BBA 26268

THE REDOX STATE OF THE MITOCHONDRIAL NAD SYSTEM IN CIRRHOSIS OF THE LIVER AND IN CHRONIC QUANTITATIVE UNDERNUTRITION IN THE RAT

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(Received September 1st, 1969)

SUMMARY

1. To determine the redox state of the mitochondrial NAD system in cirrhosis of the liver and in chronic quantitative undernutrition, the substrates of the L-glutamate dehydrogenase (EC 1.4.1.2) and D-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30) reactions were measured. The livers of animals fed *ad libitum* served as controls.

2. Assuming these substrates to be in equilibrium, the $NAD^+/NADH$ ratio calculated from the substrates of the L-glutamate dehydrogenase reaction was lower in all three groups of animals than that calculated from the D-β-hydroxybutyrate dehydrogenase reaction. On the other hand, the ratio

$$\frac{([\beta\text{-hydroxybutyrate}] \times [\alpha\text{-ketoglutarate}] \times [NH_4^+])}{([\text{acetoacetate}] \times [\text{glutamate}])}$$

was similar in all three groups of animals.

3. These data suggest that in these animals the mitochondrial NAD^+–NADH system is in equilibrium provided a H^+ concentration gradient can be postulated between the sites of D-β-hydroxybutyrate dehydrogenase and L-glutamate dehydrogenase.

INTRODUCTION

Previous studies from our laboratory[1] have suggested that in cirrhosis of the liver and in chronic quantitative undernutrition the substrates of the two major dehydrogenases of the cytosol, lactate dehydrogenase (EC 1.1.1.27) and L-α-glycerophosphate dehydrogenase (EC 1.1.1.8) are not in equilibrium owing to a more reduced state of the redox pair of the L-α-glycerophosphate dehydrogenase reaction.

The experiments reported here have been designed to determine the redox state of the mitochondrial NAD system in these nutritionally abnormal animals.

MATERIALS AND METHODS

Male rats of the Sprague–Dawley strain were placed on the cirrhogenic diet described by GRISHAM *et al.*[2]. After about 6 months on the diet they received normal

chow for 10–14 days to remove most of the accumulated lipid. Cirrhosis of the liver was verified histologically at the time of sacrifice. Animals undergoing chronic quantitative undernutrition received normal chow for the same period of time, but in restricted amounts, so that their weight curves were similar to those of the cirrhotic rats. The controls were fed *ad libitum* also for 6 months. All animals were of the same initial weight (90–110 g) and were housed in single cages. They were sacrificed under light ether anaesthesia after an overnight fast (16–18 h).

A sample of liver tissue was frozen *in situ* as described by HOHORST *et al.*[3]. The preparation of the tissue extract and assay of metabolites was then carried out according to WILLIAMSON *et al.*[4]. The concentrations of the metabolites assayed (L-glutamate, ammonia, α-ketoglutarate, β-hydroxybutyrate and acetoacetate) were corrected for blood contamination. For this purpose, the content of blood in the liver was measured as described by HOHORST *et al.*[3]. It was found that if blood and liver were removed from the same animal there was a marked increase in the concentration of L-glutamate and β-hydroxybutyrate in the liver, presumably as a result of anoxia. For this reason the concentration of metabolites in the blood were measured in different animals of the same group. The magnitude of the correction was small, averaging ± 7 %. Statistical comparisons were based on the *t* test and, where applicable, on the modification of this test by DUNCAN[5].

RESULTS

Preliminary studies in rats weighing less than 200 g were well within the range reported by WILLIAMSON *et al.*[4] for normal animals of that size. As seen in Table I, the ratios of the redox partners of the L-glutamate dehydrogenase reaction and of the D-β-hydroxybutyrate dehydrogenase reaction were lower in the animals undergoing chronic quantitative undernutrition compared with the other two groups.

TABLE I

REDOX STATE OF MITOCHONDRIAL DEHYDROGENASES

$K_{(glutamate)(NAD^+)}$ = 3.87 mM and $K_{(\beta\text{-hydroxybutyrate})}$ = 49.3 mM (pH 7.0, I = 0.25, temp. = 38°)[4].

		β-Hydroxybutyrate (*μmoles/g*)	*Acetoacetate* (*μmoles/g*)	NH_4^+ (*μmoles/g*)	*Glutamate* (*μmoles/g*)	*α-Ketoglutarate* (*μmoles/g*)
Normally fed (O), n = 13	Mean	0.424	0.0772	0.149	1.200	0.061
	S.E.	0.029	0.0078	0.019	0.063	0.008
Chronic quantitative undernutrition (U), n = 16	Mean	0.265	0.204	0.283	1.64	0.161
	S.E.	0.035	0.019	0.040	0.027	0.017
Cirrhotic (T), n = 15	Mean	0.401	0.0853	0.169	1.47	0.054
	S.E.	0.021	0.010	0.015	0.11	0.0055
Significance tests		O,T > U ($P < 0.01$)	U > T,O ($P < 0.01$)	U > O,T ($P < 0.05$)	T,U > O ($P < 0.01$)	U > O,T ($P < 0.05$)

The ratios [NAD+]/[NADH] were calculated using the equilibrium constants determined by WILLIAMSON *et al.*[4], *i.e.* on the basis that

$$\frac{[NAD^+]}{[NADH]} = \frac{1}{K} \times \frac{[\text{oxidized substrate}]}{[\text{reduced substrate}]}$$

The ratio calculated from the substrates of the L-glutamate dehydrogenase reaction was significantly lower in all three groups compared with that calculated from the D-β-hydroxybutyrate dehydrogenase reaction. These observations would suggest that the two dehydrogenases are not in equilibrium. However, if, as suggested by WILLIAMSON *et al.*[4] the equations for the equilibrium constants of the two dehydrogenases are combined, *i.e.* if the expression

$$\frac{([\beta\text{-hydroxybutyrate}] \times [\alpha\text{-ketoglutarate}] \times [NH_4^+])}{([\text{acetoacetate}] \times [\text{glutamate}])}$$

is calculated then, if the two dehydrogenase are in equilibrium, the value obtained should be constant. The last column of Table I shows that this is correct for the three groups of animals examined by us. However, the theoretical value, calculated from the ratio $K_{(\text{glutamate})(NAD^+)}/K_{(\beta\text{-hydroxybutyrate})}$ was found[4] to be 79 μM. This value is on an average 2.26 times higher than that determined by us on the basis of substrate concentration.

DISCUSSION

As pointed out by KREBS[6] the equilibrium constants were calculated on the assumption that the pH of the cell is 7.0. The value of the constant is halved when the pH rises from 7.0 to 7.3. Hence, if the pH at the L-glutamate dehydrogenase site were 7.0 and that at the D-β-hydroxybutyrate dehydrogenase about 7.4 the substrate ratios would be similar to those found by us. Conversely, if the pH at the D-β-hydroxy-

n = number of observations.

$\frac{\beta\text{-Hydroxybutyrate}}{\text{Acetoacetate}}$ (M)	$\frac{\text{Glutamate}}{\alpha\text{-Ketoglutarate} \times NH_4^+}$ (M)	$NAD^+/NADH$ from $K_{(glutamate)}$	$NAD^+/NADH$ from $K_{(\beta\text{-hydroxybutyrate})}$	$\frac{\beta\text{-Hydroxybutyrate}}{\text{Acetoacetate}} \times \frac{\alpha\text{-Ketoglutarate} \times NH_4^+}{\text{Glutamate}}$ ($mM \times 10^{-2}$)
5.21	143.9	1.93	3.47	3.62
1.02	27.5	0.63	0.27	0.44
1.37	37.7	6.84	15.79	3.63
0.22	9.34	1.03	2.04	0.52
4.43	134.9	1.62	3.92	3.28
0.51	11.8	0.14	0.48	0.76
U < O,T ($P < 0.01$)	U < O,T ($P < 0.01$)	U > O,T ($P < 0.01$)	U > O,T ($P < 0.01$)	

butyrate dehydrogenase site were 7.0, the pH at the glutamate dehydrogenase site would have to be about 6.3 for equilibrium to be maintained. Clearly the number of possibilities consistent with equilibrium is infinitely large, dependent only on the postulate that a mean H^+ concentration gradient of about 2.26 were established between the mitochondrium matrix, the site of L-glutamate dehydrogenase[7] and the intercristal space, the site of D-β-hydroxybutyrate dehydrogenase[8].

Our animals differed from those studied by WILLIAMSON *et al.*[4]. The data in this report was based on fasted animals, the liver was removed under ether anaesthesia and not from an animal killed by dislocation of the neck, and was frozen very rapidly *in situ* rather than after excision of the liver which requires an additional 10 sec (ref. 4). These factors may account for the difference between their results which is compatible with an intramitochondrial pH of 7.0 in all compartments and ours which postulate a pH gradient between compartments. Alternatively, the substrates of the dehydrogenases in the abnormal may have been distributed differently compared with the normal. Other interpretations of our findings appear less attractive because they would require major differences in ionic strength or tissue water, neither of which are likely to be compatible with life. There is no ready explanation for the observation that the redox state of both mitochondrial compartments in the animals with quantitative undernutrition was more oxidized compared with the other two groups tested.

Considerations similar to those advanced here for mitochondria could have been applied to the redox pairs of the lactate and L-α-glycerophosphate dehydrogenase reactions which had also been shown not to be in apparent equilibrium in the nutritionally abnormal rats[1]. In the cytosol, unlike the matrix, however, there is no morphological basis for postulating a pH gradient between the two dehydrogenases, and when the ratios of these substrates were calculated the values obtained were different in the normals compared with the abnormals.

If the substrates of the L-glutamate dehydrogenase reaction are in equilibrium *in vivo*, it is unlikely that the mechanism for the incorporation of ammonia into glutamate by the glutamic dehydrogenase reaction in the cirrhotic would be impaired. This is in agreement with recent studies[9] which indicate that the mechanism for the incorporation of ammonia into glutamate by mitochondria from cirrhotic rat liver is not modified, if the concentration of K^+ in the serum is normal.

ACKNOWLEDGMENTS

This study was supported in part by grant AM-07361 of the National Institutes of Health. Our thanks are due to Dr. H. Marvin Pollard for encouragement and to Miss Ruby Wells for care and feeding of the animals.

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