LONG CHAIN ACYL CoAs, ADENINE NUCLEOTIDE TRANSLOCASE AND THE COORDINATION OF THE REDOX STATES OF THE CYTOSOLIC AND MITOCHONDRIAL COMPARTMENTS

Patricia McLEAN, K.A. GUMAA and A.L. GREENBAUM

Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London W1P 5PR

and

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

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1. Introduction

The recent demonstration by Shug et al. [1] of the inhibition of the adenine nucleotide translocase of isolated rat liver mitochondria by long chain acyl CoA derivatives draws attention to another possible locus of action of such compounds which could have important implications in the regulation of metabolism. These authors point out that the inhibition of the adenine nucleotide translocase would have the effect of causing a state 3 to 4 transition (as defined by Chance and Williams [2]) and they have used this idea to provide a possible explanation for the very low respiratory activity of the mitochondria of hibernating animals. If, in fact, their observed *in vitro* inhibition of the translocase also occurred under *in vivo* conditions where raised tissue levels of long chain acyl CoAs prevailed, then it is possible to predict a sequence of changes in the tissues of animals subjected to dietary or hormonal treatments which modify the tissue content of long chain acyl CoAs. Among the consequences of an accumulation of such compounds, three may be noted as immediate effects, all arising directly as a result of the state 3 → state 4 transition: (1) an increase of the ratio ATP/ADP × Pi in the mitochondria; (2) a change of the mitochondrial NAD^+/NADH ratio in favour of the reduced form and (3) a decreased flux through the tricarboxylic acid cycle.

Marked changes in the long chain acyl CoA content of the liver occur in conditions such as starvation or starvation followed by refeeding a high fat diet [3, 4] and it should be possible to show whether alterations in the mitochondrial ATP/ADP × Pi and NAD^+/NADH ratios also occur in these conditions. It is shown below that, in those conditions where long chain acyl CoAs accumulate, there is, indeed, an increase of the mitochondrial ATP/ADP × Pi ratio and a decrease of the NAD^+/NADH ratio and it has previously been shown [5] that the flux in the tricarboxylic acid cycle also follows the predicted pattern. It has also been found that, in such animals, the normal co-ordination between the cytosolic and mitochondrial redox states, which keeps the ratio between the two compartments at approximately 150:1 (when the redox states are calculated from the reactants and equilibrium constants of lactate dehydrogenase and glutamate dehydrogenase respectively), is disrupted and that the redox states of the two compartments vary in an independent manner with the cytosolic compartment becoming relatively more oxidized than the mitochondrial.

2. Methods

The treatment of animals is as previously described [5] but may be briefly summarized as follows:

Table 1
Changes in the relative redox state of the cytosolic and mitochondrial $NAD^+/NADH$ and of the ATP/ADP × Pi ratios in relation to the liver content of long chain acyl CoA and free fatty acid.

Condition	Long chain acyl CoA mμmole/g	Free fatty acids μ equivs/g	Cytosolic ATP/ADP × Pi	Mitochondrial ATP/ADP × Pi +	Ratio Cytosolic $NAD^+/NADH$ / Mitochondrial $NAD^+/NADH$	
					LDH/HBDH[a]	LDH/GDH[b]
Control	83 ± 11	4.03 ± 0.49	780 ± 186	4.83×10^{-2}	75 ± 16	156 ± 34
Alloxan diabetic	105 ± 6	6.76 ± 1.09*	196 ± 57**	4.65×10^{-2}	62 ± 14	157 ± 36
Alloxan diabetic, treated with insulin, 3 days	73 ± 3	1.83 ± 0.34**	372 ± 40*	3.58×10^{-2}	42 ± 5	176 ± 27
Starved 3 days, refed carbohydrate, 3 days	72 ± 6	3.12 ± 0.39	1160 ± 260	4.41×10^{-2}	83 ± 16	189 ± 47
Nicotinamide-treated, 4 hr	90 ± 8	23.20 ± 2.78***	918 ± 193	3.40×10^{-2}	71 ± 17	120 ± 24
Nicotinamide-treated, 6hr	92 + 6	3.61 ± 0.61	1025 ± 220	4.30×10^{-2}	83 ± 13	190 ± 37
Starved, 3 days	131 ± 11**	5.05 ± 0.67	588 ± 143	7.03×10^{-2}	132 ± 19*	335 ± 35**
Starved, 3 days refed fat, 3 days	144 ± 4***	3.75 ± 0.57	499 ± 112	6.78×10^{-2}	196 ± 43*	437 ± 63**

Values are the means ± S.E.M. and represent groups containing not less than 6 rats.

+ Calculated from the mean value of the compartmented metabolites and redox state of each group.

Cytoplasmic $NAD^+/NADH$ calculated from the reactants and equilibrium constant of lactate dehydrogenase (LDH).

Mitochondrial $NAD^+/NADH$ calculated from, [a] the reactants and equilibrium constant of β-hydroxybutyrate dehydrogenase (HBDH) or from, [b] the reactants and equilibrium constant of glutamate dehydrogenase (GDH).

Levels of significance are: *, < 0.05; **, < 0.01; ***, < 0.001.

alloxan diabetic animals were used 3–4 weeks after the induction of diabetes: insulin treatment was for 3 days, 2 I.U. PZ1/day; starvation was for 3 days and the refeeding regime following this starvation was also for 3 days using either a high fat or a high carbohydrate diet; nicotinamide treatment was for either 4 or 6 hr at a dose level of 50 mg/100 g body weight [6].

The measurements of metabolites were as previously described [5, 6]. Direct measurement of mitochondrial ATP/ADP × Pi or of NAD^+/NADH is, of course, not practical, but both ratios may be calculated, the former by the use of the pyruvate carboxylase equilibrium as described by Krebs and Veech [7] and the latter by the use of the reactants and equilibrium constants of β-hydroxybutyrate dehydrogenase or of glutamate dehydrogenase [8]. The corresponding cytosolic values may be obtained from the substrate and product and the equilibrium constant of the combined glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglycerate kinase reaction [9] and the NAD^+/NADH from the lactate dehydrogenase reaction [8]. In the calculation of the mitochondrial ATP/ADP × Pi, use has been made of the compartmented mitochondrial contents for the relevant metabolites, the distribution of which was obtained by the procedure of Williamson [10], rather than the measured whole cell values since such compartmented values are clearly more relevant in that they relate the enzyme to the accessible substrate in the same compartment. The main bulk of the metabolite data used in the present calculations is taken from previous publications [5, 6] as are the calculated values for the mitochondrial contents of the appropriate metabolites.

3. Results and discussion

Table 1 shows the changes in the liver content of long chain acyl CoAs and free fatty acids in a number of conditions which are known to lead to considerable variations of the redox states of the two intracellular compartments. Of the conditions studied, only two, starvation and starvation followed by refeeding a high fat diet, caused a significant change in the content of long chain acyl CoAs, both treatments leading to a substantial accumulation of these compounds. The free fatty acid content of the liver varied over a wide range with significant increases found in diabetic animals and in rats treated with nicotinamide for 4 hr, and a significant fall occurred in the diabetic group treated with insulin.

Table 1 also shows the calculated values for the cytosolic and mitochondrial ATP/ADP × Pi. These values show excellent agreement with those found by Veech, Raijman and Krebs [9] and by Krebs and Veech [7]. Three points concerning the adenine nucleotide ratio emerge from this table: (1) that the cytosolic values vary over a much wider range than the mitochondrial values; (2) that no parallelism exists between the ratios in the mitochondrial and cytosolic compartments and (3) that, while the mitochondrial ratio remains remarkably constant in six of the conditions, despite their gross differences in physiological status, in the remaining two conditions, starvation and starvation followed by refeeding a high fat diet, the ratio significantly shifts towards the more phosphorylated form, i.e. the shift only occurs in those conditions where an accumulation of long chain acyl CoAs occurs. The correlation co-efficient for the relation between long chain acyl CoA content and mitochondrial ATP/ADP × Pi ratio is 0.847 and is significant at the 1–5% level.No comparable correspondence is shown between the more phosphorylated state of the mitochondrial adenine nucleotides and the free fatty acid content ($\rho = -0.340$), in keeping with the findings of Shug et al. [1] that, while the long chain acyl CoAs inhibited the adenine nucleotide translocase, free fatty acids did not.

Fig. 1 shows the redox state of the NAD couple in the cytosolic and mitochondrial compartments and the ratio of the cytosolic: mitochondrial NAD^+/NADH. As has been previously reported [11] conditions where carbohydrate is the main metabolic fuel lead to a more oxidized state of the NAD couple in the mitochondria, while conditions where carbohydrate oxidation is supplanted by fat oxidation lead to a more reduced state of the couple in the mitochondria. Krebs and Veech [11, 12] have described the systems which regulate the redox state of the two compartments by a sharing of some metabolites by equilibrium enzymes in the cytosol and mitochondria and the way in which these adjust the relative redox states so that the cytosolic

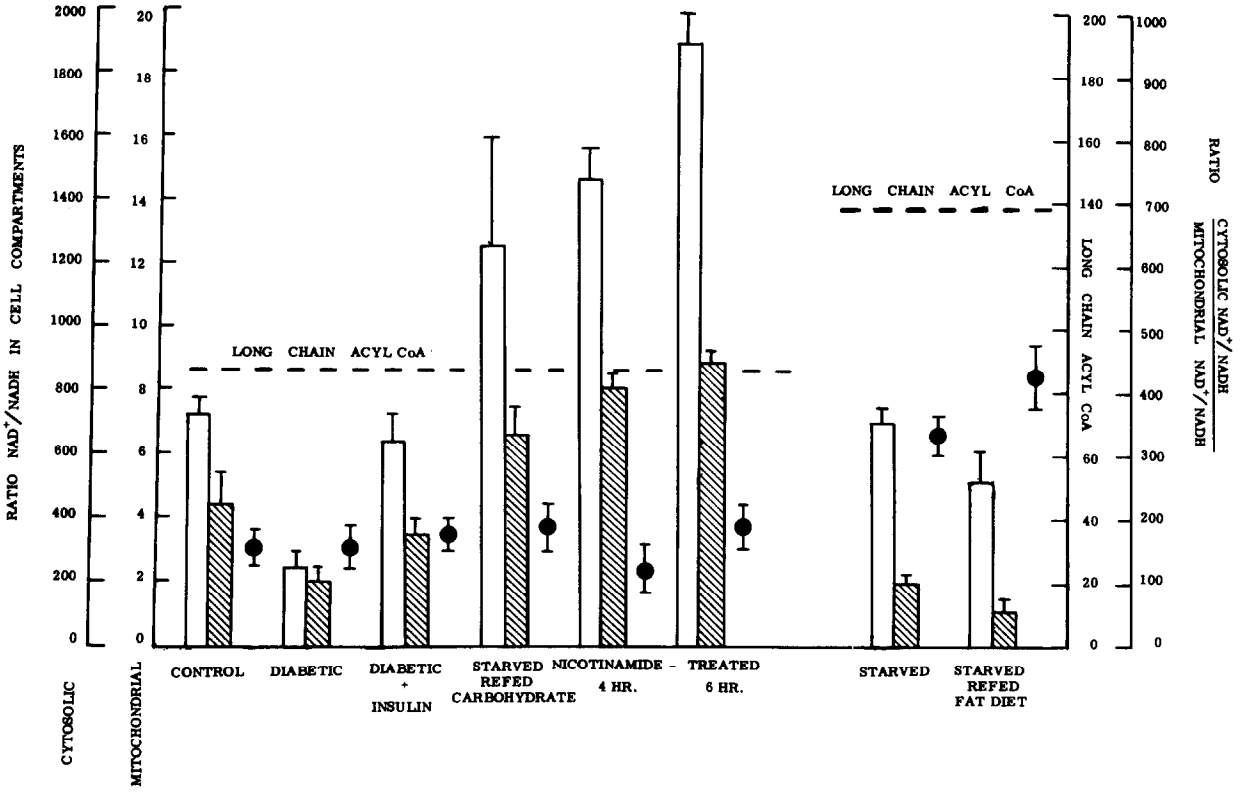

Fig. 1. Variations in the relationship of the cytosolic and mitochondrial redox states in different conditions. Open columns; cytosolic $NAD^+/NADH$; hatched columns; mitochondrial $NAD^+/NADH$; closed circles; ratio of cytosolic $NAD^+/NADH$/mitochondrial $NAD^+/NADH$. The vertical lines represent the SEM, each group contains no less than six values. The horizontal broken line is the mean value of the long chain acyl CoA content of the two populations; for individual values and SEM see table 1.

$NAD^+/NADH$ ratio exceeds that of the mitochondria by a factor of approximately 100–200 (depending on the enzyme used for the calculation). In the conditions studied here the ratio of the cytosolic $NAD^+/NADH$: mitochondrial $NAD^+/NADH$ lies between 100–200 (GDH) or 40–80 (HBDH) for 6 states, but rises to about 350 and 450 (GDH) and 130 and 200 (HBDH) in the two conditions where long chain acyl CoAs accumulate. The coorelation co-efficient for the long chain acyl CoAs against the ratio cytosolic $NAD^+/NADH$: mitochondrial $NAD^+/NADH$ is 0.734, i.e. significant at the 1–5% level. It is worth noting that, in the two groups, starved and starved, refed fat, where the ratio deviates from that found in the other six groups, the long chain acyl CoA content is some 40–50 mμmole/g above that of the mean content of the other groups. This figure takes on added significance when considered in relation to the value of 30 μM long chain acyl CoA producing a 90% inhibition of the adenine nucleotide translocase activity [1]. While it is not possible to relate the content of long chain acyl CoAs given in table 1 to concentrations (due to binding, compartmentation etc.) it may be important that the increased content found in starvation and starvation followed by refeeding high fat falls in the inhibitory range described by Shug et al. [1].

It is thus apparent that the redox states can, under certain conditions, vary in an independent manner, i.e. the linking systems for the two compartments do not achieve equilibrium across the mitochondrial membrane. The reason for this failure

to equilibrate cannot be ascribed to the free fatty acid content of the livers since the level of these is significantly raised in diabetes and in rats treated with nicotinamide for 4 hr and depressed in diabetic rats treated with insulin and in all three of these conditions the normal relationship between the cytosolic and mitochondrial compartments holds. In the two conditions where the relationship fails, the long chain acyl CoA content of the liver is significantly raised (see above). If the accumulation of long chain acyl CoA inhibits the adenine nucleotide translocase *in vivo*, as shown by Shug et al. [1] to occur *in vitro*, then it might be anticipated that the shift of the ATP/ADP X Pi to a more phosphorylated state in the mitochondria (table 1) would not be transmitted to the cytosolic compartment and, hence, a link between the redox state of the two compartments would be broken. The importance of the adenine nucleotide translocase in compartmentation and metabolism has been reviewed by Klingenberg [13] and it is possible that, if long chain acyl CoAs modify the activity of this system, then the effects of some dietary and hormonal conditions are mediated through this means.

It is possible to consider that the oxidation of these long chain acyl CoAs in the mitochondria is, itself, responsible for the shift in the relative redox states of the two compartments.

That the long chain acyl CoAs can cause a disturbance of the co-ordination of the redox state between the cytosol and mitochondria, independent of their metabolism within the mitochondria, is shown by the experiments of Williamson et al. [14] who employed the inhibitor (+) decanoylcarnitine which permits the activation of fatty acids to the CoA derivatives, but prevents their further metabolism by blocking the transfer of these derivatives across the mitochondrial membrane, thus separating the effects of formation and accumulation of long chain acyl CoAs from those following oxidation. In the experiments of Williamson et al. [14], employing a liver perfusion system, the accumulation of long chain acyl CoAs (induced by the addition of oleate to the perfusion medium) increased the ratio of cytosolic NAD^+/NADH to mitochondrial NAD^+/NADH both in the presence and absence of (+) decanoylcarnitine, i.e. the effect is observed independent of the entry of the long chain acyl CoAs into the mitochondrial compartment. One explanation of this effect is that the accumulation of long chain acyl CoAs in the cytosol is acting on the mitochondrial membrane, possibly on some transport system (s) and it is the failure of these translocases which leads to the severing of the link between the redox state of the two compartments.

An alternative hypothesis to this is to suppose that the effect of the long chain acyl CoA derivatives is not limited to the adenine nucleotide translocase but may also affect other transport mechanisms located in the same membrane. Inhibition of the 2-oxoglutarate translocase would produce a similar effect to that described above since the free movement of 2-oxoglutarate is an essential factor in the coupling of the NAD^+/NADH pools of the two compartments [12]. In this latter context it is interesting to note that, in starved rats treated with quinolinate, the equilibration between the glutamate and β-hydroxybutyrate dehydrogenase systems is disrupted [15]. Williamson et al. [15] have suggested that this may be due to the fact that, in some situations, the penetrations of 2-oxoglutarate into mitochondria may be limiting. The importance of other transport systems in regulating the relative redox states of the cytosol and mitochondria is illustrated by the data of Williamson et al. [16] who found that butylmalonate caused the cytosolic redox state to shift more oxidized relative to that of the mitochondria irrespective of the presence or absence of oleate and unrelated to whether long chain acyl CoAs accumualted or not. Since butylmalonate is thought to affect solely the malate transport system [17], these results would appear to involve malate transport as part of the system equilibrating the redox state of the two compartments. It is worthy of note that the relative shifts of the two redox states are greater when, in addition to the presence of butylmalonate, there is also an accumulation of long chain acyl CoAs [16] and this may suggest that both the malate and adenine nucleotide translocase systems contribute to the maintenance of the cytosolic/mitochondrial NAD^+/NADH ratio.

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