The source of malonyl-CoA in rat heart

The calcium paradox releases acetyl-CoA carboxylase and not propionyl-CoA carboxylase

Hans R. Scholte, Inez E.M. Luyt-Houwen, Marie-Louise Dubelaar and Willem C. Hulsmann

Department of Biochemistry I, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands

Received 17 January 1986

The formation of malonyl-CoA in rat heart is catalyzed by cytosolic acetyl-CoA carboxylase. The existence of this enzyme in heart is difficult to prove by the abundant occurrence of mitochondrial propionyl-CoA carboxylase, which is also able to catalyze the carboxylation of acetyl-CoA. We used the calcium paradox as a tool to separate cytosolic components from the remaining heart, and found that acetyl-CoA carboxylase activity was preferentially released, like lactate dehydrogenase and carnitine, while propionyl-CoA carboxylase was almost fully retained. Acetyl-CoA carboxylase activity was determined after activation by citrate ion and Mg^{2+}. The activity decreased to 64% by 48 h of fasting.

Acetyl-CoA carboxylase *Calcium paradox* *Carnitine palmitoyltransferase I* *Malonyl-CoA* *Propionyl-CoA carboxylase* *(Rat heart)*

1. INTRODUCTION

Malonyl-CoA inhibits carnitine palmitoyltransferase I in liver [1,2] and is an important factor in determinating the rate of the mitochondrial oxidation of long-chain fatty acids. The existence and regulation of acetyl-CoA carboxylase in lipogenic tissues like liver and adipose tissue is well known. Phosphorylation by a cyclic AMP-dependent protein kinase inhibits the activity, while phosphorylation at another site by insulin-dependent protein kinase stimulates the activity [3,4].

Carnitine palmitoyltransferase I is also inhibited by malonyl-CoA in non-lipogenetic tissues such as skeletal muscle and heart [5,6]. Appreciable amounts of malonyl-CoA in these tissues were detected [6]. Higher levels were found in the fed than in the fasted state. The assayed compound was without doubt malonyl-CoA, since it disappeared after treatment with purified malonyl-CoA decarboxylase. The authors were puzzled by the problem of malonyl-CoA synthesis, and gave three suggestions for its formation: (i) It is formed in the cytosol by the action of rudimentary acetyl-CoA carboxylase. (ii) It is formed in the mitochondrial matrix by the action of propionyl-CoA carboxylase on acetyl-CoA, followed by a carnitine-linked transfer of the malonyl-group to extramitochondrial coenzyme A. (iii) The inhibition by malonyl-CoA is physiologically fortuitous. In intact cells another metabolite, perhaps chemically related to malonyl-CoA serves as a regulator of fatty acid oxidation. These three suggestions are not very likely for the following reasons: (i) In guinea pig heart, acetyl-CoA carboxylase was partitioned over the subcellular fractions like propionyl-CoA carboxylase, and it was concluded that acetyl-CoA carboxylase was absent [7]. (ii) Propionyl-CoA carboxylase in heart also catalyzes, but at a much lower rate, the carboxylation of acetyl-CoA [8] but only in the matrix of the mitochondria, where the enzyme is localized [7,9]. The malonyl-CoA produced is immediately decar-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

boxylated by malonyl-CoA decarboxylase, which is localized in the same compartment [9]. The malonyl group cannot be transported as carnitine ester, since malonyl-CoA is not a substrate for carnitine acetyltransferase [10]. (iii) If this possibility is true, the question on the origin of heart and skeletal muscle malonyl-CoA remains to be answered.

In spite of earlier work indicating the absence of acetyl-CoA carboxylase in guinea pig heart [7] we decided to reinvestigate this possibility in rat heart by a better method than tissue fractionation to separate cytosolic and mitochondrial enzymes: the calcium paradox.

2. MATERIALS AND METHODS

2.1. *The calcium paradox*

Male Wistar rats (300–400 g) were decapitated and their hearts were perfused as described by Zimmerman and Hülsmann [11]. After 10 min Ca^{2+}-free perfusion, Ca^{2+} was reintroduced and this perfusate was collected for 10 min. The remaining heart (1.0–1.1 g) was minced with a pair of scissors in ice-cold perfusion medium and homogenized with a motor-driven Potter-Elvehjem homogenizer. A 10% homogenate was made. Homogenate and perfusate were stored batchwise at −70°C in small, closed plastic cups. The assays were carried out within a week.

2.2. *Assay of acetyl-CoA carboxylase*

To enable assay of the enzyme in the perfusate, a more sensitive method had to be used than in the past [7]. The water used in these carboxylation experiments was boiled and gassed with N_2. Clear solutions of 10 N KOH were used after prolonged storage to settle the K_2CO_3. $Na_2{}^{14}CO_3$ (Amersham) was diluted with $KHCO_3$, pH 9.0, to a concentration of 160 mM, 5 Ci/mol, and stored at 0–4°C with a drop of chloroform on the bottom and paraffin oil on top to prevent bacterial growth and evaporation/exchange, respectively. Triplicate 10 μl samples (removed by a Hamilton syringe) were counted with 0.2 ml Tris free base, 1 M and 5 ml Instagel, to determine the radioactivity, which did not change during prolonged storage.

50 μl of the homogenates and the perfusates were preincubated in closed plastic cups for 1 h at 37°C in 0.12 ml volume. The medium contained 66.7 mM Tris-HCl, pH 7.5; 13.3 mM potassium citrate; 1.33 mM dithiothreitol; 26.7 mM $MgCl_2$, 0.2 mg bovine serum albumin (fatty acid poor), 208 mM sucrose. 0.24 mM acetyl-CoA (pH 4.0) was put in the test tubes and the same volume of water (10 μl) in the blanks. The reaction was started by the addition of 10.7 mM $KH^{14}CO_3$ (see above) and 10.7 mM ATP (pH 7.5) from separate tubes (since part of the $^{14}CO_2$ escapes from a mixture). After mixing the tubes were closed again, and incubated for 5 min at 37°C. The total reaction volume was 0.15 ml. The reaction was terminated with 100 μl of 5 M HCl. The tube was held in a horizontal position, an acid drop placed on the vessel wall, the tube was closed, a short swing was made to allow the acid to reach the incubation mixture and the content was further mixed on a Vortex machine. The tubes were placed in a box with NaOH-pellets, and opened. After 2 h the tubes were poured into scintillation vessels, and rinsed twice with 0.5 ml of ethanol. After standing overnight under vacuum, the vessels were counted in a scintillation counter. The radioactivity of the blanks was very low (100–180 dpm).

2.3. *Assay of propionyl-CoA carboxylase*

Propionyl-CoA carboxylase was measured for 10 min at 37°C in closed plastic cups. The volume was 100 μl. The medium contained 50 mM Tricine-KOH, pH 8.5; 6 mM $MgCl_2$; 100 mM KCl; 1 mM dithiothreitol; 3 mM ATP, pH 8.5; 0.15 μM rotenone and 0.2 μg oligomycin (these inhibitors were added in a total ethanol volume of 1.2 μl); 10.4 mM $KH^{14}CO_3$, pH 9.0 (see above). 0.6 mM propionyl-CoA (pH 4.0) was put in the test tubes and the same volume of water (10 μl) in the blanks. The reaction was started by the addition of 50 μl of the heart fractions, which were pretreated with 1/40 vol. of 1% Lubrox-WX in water at 0°C, for 12 min. The reactions were terminated with 50 μl of 5 M HCl (for further details see section 2.2).

2.4. *Other assays*

Lactate dehydrogenase [12], protein [13] and total carnitine [13] were assayed as described.

3. RESULTS

The sum of the activities (amounts) in the heart fraction and in the effluent of acetyl-CoA carbox-

ylation, propionyl-CoA carboxylase, lactate dehydrogenase, protein and carnitine are given in table 1. Neither these values, nor the percentage recovered in the effluent (in parentheses) were much different in the fed and in the fasted animals. The calcium paradox worked well in releasing the cytosolic components from the hearts, since about 89% of the total carnitine and 71% of the lactate dehydrogenase activity were released. For propionyl-CoA carboxylase activity, on the other hand, less than 1% was released (with one exception), while for the acetyl-CoA carboxylation activity 32% was released (average value). This phenomenon can only be explained by the existence of acetyl-CoA carboxylase activity in rat heart.

The activity ratios of acetyl-CoA carboxylation/propionyl-CoA carboxylase in the 8 heart homogenates and in the effluents were 0.014 ± 0.005 and 3.4 ± 1.9, respectively, a reflection of the preferential release of acetyl-CoA carboxylase activity. Because of the high activity of propionyl-CoA carboxylase in the heart fraction, the acetyl-CoA carboxylation activity of this fraction is caused by propionyl-CoA carboxylase activity with acetyl-CoA and proper acetyl-CoA carboxylase activity retained in the heart. In the effluent the acetyl-CoA carboxylation activity can only be catalyzed by acetyl-CoA carboxylase, since the propionyl-CoA carboxylase activity in this fraction is modest. We calculated the total heart acetyl-CoA carboxylase by assuming that the same percentage of this enzyme was released as that of the cytosolic marker lactate dehydrogenase. The average activity in fed rats was 3.3 ± 1.0 nmol $^{14}CO_3^{2-}$/min per g wet wt and decreased in the 48 h fasted rats to 2.1 ± 0.6.

When the acetyl-CoA carboxylation activity was determined without citrate in the preincubation step, the activity in the homogenate fell to about 1/3 and to 1/10 in the effluent.

Table 1

Activities of acetyl-CoA carboxylation, acetyl-CoA carboxylase, propionyl-CoA carboxylase, lactate dehydrogenase, and amounts of protein and total carnitine in heart homogenate plus 10 min effluent after the calcium paradox

	Acetyl-CoA carboxylation	Acetyl-CoA carboxylase	Propionyl-CoA carboxylase	Lactate dehydrogenase	Protein	Carnitine
Fed rats						
1	9.0 (36)	4.3 (77)	266 (0.36)	317 (77)	141 (14)	1.10 (92)
2	3.7 (42)	2.6 (58)	315 (0.10)	236 (58)	184 (8)	1.19 (81)
3	8.0 (40)	4.0 (80)	258 (0.88)	352 (80)	132 (13)	1.22 (93)
4	5.1 (25)	2.1 (60)	331 (0.13)	247 (60)	154 (13)	1.54 (92)
Average	6.4 (35)	3.3 (68)	293 (0.37)	288 (68)	153 (10)	1.26 (90)
± SD	2.5 (7)	1.0 (11)	36 (0.36)	57 (11)	23 (5)	0.19 (6)
Fasted rats						
1	5.8 (16)	1.5 (62)	312 (2.18)	415 (62)	134 (10)	1.24 (77)
2	5.9 (31)	2.6 (69)	293 (0.16)	199 (69)	133 (9)	0.94 (89)
3	5.4 (40)	2.5 (84)	330 (0.18)	254 (84)	145 (14)	1.35 (95)
4	5.6 (26)	1.8 (80)	309 (0.10)	291 (80)	107 (17)	1.30 (92)
Average	5.7 (28)	2.1 (74)	311 (0.65)	290 (74)	130 (12)	1.21 (88)
± SD	0.2 (10)	0.6 (10)	15 (1.02)	92 (10)	16 (4)	0.18 (8)

Percentage of the released activities and amounts in the effluent are given between parentheses. The activities are given in nmol $^{14}CO_3^{2-}$/g wet wt for the carboxylations, μmol pyruvate/min per g for lactate dehydrogenase, mg/g for protein and μmol/g for total carnitine. The acetyl-CoA carboxylase activity was calculated by making use of the partition of the cytosol marker lactate dehydrogenase. The total cardiac acetyl-CoA carboxylase activity was calculated by multiplying the acetyl-CoA carboxylase activity in the effluent by 100/percentage lactate dehydrogenase in the effluent. The acetyl-CoA carboxylase activity decreased significantly ($P < 0.05$, Student's t-test) by fasting. The volume of the effluent was 60 ± 15 ml in the fed rats and 69 ± 17 ml in the fasted rats (per g heart ± SD)

4. DISCUSSION

With the aid of the calcium paradox, a good tool to separate cytosolic from particle-confined enzyme and substances (see also [14]), we were able to prove the existence of cytosolic acetyl-CoA carboxylase activity in rat heart. This activity decreases to 64% by 48 h of fasting, while it then almost disappears in lipogenetic tissues. In rat heart, if no de novo fatty acid synthesis and microsomal elongation from malonyl-CoA occurs, as in guinea pig heart [7], it is likely that the only function of the enzyme is the production of malonyl-CoA for the regulation of the activity of carnitine palmitoyltransferase I. The regulation of acetyl-CoA carboxylase could be identical to that in lipogenetic tissues of insulin and stress hormones. Malonyl-CoA is not the only determinant of the carnitine palmitoyltransferase I activity. In rat hepatocytes an independent cyclic AMP-dependent phosphorylation mechanism of carnitine palmitoyltransferase has been detected [15]. Another important factor which affects the rate of mitochondrial oxidation of long-chain fatty acids is the rate of lipolysis in adipose tissue. In human pathology other factors like the oxygen availability, deficiencies of carnitine or carnitine palmitoyltransferase II and respiratory chain defects become important.

REFERENCES

[1] McGarry, J.D. and Foster, D.W. (1980) Annu. Rev. Biochem. 49, 395–420.
[2] Bremer, J. (1981) Biochim. Biophys. Acta 665, 628–631.
[3] Brownsey, R.W. and Denton, R.M. (1982) Biochem. J. 202, 77–86.
[4] Holland, R., Hardie, D.G., Clegg, R.A. and Zammit, V.A. (1985) Biochem. J. 226, 139–145.
[5] Veerkamp, J.H. and Van Moerkerk, H.T.B. (1982) Biochim. Biophys. Acta 710, 252–255.
[6] McGarry, J.D., Mills, S.E., Long, C.S. and Foster, D.W. (1983) Biochem. J. 214, 21–28.
[7] Wit-Peeters, E.M., Scholte, H.R. and Elenbaas, H.L. (1970) Biochim. Biophys. Acta 210, 360–370.
[8] Hülsmann, W.C. (1966) Biochim. Biophys. Acta 125, 398–400.
[9] Scholte, H.R. (1969) Biochim. Biophys. Acta 178, 137–144.
[10] Scholte, H.R. (1973) Biochim. Biophys. Acta 309, 457–465.
[11] Zimmermann, A.N.E. and Hülsmann, W.C. (1966) Nature 211, 646–647.
[12] Kornberg, A. (1955) Methods Enzymol. 1, 441–443.
[13] Barth, P.G., Scholte, H.R., Berden, J.A., Van der Klei-Van Moorsel, J.M., Luyt-Houwen, I.E.M., Van 't Veer-Korthof, E.T., Van der Harten, J.J. and Sobotka-Plojhar, M.A. (1983) J. Neurol. Sci. 62, 327–355.
[14] Hülsmann, W.C. (1983) Eur. Heart J. 4, suppl. H., 57–61.
[15] Harano, Y., Kashiway, A., Kojima, H., Suzuki, M., Hashimoto, T. and Shigeta, Y. (1985) FEBS Lett. 188, 267–272.