LACK OF DIETARY DEPENDENCE OF LIVER ADENYLATE KINASE LEVELS

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1. Introduction

It is very important for the regulation of cellular metabolism that a balance of the concentrations of adenine nucleotides be maintained in any physiological condition [1]. The activity of rat liver adenylate kinase, a key enzyme for such equilibrium, is high enough in any metabolic situation to effeciently maintain the necessary balance of adenine nucleotides. Therefore, the existence of a control of adenylate kinase activity which depends on the metabolic condition of the animal seems unlikely. Nevertheless, an almost 3-fold increase in rat liver adenylate kinase activity after 48 h fasting and a drop to 30% normal-feed levels when 48 h fasting was followed by 24 h refeeding with a glucose-rich diet was reported [2]. A critical examination of the significance of this observation is reported here.

2. Materials and methods

Male white rats were fed ad libitum with a standard diet, fasted or fed a glucose-rich diet [3]. The animals were killed by decapitation. The livers were excised, weighed and homogenized with 4 vol. cold 0.25 M sucrose in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 400 × *g* for 1 min to remove cellular debris, and the supernatant, referred to as 'crude homogenate', was used for enzyme analysis. The crude homogenate was further centrifuged at 13 000 × *g* for 15 min to sediment mitochondria and nuclei, and the supernatant, containing cytosol plus microsomes, was also used for enzyme assay. When indicated, aliquots of each fraction were incubated 5 min at 0°C with 1% Triton X-100 before assay of adenylate kinase activity. All operations were carried out at 0–4°C.

Adenylate kinase activity was assayed at 25°C in the presence of glucose, $NADP^+$, hexokinase, glucose-6-phosphate dehydrogenase and ADP, as in [4]. Enzyme activities are expressed as μmol ATP formed/min/g liver × 2.

3. Results

As can be seen in table 1, the activity of adenylate kinase doubled in rat liver after 48 h fasting when measured in crude homogenate. When rats which had fasted for 24 h were refed for another 24 h with the glucose-rich diet, assays of adenylate kinase activity in crude homogenates gave results similar to those obtained with normally-fed animals. Treatment of crude homogenate with Triton X-100 caused adenylate kinase activity to increase, as compared with the same untreated fraction, in livers obtained from normally fed rats and from those in which 48 h fasting was followed by 24 h glucose-rich diet, but not in those obtained from 48 h fasted rats. The values obtained for adenylate kinase activity in the three dietary conditions tested were not significantly different in homogenates treated with Triton X-100.

About 50% crude homogenate activity was found to be located in the 13 000 × *g* supernatant fraction.

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Table 1
Effect of diet on adenylate kinase activity in rat liver

Dietary condition	Body wt (g)	Liver wt (g)	No. animals	Adenylate kinase activity (units/g liver)			
				Crude homog.	Crude homog. + Triton X-100	13 000 × *g* supernatant	13 000 × *g* supernatant + Triton X-100
Normally fed	343 ± 96	11.1 ± 5.1	12	35.5 ± 15.9	58.2 ± 27.8	18.4 ± 10.4	20.4 ± 10.9
48 h fasted	365 ± 24	8.2 ± 1.3[a]	4	72.8 ± 7.9[b]	74.8 ± 17.7	28.3 ± 7.4	28.4 ± 1.3
48 h fasted + 24 h refed with glucose-rich diet	292 ± 84	10.7 ± 5.1	12	34.1 ± 17.7	57.9 ± 29.6	16.2 ± 7.8	18.1 ± 8.9

[a] $p < 0.05$
[b] $p < 0.001$

Preparation of liver tissue, enzyme activity assay and dietary conditions are described in text. Results are given as means ± SD. Significances of differences, as compared with normally fed, have been calculated by means of the Student's *t*-test

4. Discussion

Adenylate kinase activity has been reported to be associated with different homogenate fractions [2,5,6]. Such findings have been related to different homogenizing conditions [4]. Adenylate kinase activity was assayed [2] in liver extract supernatants after 13 000 X *g* centrifugation for 15 min. In the results shown here, about 50% activity measured in crude homogenate was lost in the particulate fraction. When the extraction conditions in [2] were used, we could partly reproduce the results reported [2] in crude homogenate; nevertheless, upon releasing criptic enzyme activity in the presence of Triton X-100, we found that total liver adenylate kinase activity was the same for all the dietary conditions tested.

We therefore conclude that liver adenylate kinase is not inducible by diet, a conclusion which is to be expected, given the high activity and the biological importance of this enzyme. In view of the results shown here and in [2], it seems possible that adenylate kinase could be included within the recently proposed category of ambiquitous enzymes [7].

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