THE INFLUENCE OF ALANINE ON THE GLUTAMATE/OXOGLUTARATE × NH_4^+ RATIO IN THE GUINEA-PIG LIVER

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1. Introduction

Klingenberg and von Häfen [1] introduced the 3-hydroxybutyrate/acetoacetate ratio as an indicator of the mitochondrial redox state, since 3-hydroxybutyrate dehydrogenase was found in mitochondrial cristae. Williamson et al. [2] used the glutamate dehydrogenase system to calculate the mitochondrial redox state, since glutamate dehydrogenase is located inside the mitochondrial matrix. These authors found agreement between the redox potential estimated from both systems and suggested that they share a common NADH pool in rat liver.

Willms et al. [3] presented some evidence that, in the guinea-pig liver, the mitochondrial redox state, calculated from both systems, did not sufficiently agree under different treatments of the animals. We reported recently [4] that, after addition of 0.02 mM *N*-2-phenylethylbiguanide, the glutamate/2-oxoglutarate × NH_4^+ ratio increased by about 300% whereas that of 3-hydroxybutyrate/acetoacetate and lactate/pyruvate were unchanged.

In the present communication, experimental conditions are reported in which the glutamate/2-oxoglutarate × NH_4^+ ratio was markedly increased in perfused guinea-pig liver although the mitochondrial redox state might not have been significantly altered.

2. Methods

Our modified perfusion procedure was that of Miller et al. as described elsewhere [5]. Male albino guinea-pigs (random breed, weighing between 250 and 350 g) were fasted 48 hr prior to the experiments. A pH electrode was installed in the perfusate reservoir and the pH kept at 7.4 by the addition of sodium bicarbonate if necessary. All perfusions were stopped after 120 min when liver samples were taken for the analysis of metabolites [5] according to Wollenberger et al. [6]. In some experiments, alanine was added to the medium after 60 min.

The assay mixture for ammonia contained: 0.1–0.2 ml liver extract, 0.2 mg glutamate dehydrogenase (in glycerol, Boehringer, Mannheim), 2.5 mM 2-oxoglutarate, 2.4 mM ADP, 0.2 M triethanolamine buffer (pH 7.4) in a final volume of 1.0 ml. All solutions were prepared freshly to avoid contamination with exogenous ammonia. Duplicates, including blanks, were run each time. Other metabolites were determined as previously described [5].

3. Results and discussion

After addition of alanine, the hepatic glutamate concentration rose by about 800% whereas that of 2-oxoglutarate decreased by more than 80%. The sum of lactate and pyruvate increased by about 230% (table 1). These data indicate, that glutamate accumulated in the liver cell. 2-Oxoglutarate concentration apparently diminished because of transamination of alanine to pyruvate.

Calculation of the free NADH/NAD^+ ratio from the concentration of metabolites considered to be in equilibrium with this ratio anticipates that these metabolites are evenly distributed throughout the tissue. Williamson et al. [2] had found evidence that

Table 1
The influence of alanine on some redox couples and the ATP/ADP ratio of perfused guinea-pig livers.

Alanine	–	10 mM
ATP/ADP	3.25 ± 0.31 (4)	3.21 ± 0.24 (10)
3-OH-Butyrate/acetoacetate	0.18 ± 0.09 (4)	0.20 ± 0.05 (10)
Glutamate	1600 ± 105 (4)*	13240 ± 2490 (10)
2-Oxoglutarate	380 ± 190 (4)	67 ± 17 (10)
NH_4^+	760 ± 130 (4)	1830 ± 610 (10)
Glutamate/2-oxoglut. × NH_4^+	5.5	108
Lactate	267 ± 180 (4)	608 ± 120 (10)
Pyruvate	30 ± 2 (4)	69 ± 18 (10)
Lactate/pyruvate	8.9	8.8

* nmoles/g wet weight, with standard deviation and the number of experiments in parentheses.

this may be the case for ammonia. The application of ammonia to rats caused a parallel increase in the ratio of glutamate/2-oxoglutarate × NH_4^+ and 3-hydroxybutyrate/acetoacetate. However, we conclude from our data, that glutamate and perhaps 2-oxoglutarate did not fulfil this requirement.

In the presence of alanine, the glutamate/2-oxoglutarate × NH_4^+ ratio increased about 20-fold. However, the intramitochondrial redox state cannot be expected to have been enhanced to this extent, since the ratios of 3-hydroxybutyrate/acetoacetate and lactate/pyruvate (table 1) were unchanged under these conditions. Although the lactate dehydrogenase system is not in equilibrium with the mitochondrial NADH pool, it is known that the lactate/pyruvate ratio is altered in perfused rat livers if the mitochondrial redox state is increased during cycles of anoxia [7]. The ATP/ADP ratio was not altered by the addition of alanine (table 1).

In summary, under our experimental conditions, the mitochondrial redox state cannot be calculated from the glutamate dehydrogenase system in the perfused guinea-pig liver.

References

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