instead of acetyl-CoA. Regardless on the mechanism of this reduction of the free CoA-SH level, it appears likely that such a reduction plays also an important role in the inhibitory effect of 2-mercaptoacetate on β-oxidation. A reduction of the intramitochondrial CoA-SH pool would indeed reduce the activity of the mitochondrial fatty acid thiokinase and, as suggested in heart mitochondria [21], of 3-ketoacyl-CoA thiolase.

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References

[1] Nordmann, R. and Nordmann, J. (1971) Rev. Et. Clin. Biol. 16, 564–566.
[2] Nordmann, R. and Nordmann, J. (1971) Biochimie 53, 705–708.
[3] Sabourault, D., Giudicelli, Y., Nordmann, R. and Nordmann, J. (1976) Biochim. Biophys. Acta 431, 241–248.
[4] Sabourault, D., Giudicelli, Y., Nordmann, J. and Nordmann, R. (1977) Lipids 12, 641–644.
[5] Nordmann, R., Sabourault, D. and Nordmann, J. (1971) CR Soc. Biol. 165, 1262–1265.
[6] Goldberg, L. and Rydberg, U. (1969) Biochem. Pharmacol. 18, 1749–1762.
[7] Mc Garry, J. D. and Foster, D. W. (1969) Biochim. Biophys. Acta 177, 35–41.
[8] Williamson, D. H., Mellanby, J. and Krebs, H. A. (1969) Biochem. J. 82, 90–96.
[9] Stadtman, E. R., Novelli, G. D. and Lipman, F. (1951) J. Biol. Chem. 191, 365.
[10] Decker, K. (1974) Methods Enz. Anal. 4, 1988–1993.
[11] Garland, P. B. (1974) Methods Enz. Anal. 4, 2015–2016.
[12] Czok, R. and Lamprecht, W. (1974) Methods Enz. Anal. 3, 1446–1451.
[13] Bergmeyer, H. U. and Bernt, E. (1974) Methods Enz. Anal. 3, 1205–1215.
[14] Senior, A. E., Robson, B. and Sherratt, H. S. A. (1968) Biochem. J. 110, 511–519.
[15] Holland, P. C., Senior, A. E. and Sherratt, H. S. A. (1973) Biochem. J. 136, 173–184.
[16] Sherratt, H. S. A. and Osmundsen, H. (1976) Biochem. Pharmacol. 25, 743–750.
[17] Halestrap, A. P. (1978) Biochem. J. 172, 377–387.
[18] Mc Clure, W. R. and Lardy, H. A. (1971) J. Biol. Chem. 246, 3591–3596.
[19] Utter, M. F. and Keech, D. B. (1963) J. Biol. Chem. 238, 2604–2608.
[20] Pettit, F. H., Pelley, J. W.A. and Reed, L. J. (1975) Biochem. Biophys. Res. Commun. 65, 575–582.
[21] Fong, J. C. and Schulz, H. (1978) J. Biol. Chem. 253, 6917–6922.