EFFECT OF (−)-HYDROXYCITRATE ON ETHANOL METABOLISM

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Received 18 August 1973

1. Introduction

In non-ruminant mammals pyruvate derived from carbohydrate, and fatty acids derived from fat, are converted to acetyl-CoA by intramitochondrial enzyme systems. The de novo synthesis of fatty acids is catalyzed by an extramitochondrial enzyme system. The transfer of the acetyl group of intramitochondrial acetyl-CoA into the extramitochondrial space is thus an obligatory step in the de novo synthesis of fatty acids. The transfer of the acetyl group of intramitochondrial acetyl-CoA into the extramitochondrial space occurs in the form of citrate [1–3]. The reactions involved in the transfer of the acetyl group are as follows (where I.M. and E.M. refer to intra- and extra-mitochondrial, respectively):

I.M. acetyl CoA + oxaloacetate + H_2O → citrate + CoA (1)

$\text{citrate}_{\text{I.M.}} \rightarrow \text{citrate}_{\text{E.M.}}$ (2)

E.M. citrate + ATP + CoA → acetyl CoA + oxaloacetate + ADP + P_i (3)

E.M. oxaloacetate + DPNH + H^+ → malate + DPN^+ (4)

$\text{malate}_{\text{E.M.}} \rightarrow \text{malate}_{\text{I.M.}}$ (5)

I.M. malate + DPN^+ → oxaloacetate + DPNH + H^+ (6)

Carbon balance: $\text{acetyl}_{\text{I.M.}} \rightarrow \text{acetyl}_{\text{E.M.}}$ (7)

Hydrogen balance: $2H_{\text{E.M.}} \rightarrow 2H_{\text{I.M.}}$ (8)

The equations take into account the observation that rat liver mitochondria are, for physiological purposes, impermeable to oxaloacetate [4]. The carbon skeleton of oxaloacetate generated in reaction 3 must be returned to the intramitochondrial space, otherwise the mitochondria would become drained of dicarboxylates while the cytoplasm would become filled with them. Oxaloacetate can be converted either to malate or to aspartate, either of which can penetrate the inner mitochondrial membrane via specific carrier mechanisms [4]. Equations 1–8 show that the export of one acetyl group from the mitochondria in the form of citrate can be associated with the entry of one pair of hydrogens into the mitochondria in the form of malate. We wish to present evidence that a citrate–malate shuttle operates in rat liver, and that this shuttle may, under some conditions, account for the oxidation of approximately one-half of the extramitochondrial DPNH generated during aerobic glycolysis.

2. Materials and methods

[1-^{14}C]ethanol was obtained from New England Nuclear Corporation, Boston. The dietary regimen and the perfusion method were as described previously [5].

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3. Results and discussion

(−)-Hydroxycitrate is a powerful inhibitor of citrate cleavage enzyme (ATP: citrate lyase) *in vitro* [6], and an inhibitor of fatty acid synthesis *in vivo* [2]. (−)-Hydroxycitrate is believed to exert its effect on fatty acid synthesis by inhibiting citrate cleavage enzyme (reaction 3). If this interpretation is correct, then fatty acid synthesis from acetate and ethanol should not be inhibited by (−)-hydroxycitrate, since acetate and ethanol can give rise to extra-mitochondrial acetyl-CoA without involving the citrate cleavage reaction. No hydroxycitrate inhibition of acetate or ethanol incorporation was observed. Indeed we found that while hydroxycitrate inhibits fatty acid synthesis from carbohydrate, it actually enhances fatty acid synthesis from acetate or ethanol (Brunengraber and Lowenstein, to be published).

We have measured the [lactate]/[pyruvate] ratio (L/P) and the [β-hydroxybutyrate]/[acetoacetate] ratio (hereafter the B/A ratio) in the perfusates of livers perfused with acetate or ethanol, in the presence and absence of (−)-hydroxycitrate (tables 1 and 2).

Table 1
[Lactate]/[Pyruvate] ratios in liver perfusate.

Additions	[Lactate]/[pyruvate] in perfusate during test period
None	8.05 ± 0.25
Acetate	7.50 ± 0.42
Acetate and hydroxycitrate	10.5 ± 0.62
Ethanol	26.2 ± 5.0
Ethanol and hydroxycitrate	98 ± 13

The livers were obtained from rats which had been schedule-fed a diet high in glucose that lacked fat. The diet and perfusion conditions were identical to those described in ref. [1]. All perfusates contained 25 mM glucose. No amino acids were added to the perfusate. Where indicated acetate or ethanol was added to the reservoir 58 min after starting the perfusion, to yield an initial perfusate concentration of 10 or 7 mM respectively. Where indicated (−)-hydroxycitrate was added to the reservoir 58 min after starting the perfusion to yield a final concentration of 2 mM. Samples of perfusate were withdrawn for analysis of lactate and pyruvate at 15, 30, 45, and 57 min (control period), and at 75, 90, 105, and 120 min (test period). The control group consisted of 29 perfusions; the test groups each consisted of 6 perfusions. The [lactate]/[pyruvate] ratio during the control period was 7.05 ± 0.15 (S.E.). [Lactate] plus [pyruvate] production during the control period was 4.43 ± 0.20 mM.

Table 2
[β-Hydroxybutyrate]/[Acetoacetate] ratios in liver perfusate.

Additions	[β-Hydroxybutyrate]/[acetoacetate] in perfusate during test period
None	0.82 ± 0.16
Acetate	0.55 ± 0.09
Acetate and hydroxycitrate	0.62 ± 0.05
Ethanol	0.80 ± 0.15
Ethanol and hydroxycitrate	0.76 ± 0.19

Conditions were as described in table 1.

Addition of acetate to the perfusate does not affect the L/P ratio. Addition of ethanol causes the well known increase [7–11] in the L/P ratio from about 8 in the controls to about 26 in the presence of ethanol (table 1). An interesting point is that hydroxycitrate causes a further increase in the L/P ratio. The effect is slight in the presence of acetate, the L/P ratio being increased from about 8–10.5. However, the increase is large in the presence of ethanol, the L/P ratio being increased from 8 to about 100.

Table 2 shows the [β-hydroxybutyrate]/[acetoacetate] ratio (B/A ratio) under various conditions. It is not significantly affected by the addition of acetate or ethanol, in the presence or absence of (−)-hydroxycitrate.

Our interpretation of these results is as follows. The reducing equivalents generated in the cytoplasm during glycolysis are transferred to the mitochondria in part through a citrate–malate shuttle (reactions 1–6). In this shuttle, citrate leaves the mitochondria and is cleaved to acetyl-CoA and oxaloacetate in the cytoplasm via the citrate cleavage reaction. The acetyl-CoA is used for fatty acid synthesis. The oxaloacetate made in the same reaction serves to oxidize one molecule of DPNH via the cytoplasmic malate dehydrogenase reaction, and then malate passes back into the mitochondria. It can be calculated that under conditions when the rate of fatty acid synthesis is high [5], that the citrate–malate shuttle can oxidize at least 45% of the extra-mitochondrial DPNH generated during glycolysis. Addition of ethanol puts a new burden on the shuttles responsible for the oxidation of cytoplasmic DPNH, and leads to a higher L/P ratio. Addition of hydroxycitrate on top of the ethanol inhibits not only fatty acid synthesis, but also the generation

of oxaloacetate from citrate. In other words, one of the major hydrogen shuttles becomes strongly inhibited and this results in a large increase in the L/P ratio. The observation that the B/A ratio remains unaffected indicates that the rate limiting step(s) occur(s) before the formation of intramitochondrial DPNH.

Acknowledgements

This work was supported by the John A. Hartford Foundation, and by the Fonds de la Recherche Scientifique Médicale of Belgium.

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