THE ROLE OF 3-OXO ACID-CoA TRANSFERASE IN THE REGULATION OF KETOGENESIS IN THE LIVER

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1. Introduction

It has usually been accepted that liver cannot utilise ketone bodies since the activity of 3-oxo acid-CoA transferase is either very low or non-detectable [1,2]. In a limited comparative study, 3-oxo acid-CoA transferase activity was detected in the livers of some fish, but it was suggested that the enzyme was involved in ketone body production rather than utilisation [3]. In order to investigate the possible role of this enzyme in ketone body metabolism in the liver, the activities of the HMG-CoA synthase (EC 4.1.3.5), HMG-CoA lyase (EC 4.1.3.4), acetoacetyl CoA hydrolase (EC 3.1.2.11), 3-oxo acid-CoA transferase (EC 2.8.3.5) and 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) have been measured in livers from 13 different vertebrates. The results are presented and discussed here.

2. Materials and methods

Animals, chemicals and enzymes were obtained from sources described in [4–6]. Animals were killed and livers were homogenized in ground glass homogenizers with 5 vol. extraction medium at 0°C. The extraction medium for 3-oxo acid-CoA transferase consisted of 10 mM Tris/HCl, 1 mM mercaptoethanol, 1 mM EDTA and 3 mM $MgCl_2$ at pH 7.4. The extraction medium for 3-hydroxybutyrate dehydrogenase consisted of 50 mM triethanolamine, 1 mM EDTA, 2 mM $MgCl_2$ and 30 mM mercaptoethanol at pH 7.5. For the assay of 3-hydroxybutyrate dehydrogenase the homogenate was sonicated at 0°C for two 15 s periods with a microprobe of an MSE 100 W ultrasonic disintegrator operating at an amplitude of 6 μm. For the assays of 3-oxo acid-CoA transferase and acetoacetyl-CoA thiolase the homogenates were treated with Triton X-100: a volume (100 μl) of a 1% (v/v) solution of Triton X-100 was added to the cuvette immediately prior to assay. HMG-CoA lyase, acetoacetyl-CoA hydrolase and, for all animals except the fish, HMG-CoA synthase were assayed as in [7]. For fish, HMG-CoA synthase was assayed spectrophotometrically as in [8]. Preliminary experiments established that very similar activities were obtained when using either of the assays for synthase. 3-Hydroxybutyrate dehydrogenase was assayed in the direction hydroxybutyrate oxidation [9] for all animals except the fish. 3-Hydroxybutyrate dehydrogenase in fish was assayed in the direction of hydroxybutyrate formation [10]. Preliminary experiments indicated that the activities in either direction were very similar. 3-Oxo acid-CoA transferase was assayed in the direction of acetoacetate formation by following the decrease in A_{303} caused by acetoacetyl-CoA cleavage [11].

3. Results and discussion

Here, the activities of 3-oxo and CoA-transferase were found in livers from very different animals ranging from fish to mammals and, particularly in the teleost fish, the activities were very high (e.g., trout, herring, bass; see table 1). The enzyme was suggested [3] to be involved in the production of ketone bodies in livers in which the capacity of the HMG-

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Table 1

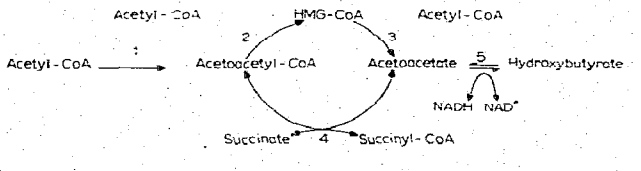
Maximal Activities of HMG-CoA Synthase, HMG-CoA lyase, acetoacetyl-CoA hydrolase, 3-hydroxybutyrate dehydrogenase and 3-oxo acid-CoA transferase in livers from vertebrates

Animal	Enzyme activities (μmol substrate utilized $.min^{-1}$ .g fresh wt $tissue^{-1}$				
	HMG-CoA synthase	HMG-CoA lyase	Acetoacetyl-CoA hydrolase	3-hydroxybutyrate dehydrogenase	3-Oxo acid-CoA transferase
Pisces					
Rainbow trout	0.41	1.3	0.34	–	10.0
(*Salmo gairdneri*)	(0.36–0.51)	(1.0–1.5)	(0.30–0.40)		(8.5–12.2)
	(7)	(5)	(5)		(3)
Mackerel[a]	0.14	0.70		< 0.01	4.0
(*Scombrus scombrus*)	(0.08–0.20)	(0.31–1.30)	–		(3.5–4.7)
	(3)	(6)		(4)	(6)
Herring[a]	0.13	1.9		< 0.01	14.4
(*Clupea harengus*)	(0.08–0.21)	(1.9–1.9)	–		(14.1–14.5)
	(3)	(2)		(3)	(3)
Bass[a]	0.25	0.45	–	< 0.01	9.15
(*Dicentrarcus labrax*)	(0.22–0.48)	(0.40–0.53)			(8.7–9.8)
	(8)	(3)		(3)	(3)
Plaice[a]	0.22	0.47	–	< 0.01	4.8
(*Pleuronecthes*	(0.10–0.33)	(0.40–0.50)			(4.3–5.1)
platessa)	(5)	(3)		(2)	(3)
Dogfish[a]	0.41	0.55	< 0.01	0.16	0.80
(*Scylliorhinus canicula*)	(0.22–0.61)	(0.51–0.58)	(6)	(0.06–0.32)	(0.44–1.1)
	(8)	(3)		(3)	(3)
Ray[a]	0.32	0.71	–	–	0.21
(*Raja clavata*)	(0.27–0.35)	(0.65–0.76)			(0.15–0.27)
	(3)	(3)			(4)
Reptilia					
Green lizard	0.51	3.6	< 0.01	1.5	0.68
(*Lacerta viridis*)	(0.43–0.60)	(3.0–4.3)		(1.3–1.7)	(0.60–0.77)
	(2)	(2)	(2)	(2)	(2)
Aves					
Domestic pigeon	1.7	8.2	0.26	0.15	1.5
(*Columba livia*)	(1.5–1.9)	(5.6–11.1)	(0.20–0.45)	(0.13–0.17)	(1.0–1.7)
	(10)	(5)	(3)	(5)	(8)
Domestic fowl	1.7	4.3	0.33	0.22	1.0
(*Gallus gallus*)	(1.5–2.0)	(3.9–5.0)	(0.20–0.42)	(0.19–0.29)	(0.9–1.4)
	(7)	(4)	(6)	(6)	(6)
Mammalia					
Laboratory mouse	1.1	3.8	0.44	2.3	0.86
	(0.9–1.5)	(3.6–4.0)	(0.39–0.50)	(1.9–2.7)	(0.52–1.2)
	(6)	(4)	(8)	(4)	(5)
Laboratory rat	1.8	9.8	0.21	12.4	0.46
	(1.6–2.1)	(8.0–12.7)	(0.18–0.24)	(9.4–20.3)	(0.39–0.52)
	(6)	(5)	(6)	(6)	(5)
Rabbit	0.58	2.8	0.05	2.3	0.21
	(0.37–0.70)	(2.4–3.3)	(0.04–0.06)	(1.7–2.8)	(0.16–0.32)
	(16)	(5)	(6)	(7)	(10)

Enzyme activities are presented as means, with the ranges of activities and the number of separate animals given in parentheses. Enzyme activities were measured at 25°C except for enzymes from a number of fish[a] which were measured at 10°C

CoA pathway was low. However, 3-oxo acid-CoA transferase is present in livers in which the activities of the enzymes of the HMG-CoA pathway are high (e.g., birds; see table 1). Furthermore, during starvation, large quantitites of ketone bodies are produced in the dogfish, in which 3-oxo acid-CoA transferase activity is low, whereas ketone bodies are not produced in the bass during starvation despite high activities of the transferase [6]. Consequently, it is suggested that, as for muscle, the net flux through the transferase reaction is in the direction of ketone body utilisation.

It is proposed that the role of 3-oxo acid-CoA transferase in liver is to produce a substrate cycle between acetoacetyl CoA and acetoacetate as follows:



[Reactions 1,2,3,4 and 5 are catalysed by acetoacetyl CoA thiolase, HMG-CoA synthase, HMG-CoA lyase, 3-oxo acid-CoA transferase, and 3-hydroxybutyrate dehydrogenase, respectively.] The maximum rate of ketogenesis in the liver of the fed rat is calculated to be 0.4 μmol $\cdot min^{-1} \cdot g^{-1}$ at 25°C [13] whereas the activity of the transferase is ~0.5 μmol $\cdot min^{-1} \cdot g^{-1}$ which suggests that the activity of the transferase is sufficient to restrict acetoacetate formation via the operation of a substrate cycle. The activity of a transferase will increase the steady state concentration of acetoacetyl-CoA which is both a product inhibitor of acetoacetyl-CoA thiolase [14] and a substrate inhibitor of HMG-CoA synthase [8]. Indeed it has been shown that the rate of ketogenesis in isolated mitochondria is very sensitive to the physiological concentration of acetoacetyl-CoA [15]. Thus, the presence of the transferase in liver will reduce the rate of ketogenesis directly by the removal of acetoacetate and indirectly by inhibition of flux through the HMG-CoA synthase reaction.

If 3-oxo acid-CoA transferase catalyses a near-equilibrium reaction, the concentrations of CoASH, succinyl-CoA and/or acetoacetate could regulate the enzyme activity. Since the concentration of acetoacetate is dependent upon the intramitochondrial $NAD^+/NADH$ concentration ratio, via the equilibrium catalysed by hydroxybutyrate dehydrogenase, this provides an explanation for the existence of two ketone bodies, acetoacetate and hydroxybutyrate. A low $NAD^+/NADH$ ratio in the mitochondria of the liver will favour the formation of hydroxybutyrate and reduce the concentration of acetoacetate, which will decrease the activity of the transferase, lower the concentration of acetoacetyl-CoA and lead to an increased rate of ketogenesis. On the other hand, a high $NAD^+/NADH$ ratio will increase the concentration of acetoacetate, which will increase the activity of the transferase and lead to a decrease in the rate of ketogenesis. There is, in fact, some experimental evidence to support these suggestions. It has been shown that in a mitochondrial extract of rat liver, a low $NAD^+/NADH$ concentration ratio increases the rate of ketogenesis and lowers the concentration of acetoacetyl-CoA: an increase in the $NAD^+/NADH$ concentration ratio had the opposite effects [15]. Thus the hypothesis proposes that hydroxybutyrate dehydrogenase is present in liver to provide a means of controlling the rate of ketogenesis via the $NAD^+/NADH$ concentration ratio, and as a consequence of this and the diffusability of acetoacetate, both ketone bodies are produced by the liver. The fact that high rates of ketogenesis and high levels of ketone bodies in the blood are associated with elevated 3-hydroxybutyrate/acetoacetate concentration ratios (and therefore lower $NAD^+/NADH$ ratios in the liver) [13,16] is also consistent with this hypothesis.

References

[1] Williamson, D. H. and Hems, R. (1970) in: Essays in Cell Metabolism (Bartley, W. et al. eds) pp. 257–281, Wiley Interscience, London.
[2] Greville, G. D. and Tubbs, P. K. (1968) Essays Biochem. 4, 155–212.
[3] Phillips, J. W. and Hird, F. J. R. (1977) Comp. Biochem. Physiol. 57B, 133–138.
[4] Sugden, P. H. and Newsholme, E. A. (1973) Biochem. J. 134, 97–101.
[5] Alp, P. R., Zammit, V. A. and Newsholme, E. A. (1976) Biochem. J. 154, 689–700.
[6] Zammit, V. A. and Newsholme, E. A. (1979) submitted.
[7] Williamson, D. H., Bates, M. W. and Krebs, H. A. (1968) Biochem. J. 108, 353–361.

[8] Reed, W. D., Clinkenbeard, K. D. and Lane, D. M. (1975) J. Biol. Chem. 250, 3117–3123.
[9] Lehninger, A. L., Sudduth, H. C. and Wise, J. B. (1960) J. Biol. Chem. 235, 2450–2455.
[10] Williamson, D. H. and Kuenzel, P. (1971) Biochem. J. 121, 569–570.
[11] Williamson, D. H., Bates, M. W., Page, M. A. and Krebs, H. A. (1971) Biochem. J. 121, 41–47.
[12] Beis, A. (1977) PhD Thesis, Oxford University.
[13] Krebs, H. A. (1966) Adv. Enzyme Reg. 4, 339–353.
[14] Huth, W., Jones, R., Wunderlich, I. and Seubert, W. (1975) Eur. J. Biochem. 59, 475–489.
[15] Huth, W., Stermann, R., Holze, G. and Seubert, W. (1978) in: Biochemical and Clinical Aspects of Ketone Body Metabolism. (Söling, H. D. and Seufert, C. D. eds) G. Thiene, Stuttgart.
[16] Cahill, G. F., Herrera, M. G., Morgan, A. P., Soldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A. and Kipnis, D. M. (1966) J. Clin. Invest. 45, 1751–1769.