ON THE MECHANISM OF THE ANTIKETOGENIC ACTION OF PROPIONATE AND SUCCINATE IN ISOLATED RAT LIVER MITOCHONDRIA

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1. Introduction

The antiketogenic action of propionate and its inhibitory effect on fatty acid and acetate oxidation has been known for many years [1–3]. However, discordant interpretations have been proposed concerning the mechanism of this action [1–3]. The results reported in the present paper strongly suggest that the antiketogenic action of propionate can be attributed predominantly to an inhibition of fatty acid activation while that of succinate to an improved incorporation of acetylCoA into citrate.

2. Materials and methods

Mitochondria were prepared from liver of Wistar strain albino rats according to the procedure of Schneider and Hogeboom in 0.25 M sucrose [4]. Incubation was carried out in Barcroft vessels at 25°. ^{14}C activity was determined by liquid scintillation counting. Acetoacetate was measured enzymatically according to Williamson et al. [5].

The concentrations of ATP, ADP and AMP in mitochondria were measured enzymatically as previously described [6].

Uniformly labelled ^{14}C-oleate was obtained from Radiochemical Centre, Amersham.

Reagents were analytical grade, L-propionylcarnitine and L-palmitoylcarnitine were kindly supplied by Italseber Farmaceutici (Milano, Italy).

3. Results

The results reported in table 1 show that propionate strongly inhibited oleate oxidation by rat liver mitochondria, as can be deduced from the sharp decrease in metabolites produced by oxidation and represented by the sum of ^{14}C-labelled soluble metabolites plus $^{14}CO_2$. Propionylcarnitine, on the contrary, increased ^{14}C-oleate oxidation. Since carnitine also evoked a similar stimulation, the propionylcarnitine effect seems to be due mainly to the release of free carnitine during its conversion into propionylCoA [7].

A study of mitochondrial endogenous adenine nucleotides indicated that propionate, unlike succinate and propionylcarnitine, induced a remarkable decrease of ATP/AMP ratio. This decrease, as was previously reported [8], is a very unfavourable condition for the activity of ATP dependent acylCoA synthetase (Acid:CoA ligase AMP) which is sensitive to AMP [8].

From the results reported in table 2 it can be observed that propionate, as well as succinate, inhibited the formation of acetoacetate by rat liver mitochondria incubated in the presence of oleate. Propionylcarnitine, on the contrary, did not decrease, but rather increased acetoacetate formation. Furthermore, the results of table 2 show that the inhibition of acetoacetate formation by succinate was completely abolished by the addition of malonate, which, however, did not remove the inhibition produced by propionate.

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Table 1
Action of propionate and succinate on oleate utilization in rat liver mitochondria.

Additions	Cpm per mg per 5 min $^{14}CO_2$ + acid soluble activity	(nmoles/mg Prot) ATP	ADP	AMP
1. Oleate	4,600	4.3	5.5	1.1
2. Oleate + 5 mM propionate	990	1.4	5.1	4.1
3. Oleate + 5 mM succinate	4,800	5.5	4.2	1.5
4. Oleate + 5 mM propionylCn	8,900	5.3	6.2	1.5
5. Oleate + 5 mM carnitine	8,550	7.0	2.3	1.5

The incubation mixture contained in a final volume of 1 ml: 15 mM phosphate buffer pH 7.4, 20 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 25 mM sucrose, 0.1 mM ^{14}C-oleate (U) (2.1×10^6 cpm/μmole), 8 mg mitochondrial protein. $^{14}CO_2$ was trapped by 0.2 ml of 1 M hyamine hydroxide. After 5 min of incubation the reaction was stopped by the addition of 0.2 ml of 20% H_2SO_4 (v/v). The shaking was continued for an additional 15 min. After the addition of 0.1 ml of conc. $HClO_4$, both hyamine $^{14}CO_2$ and the $HClO_4$-soluble activity were determined by liquid-scintillation counting. The acid-soluble activity represents the metabolites derived from the acid-insoluble oleate. Temperature 25°. Time 5 min.

Moreover, when palmitoylcarnitine, whose oxidation does not require a preliminary activation, was substituted for oleate, succinate, unlike propionate, substantially inhibited acetoacetate formation. Here again malonate abolished such an inhibition.

4. Discussion

For a better understanding of the results reported here, it is worthwhile mentioning that propionate is activated inside the inner membrane at the expense of endogenous ATP [9, 10]. Also, oleate under the conditions reported here is activated in the same space and by the same mechanism [11].

In the light of these circumstances it is clear why a high AMP level builds up within the mitochondria when oleate and propionate are both present in the incubation medium (see table 2).

It has been previously reported that intramitochondrially formed AMP inhibits the ATP dependent thiokinase (Acid:CoA ligase AMP 6.2.1.3) [8]. Consequently, it seems reasonable that the observed inhibition of oleate oxidation induced by propionate (as can be deduced from the sharp decrease in $^{14}CO_2$ and acid soluble activity deriving from ^{14}C-oleate) can be attributed to a high level of AMP in the presence of oleate plus propionate.

That the antiketogenic action of propionate is due to an inhibition of fatty acid oxidation is also demon-

Table 2
Action of propionate and succinate on acetoacetate formation from oleate and palmitoylcarnitine in the presence and in the absence of malonate.

Additions	Acetoacetate (nmoles/mg prot/min) –	+ 10 mM malonate
0.1 mM oleate	1.60	2.20
+ 5 mM propionate	0.50	0.80
+ 5 mM succinate	0.10	2.00
+ 5 mM propionylcarnitine	3.00	2.30
0.1 mM Palmitoylcarnitine	2.10	2.30
+ 5 mM propionate	1.60	2.10
+ 5 mM succinate	0.20	2.00
+ 5 mM propionylcarnitine	2.90	2.00

Time, temperature and incubation medium, without ^{14}C-oleate (U), as in table 1.

strated by the observation that propionylcarnitine, an already "activated" substrate, does not modify intramitochondrial ATP/AMP ratio and does not inhibit either fatty acid oxidation or acetoacetate formation. Furthermore, the failure of propionate to inhibit acetoacetate formation when oleate is replaced by palmitoylcarnitine is additional evidence for the proposed mechanism.

In the case of succinate an increased oxaloacetate formation seems to be the main factor involved in the inhibition of acetoacetate formation [12].

Oxaloacetate favours the incorporation of acetyl-CoA into citrate, thus decreasing its condensation into acetoacetate. In fact, the effect of succinate, unlike that of propionate, is completely abolished by malonate.

All these observations make reasonable the conclusion that the antiketogenic action of propionate is predominantly a consequence of an inhibition of fatty acid oxidation, while that of succinate seems entirely attributable to an enhancement of acetylCoA utilization for the citrate synthesis.

References

[1] K. Lang and K.M. Bassler, Klin. Wochenschrift 31 (1953) 675.
[2] E.J. Masoro, J.M. Felts, S.S. Panagos and D. Rapport, Arch. Biochem. Biophys. 68 (1957) 270.
[3] R.J. Pennington and J.M. Appleton, Biochem. J. 69 (1958) 119.
[4] W.C. Schneider and G.H. Hogeboom J..Biol. Chem. 183 (1950) 123.
[5] D.H. Williamson, J. Mellanby and H.A. Krebs, Biochem. J. 82 (1962) 90.
[6] Z. Drahota, A. Alexandre, C.R. Rossi and N. Siliprandi, Biochim. Biophys. Acta 205 (1970) 491.
[7] I.B. Fritz, in: Cellular Compartmentation and Control of Fatty Acid Metabolism, ed. J. Bremer (Academic Press, London, 1968) p. 39.
[8] S.V. Pande and J.F. Mead, J. Biol. Chem. 243 (1968) 352.
[9] H.R. Scholte, E.M. Wit-Peeters and J.C. Bakker, Biochim. Biophys. Acta 231 (1971) 479.
[10] C. Barth, M. Sladek and K. Decker, Biochim. Biophys. Acta 260 (1972) 1.
[11] C.R. Rossi, L. Galzigna, A. Alexandre and D.M. Gibson, J. Biol. Chem. 242 (1967) 2102.
[12] J. Bremer, Biochim. Biophys. Acta 116 (1966) 1.