EFFECT OF (−)-HYDROXYCITRATE ON KETONE PRODUCTION BY THE PERFUSED LIVER

Henri BRUNENGRABER and John M. LOWENSTEIN

Laboratoire de Chimie Médicale, Université Libre de Bruxelles, Belgium and Graduate Department of Biochemistry, Brandeis University Waltham, Mass. 02154, USA

Received 31 March 1976

1. Introduction

The enzymes responsible for the synthesis of acetoacetate by the liver occur both in the intra- and extramitochondrial compartments of the cell [1,2]. However, there is no direct evidence for the actual operation of an extramitochondrial pathway of acetoacetate synthesis. The bimodal distribution of acetoacetyl CoA thiolase and β-hydroxy-β-methylglutaryl CoA synthase has been confirmed by the isolation of distinct intra- and extramitochondrial forms of the enzymes [3–5]. The cytoplasmic activity of β-hydroxy-β-methylglutaryl CoA lyase was reported to about 8% of the total activity of homogenates prepared from livers of animals on a normal diet. This amount increased more than two-fold in animals that were starved for 48 h. In the same experiments only 3 to 4% of the total glutamate dehydrogenase activity was found in the cytoplasm, indicating that part of the lyase activity is of extramitochondrial origin [2,6]. However, other workers have concluded that the lyase occurs only as an intramitochondrial enzyme, that the extramitochondrial activity is an artifact of mitochondrial damage [7], and that synthesis of extramitochondrial β-hydroxy-β-methylglutaryl CoA occurs exclusively for the purpose of cholesterol synthesis [3]. Possible functions of a complete, extramitochondrial pathway of acetoacetate synthesis have been considered [2,6]. In brief, it was postulated that extramitochondrial acetoacetate synthesis occurs when lipogenesis is depressed and extramitochondrial acetyl CoA accumulates.

The present report demonstrates that ketone* production by perfused livers obtained from fed rats is inhibited by hydroxycitrate**. Ketone production by livers from starved rats, which occurs at a considerably greater rate, is not inhibited by hydroxycitrate. Our observations constitute evidence for the operation of an extramitochondrial pathway of ketogenesis in the fed rat, and for the operation of the intramitochondrial pathway in the starved rat.

2. Results and discussion

When livers obtained from fed rats are perfused with 25 mM glucose, they produce ketones at a rate of about 35 μmol per g dry wt./h. Hydroxycitrate inhibits ketone production under these conditions (table 1). The inhibition is progressive, being most pronounced 45 to 60 min after addition of hydroxycitrate. The inhibitor is much less effective when livers from fed rats perfused with 4 mM glucose.

Addition of 2 mM oleate to the perfusate containing 25 mM glucose increases ketone production to 109 μmol per g dry wt./h. Again this higher rate is not inhibited by hydroxycitrate (not shown in table 1). Livers from animals that have been starved for two days produce ketones at a rate of about 76 μmol per g dry wt./h when they are

* The term *ketone* refers to acetoacetate plus β-hydroxybutyrate.

** (−)-Hydroxycitrate is (2R,3S)-2-hydroxycitrate [8]. In this paper it is referred to simply as hydroxycitrate.

Table 1
Ketone production by perfused rat liver

Time interval (min)	25 mM Glucose in perfusate			4 mM Glucose in perfusate		
	Control	Plus 2 mM hydroxy-citrate	Inhibition	Control	Plus 2 mM hydroxy-citrate	Inhibition
	μmol/g dry wt.		%	μmol/g dry wt.		%
60– 75	13.9	6.56	53	8.79	6.90	21
75– 90	8.62	2.96	66	7.90	6.92	12
90–105	7.37	2.07	72	5.39	5.39	0
105–120	5.84	0.90	85	3.14	2.69	14

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 as described in [9]. 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 β-hydroxybutyrate and acetoacetate at 15, 30, 45, and 57 min (pre-test period) and at 60, 75, 90, 105, and 120 min (test period). No amino acids were added to the perfusate. Rates of ketone production by the control and hydroxycitrate groups were identical from 0 to 57 min. The number of perfusions (n) and standard errors of the means (SE) were as follows: 25 mM glucose controls n = 9, SE = ± 5%; 25 mM glucose plus hydroxycitate n = 13, SE = ± 9%; 4 mM glucose controls n = 5, SE ± 19%; 4 mM glucose plus hydroxycitrate n = 5, SE = ± 19%.

perfused with 4 mM glucose. Subsequent addition of 2 mM oleate increases this rate to 248 μmol per g dry wt./h. However, ketogenesis by livers obtained from starved rats is not inhibited by hydroxycitrate, either in the presence or the absence of oleate (not shown in table 1). In these experiments oleate was added to the reservoir of the perfusion apparatus in the form of 7.5 ml 40 mM sodium oleate in 20% dialysed bovine serum albumin. The total volume of perfusate was 150 ml. Control experiments showed that addition of an identical amount of bovine albumin alone had no effect on ketone production.

Under the conditions described in table 1, addition of oleate caused an increase in the ratio [β-hydroxybutyrate]/[acetoacetate] from 0.9 to 1.9, but this increase was transient, and the ratio returned to 0.9 in about 15 min. Addition of oleate also caused an increase in the ratio [lactate]/[pyruvate] from 10 to 20, but this increase too was transient.

Hydroxycitrate inhibits citrate cleavage enzyme (ATP: citrate lyase) [10], the enzyme chiefly responsible for the production of extramitochondrial acetyl CoA in non-ruminant mammals [11]. Our results indicate that extramitochondrial synthesis of acetoacetate occurs under conditions when the rate of lipogenesis is maximum. When lipogenesis is diminished by lowering the glucose concentration, or by starving the animal, or by adding oleate to the perfusate, the extramitochondrial pathway becomes diminished or stops, and the intramitochondrial pathway takes over.

Livers from fed animals produce ketones in the absence of oleate at a rate which is 100 × 35/248 = 14% of the maximum rate observed with livers from starved animals in the presence of oleate. This is about the same as the percentage of extramitochondrial β-hydroxy-β-methylglutaryl CoA lyase found by Williamson et al. [2]. These workers postulated that the extramitochondrial pathway comes into play when lipogenesis becomes diminished and there is an excess of acetyl CoA.

The interpretation of the effect of hydroxycitrate on ketogenesis rests on the assumption that hydroxycitrate acts chiefly or solely by inhibiting citrate cleavage, the result being a diminished rate of production of extramitochondrial acetyl CoA and hence of ketones via the extramitochondrial pathway of ketogenesis. However, other explanations are not ruled out. For example in the fed liver ketogenesis may occur only from fatty acids synthesized de novo. In this case hydroxycitrate would inhibit intra- and extramitochondrial ketogenesis by inhibiting fatty

acid synthesis, and our experiments would not shed light on the operation of an extramitochondrial pathway. Another possibility is that hydroxycitrate inhibits one of the steps of ketogenesis from acetyl CoA. To be correct, this would require inhibition of the extramitochondrial but not of the intramitochondrial pathway of ketogenesis.

It was shown previously that under conditions when fatty acid synthesis is maximum, the perfused liver of rat converts acetyl groups into ketones at 17% the rate that it converts them into long chain fatty acids [9]. The role of ketones as a circulating metabolic fuel is now generally acepted [1,12,13]. There is no obvious reason why ketones should not also be produced and function as fuel in the fed state.

Acknowledgements

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

References

[1] Wieland, O. (1968) in: Advances in Metabolic Disorders (Levine, R. and Luft, R., eds.) Vol. 3, pp. 1–47, Academic Press, New York.
[2] Williamson, D. H., Bates, M. W. and Krebs, H. A. (1968) Biochem. J. 108, 353–361.
[3] Clinkenbeard, K. D., Reed, W. D., Mooney, R. A. and Lane, M. D. (1975) J. Biol. Chem. 250, 3108–3116.
[4] Reed, W. D., Clinkenbeard, K. D. and Lane, M. D. (1975) J. Biol. Chem. 250, 3117–3123.
[5] Clinkenbeard, K. D., Sugiyama, T., Reed, W. D. and Lane, M. D. (1975) J. Biol. Chem. 250, 3124–3135.
[6] Sauer, F. and Erfle, J. D. (1966) J. Biol. Chem. 241, 30–37.
[7] Bucher, N. L. R., Overath, P. and Lynen, F. (1960) Biochim. Biophys. Acta 40, 491–501.
[8] Lewis, Y. S. (1969) Methods in Enzymol. 13, 613–619.
[9] Brunengraber, H., Boutry, M. and Lowenstein, J. M. (1973) J. Biol. Chem. 248, 2656–2669.
[10] Watson, J. A., Fang, M. and Lowenstein, J. M. (1969) Arch. Biochem. Biophys. 135, 209–217.
[11] Lowenstein, J. M. (1967) Biochem. Soc. Symp. 27, 61–86.
[12] McKay, E. M. (1943) J. Clin. Endocrinol. Metab. 3, 101–110.
[13] Bates, M. W., Krebs, H. A. and Williamson, D. H. (1968) Biochem. J. 110, 655–661.