THE ROLE OF ACETOACETATE IN THE TRANSFER OF ACETYL UNITS OUTSIDE THE MITOCHONDRIA IN LIVER AND ADIPOSE TISSUE OF RATS OR MICE

S. ROUS and P. FAVARGER
Département de Biochimie médicale, Université de Genève, Suisse

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1. Introduction

It was recently proposed that acetoacetate could act as a carrier of acetyl-CoA outside the mitochondria for fatty acid synthesis in brain [1]. An acetoacetyl-CoA synthetase activity has also been found in the liver of fed rats [2]. However, the mode of transfer of acetyl-CoA in organs where lipogenesis is very intensive, e.g. liver or adipose tissue, has not been determined so far. If citrate is an effective carrier of acetyl-CoA in the livers of rats [3–13] its role seems to be more modest in that of mice and of little effect in adipose tissues of both these species [14]. Therefore, this work was carried out in order to determine whether acetoacetate could compete with citrate for transporting acetyl-CoA into liver and adipose tissue of mice or rats.

To this end, we have compared the incorporations of [2-^{14}C]leucine or [U-^{14}C]leucine into fatty acids of rats and mice. After degradation, [2-^{14}C]leucine yields only labeled acetyl-CoA while [U-^{14}C]leucine is converted in the mitochondria into both radioactive acetoacetate and acetyl-CoA. Thus, if acetyl-CoA emerges from mitochondria in the form of acetoacetate a similar or a better incorporation of [U-^{14}C]leucine than of that [2-^{14}C]leucine should be obtained.

Our results demonstrate that, except in rat liver, where it is known that citrate cleavage enzyme plays an important role in fatty acid synthesis, [U-^{14}C]leucine is better incorporated than [2-^{14}C]leucine. This clearly indicates an important role of acetoacetate for the diffusion of acetyl-CoA.

2. Experimental

15 fed mice (30–35 g) or fed rats (250–300 g), received an intravenous injection of [2-^{14}C]L leucine (46 mCi/mM) (CEA France), or [U-^{14}C]L leucine (10 mCi/mM) (Radiochemical Center Amersham). The first was diluted to obtain a same millimolar radioactive concentration than [U-^{14}C]leucine. The animals were killed 10 min (mice) or 15 min (rats) later. Liver and adipose tissue were quickly excised and fixed with alcoholic KOH. After elimination of unsaponifiable matter, fatty acids were extracted with petroleum ether. A washing-out with unlabeled leucine and three washings with ethanol/water (1:1) were carried out. The radioactivity of fatty acids was determined in a liquid scintillation counter (Intertechnique).

3. Results and discussion

The comparison of 2-^{14}C and U-^{14}C seemed to us adequate for estimating the proportion of mitochondrial acetyl-CoA which can leave the mitochondria in the form of acetoacetate or acetyl-CoA since [2-^{14}C]leucine is converted solely to acetyl-CoA while [U-^{14}C]leucine produces both acetoacetate and acetyl-CoA. If the acetoacetate part derived from leucine leaves the mitochondria to the same extent as acetyl-CoA, then in no case, should the value found for the incorporation of [U-^{14}C]leucine be more than 5/6 of that of [2-^{14}C]leucine since one of the six carbons of [U-^{14}C]leucine is lost during its degradation into acetate and acetyl-CoA. A better incorporation of [U-^{14}C]leucine than that of [2-^{14}C]leucine would

Table 1
Incorporation of [2-^{14}C] or [U-^{14}C]leucine into liver or adipose tissues fatty acids of rats or mice.

	[2-^{14}C]leucine		[U-^{14}C]leucine	
	Liver	Adipose tissue	Liver	Adipose tissue
		$p < 0.01$		
Rats	80 330	10 160	54 300	13 520
	$p < 0.001$			
		$p < 0.01$		
Mice	42 220	12 045	76 000	18 350
	$p < 0.001$			

These results represent the total radioactivity of fatty acids expressed in dpm for a dose of 10 μCi of each precursor.

mean that acetoacetate diffuses outside the mitochondria more easily than does acetyl-CoA in the form for example of citrate or acetylcarnitine.

In rats, the radioactivity of liver fatty acids is about double when animals received [2-^{14}C]leucine than [U-^{14}C]leucine. On the contrary the incorporations of U-^{14}C is slightly higher than that of 2-^{14}C in adipose tissues (cf. table 1).

In mice, [U-^{14}C]leucine is better incorporated than [2-^{14}C]leucine (cf. table 1) both in liver and adipose tissue fatty acids.

These results are in agreement with our earlier report concerning the importance of citrate in fatty acid synthesis. It is quite normal that in rat liver [U-^{14}C]leucine did not show better incorporation than [2-^{14}C]leucine into fatty acids, since in this organ citrate plays an important role for the transfer of acetyl-CoA. In mice, we found that the participation of citrate was weaker. Thus, acetoacetate could substitute for citrate in the transfer of acetyl units outside the mitochondria, in mice liver as well as in adipose tissue of rats and mice.

Nevertheless this work does not assume that only citrate and acetoacetate play a role in the transfer of acetyl-CoA for fatty acid synthesis. Other pathways have been envisaged like acetylcarnitine [15, 16], free acetate [17], acetylaspartate [18]. Our results point to the fact that this problem is much more complex and that several systems function simultaneously for the diffusion of acetyl-CoA outside the mitochondria. It is difficult to ascribe an order of importance to either of these mechanisms since efficiency of the various carriers are probably different in liver and adipose tissue. Moreover if citrate plays a relatively important role in liver, then acetoacetate is likely the most effective carrier in adipose tissue. This pathway could be important in human adipose tissue where the activity of the citrate cleavage enzyme is very low [19, 20]. Studies are in progress to verify this hypothesis.

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