Volume 215, number 1, 13–15 FEB 04640 May 1987

Temporal relationships in the effects of protein-free diet on the activities of rat liver branched-chain ketoacid dehydrogenase complex and kinase

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Received 19 February 1987

Feeding rats 0% casein diet decreased liver activities of branched-chain ketoacid dehydrogenase complex (active form) and of activator protein (complete within 4 days), and increased activity of branched-chain ketoacid dehydrogenase kinase (complete within 9–10 days). Refeeding normal diet to rats fed 0% casein diet for 10 days resulted in a rapid and partial (approx. 50%) reversal of the above effects within 24 h; complete reversal required 20–30 days of refeeding.

Protein-free diet; Branched-chain ketoacid dehydrogenase complex; Branched-chain ketoacid dehydrogenase kinase

1. INTRODUCTION

The activity of the mitochondrial branched-chain 2-oxoacid dehydrogenase complex of animal tissues studied (branched-chain complex) is regulated by reversible phosphorylation, catalysed by a specific kinase, intrinsic to the complex, and by a phosphatase which is readily separated from the complex (phosphorylation is inactivating) (review [1]). In addition, a protein (activator protein) which is a free E1 component of the complex activates phosphorylated branched complex without dephosphorylation [2,3].

In rats fed normal diet, approx. 50% of the branched-chain complex in liver is in the active dephosphorylated form. Feeding protein-free diet (0% casein diet) for 10 days decreases the percentage of branched-chain complex in the active form to approx. 3% and the activity of activator protein to approx. 15% of the control [4,5]. The concentration of total complex (sum of active and inactive forms) remains unaltered [6], suggesting that the effect of 0% casein diet is mediated solely by phosphorylation of the complex. We have shown previously that the activity of branched chain kinase is increased approx. 4-fold in rat liver [7] on feeding 0% casein diet. This increase in branched-chain kinase activity intrinsic to the complex was stable to gel filtration or purification of the complex by fractional precipitation. The mechanism is not known but the major possibilities appear to be an increase in kinase concentration or covalent modification.

The temporal relationships between the effect of 0% casein diet on the activity of branched-chain kinase and percentage of branched-chain complex in the active form and reversibility upon refeeding normal diet are not known. The results of such studies are given here.

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2. EXPERIMENTAL

Details relating to male Wistar rats (source, feeding, normal and 0% casein diets), sources of chemicals and biochemicals were as in [6,7]. Rat liver mitochondria were prepared, incubated and

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

extracted as described in [6]. Activator protein and active and total forms of branched-chain complex were assayed as described in [5,6] and branched-chain kinase was assayed in extracts of mitochondria as in [7]. In computing the percentage of branched-chain complex in the active form, recoveries of mitochondria were checked by assay of citrate synthase as a marker enzyme which averaged (mean ± SE) 166 ± 7 units/g mitochondrial protein. An age-matched control rat fed normal diet was included in each experiment. All results were normalised against this and means calculated. Final results were expressed in terms of the controls for the whole series.

3. RESULTS AND DISCUSSION

It has been shown previously that 10 days feeding of 0% casein diet is required for the maximum effects of this diet [5]. The objective was to follow the time course of the effects of feeding 0% casein diet and subsequent refeeding on active branched-chain complex and to correlate this with observed changes in activator protein and branched-chain kinase.

3.1. *Effects of 0% casein diet*

After 4 days on diet, active branched-chain complex decreased from 58% total to 6% ($P < 0.001$) and further to below 4% by 10 days (fig.1a). Branched-chain kinase activity increased approx. 2-fold after 4 days on diet ($P < 0.001$); the maximum increase (4.2-fold) was attained by day 10 ($P < 0.001$) (fig.1b). The decrease in activity of activator protein paralleled that of active branched-chain complex, decreasing to 25% of control by 4 days ($P < 0.01$) and 16% by 10 days ($P < 0.001$) (fig.1c).

3.2. *Effects of refeeding normal diet*

Within 24 h of refeeding normal diet, the percentage of branched-chain complex in the active form increased from 4 to 27% ($P < 0.01$ for difference from 10 days of 0% casein diet) and subsequently returned to the control value after 30 days refeeding ($P < 0.05$) (fig.1a). There was a parallel increase in the activity of activator protein over this time which returned to the control value after 20 days of refeeding normal diet ($P > 0.05$ for difference from controls) (fig.1c). On refeeding

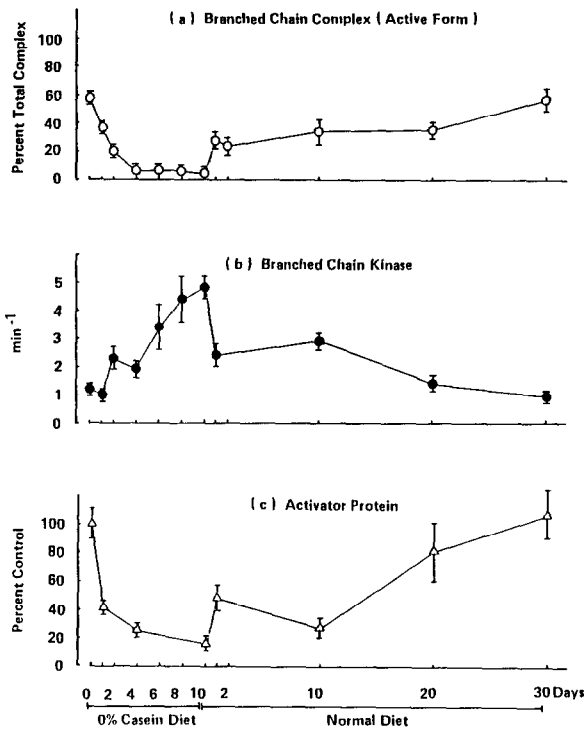

Fig.1. Time course of effects of feeding 0% casein diet and of refeeding normal diet. (a) Active branched-chain complex is expressed as percentage of complex in the active form. (b) Branched-chain kinase activities are expressed as apparent first-order rate constants for ATP-dependent inactivation of branched-chain complex at 30°C. (c) Activities of activator protein are expressed as a percentage of the control fed normal diet and computed from relative K_m values as in [5]. The K_m value for controls (mean ± SE for 14 rats was 7.20 ± 0.80 mg mitochondrial protein). Individual results are means ± SE for 3–8 animals fed 0% casein diet and for 14–25 animals fed only normal diet.

normal diet, a 51% decrease in branched-chain kinase activity occurred within 24 h ($P < 0.001$ for difference from 10 days diet) with return to the control after 20 days refeeding ($P > 0.05$) (fig.1b). Total branched-chain complex activity (mean ± SE for all observations) was 11.3 ± 0.4 mU/mg mitochondrial protein and was not affected by diet, as shown previously [6].

4. CONCLUSIONS

We have shown previously that the 10-fold decrease in percentage of active branched-chain

complex observed after 10 days on a protein-free diet is associated with a stable and 4-fold increase in the activity of branched-chain kinase [7]. It has been shown in the present study that the increase in the activity of the kinase may only parallel the decrease in percentage of active complex between days 1 and 4 of protein-free diet. Thus, the percentage of active complex decreased to its lowest level by the fourth day on diet during which time kinase activity doubled. In the first 24 h on protein-free diet the percentage of active complex decreased by about one-third whereas there was no significant change in branched-chain kinase activity. This suggests that some mechanism other than the stable increase in branched-chain kinase activity must have been responsible for the decrease in percentage of active complex over this period. Between days 4 and 10 on protein-free diet the activity of the kinase was further increased (2-fold) whereas the percentage of active complex did not change significantly. However, the activity of branched-chain complex (active form) was so low after 4 days of protein-free diet that a further decrease between 4 and 10 days would be very difficult to detect with current assays. The decrease in activity of activator protein paralleled closely the decrease in percentage of active complex. The reversal of the effects of protein-free diet by feeding normal diet appeared to be biphasic. Over the first 24 h there was a rapid but partial (approx. 50%) return towards control values in respect of the activities of branched-chain complex (active form), branched-chain kinase, and activator protein. Complete reversal of the effects of protein-free diet required some 30 days of normal diet for each of these parameters in clear contrast to the effects of protein-free diet which were largely complete by 4 days. During refeeding of normal diet the parallelism between the three parameters appeared to be much closer than during feeding of low-protein diet.

The outstanding problems to be addressed in respect of effects of protein-free diet include the mechanisms of the stable increase in branched-chain kinase activity (possibly covalent modification or increased concentration of kinase), of the decrease in activity of activator protein (free E1; possibly phosphorylation or decreased concentration), and of the unknown mechanism which decreases active branched chain complex in the first 24 h (possibilities include allosteric regulation of branched-chain kinase or altered phosphatase activity). The signals which mediate such changes are not known and a tissue culture model is being sought.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Medical Research Council and the British Diabetic Association; M.B. holds a Medical Research Council Studentship.

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