Molecular biological and quantitative abnormalities of ADP/ATP carrier protein in cardiomyopathic hamsters

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Introduction

The adenine nucleotide translocator or ADP/ATP carrier protein (AAC) is an integral protein present in the inner mitochondrial membrane, which performs the exchange of cytoplasmic and intramitochondrial ADP and ATP. The myocardial AAC content was studied in J-2-N cardiomyopathic hamsters. The AAC content was found to be significantly decreased in J-2-N hamsters. For molecular biological analysis, hamster AAC (T1 isoenzyme) cDNA was cloned by the plaque hybridization method. This AAC cDNA hybridized specifically with AAC mRNA, so RNA dot-blot hybridization was performed. The highest AAC mRNA level was observed in control hamsters followed by J-2-N hamsters with mild myocardial damage, J-2-N hamsters with severe myocardial damage and Bio 14-6 cardiomyopathic hamsters. These results suggest that a decreased AAC content may contribute to the pathogenesis of cardiomyopathy and that a decrease of AAC mRNA levels may explain the abnormalities of AAC in J-2-N cardiomyopathic hamsters.

Animal models

For the animal model we used J-2-N cardiomyopathic hamsters bred in our laboratory. Ten-week-old J-2-N hamsters underwent electrocardiography (ECG) including both limb and chest leads, and then laparotomy was done under pentobarbital anaesthesia. Blood was collected from the inferior vena cava and serum was separated for biochemical analysis.

Golden hamsters were used as healthy controls without cardiomyopathy, and compared to J-2-N hamsters as cardiomyopathic animal models. Because the serum creatine kinase (CK) level correlates well with myocardial damage in J-2-N hamsters, it was used to classify the severity of myocardial damage.

The J-2-N hamsters were divided into a very high serum CK group (>10 000 mU.ml⁻¹; J-2-N(V)), a high CK group (3000–10 000 mU.ml⁻¹; J-2-N(IV)), a moderate CK group (1000–3000 mU.ml⁻¹; J-2-N(III)), and a slightly raised CK group (<1000 mU.ml⁻¹; J-2-N(II)). A Bio 14-6 cardiomyopathic hamster group was also investigated.

Methods

DETERMINATION OF THE AAC CONTENT

The left ventricle of each hamster was minced, homogenized and centrifuged to separate the mitochondrial membrane fraction according to the method of Tzagoloff et al. N-Ethylmaleimide (NEM) was added to the mitochondrial membrane protein, followed by eosin-5-maleimide (EMA), after adding dithiothreitol, and the membrane proteins were isolated by centrifugation. The mitochondrial membrane proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and a single spot of fluorescence was observed under UV light. The gels were then stained with Coomassie blue. Finally, the AAC content ratio and AAC content ratio were determined using a densitometer.

CLONING OF HAMSTER AAC CDNA

Cardiac muscle was minced and homogenized, RNA was extracted by the guanidine thiocyanate method, and mRNA
was separated on an oligo-dT cellulose column. cDNA was synthesized from 5 µg of mRNA using a cDNA Synthesis System Plus, and a hamster cDNA library for each group was prepared from 1 µg of cDNA using an λ gt10 cDNA cloning kit. A cDNA clone of human AAC was prepared from human fibroblast cDNA by the polymerase chain reaction (PCR) using a primer designed from the base sequence of human AAC cDNA, as reported by Cozens et al. This clone was used as a probe for cloning hamster AAC cDNA from the various hamster cDNA libraries by the plaque hybridization method.

NORTHERN BLOT HYBRIDIZATION
To determine whether or not Golden hamster AAC cDNA hybridized specifically with AAC mRNA, Northern blot hybridization was performed. Hamster RNA (2 µg) from each group was subjected to electrophoresis on agarose gel, transferred to nylon membranes, and fixed under UV light. These membranes were then analysed by Northern blot hybridization using the same probe as for the dot-blot analysis.

RNA DOT-BLOT HYBRIDIZATION
To investigate the expression of hamster AAC at the mRNA level, RNA dot-blot hybridization was performed. RNA was extracted from the cardiac muscle of hamsters in each group as mentioned above, and 5 µg was taken as determined by spectrophotometry. Heat denaturation was performed in SSC buffer at 65°C for 15 minutes. The mixtures were then diluted serially to four concentrations from 1-5 to 0.19 µg dot-blotted onto nylon membranes, and analysed by dot-blot hybridization using Golden hamster AAC cDNA as the probe.

Results
The mitochondrial AAC content ratios for 10-week-old J-2-N hamsters and control hamsters are shown in Fig. 1. The AAC content ratios of J-2-N hamster groups (I), (II) and (III) were significantly lower than that of the control hamsters. The lowest AAC content was seen in the J-2-N cardiomyopathic hamsters with a very high serum CK group (J-2-N(I)) or high CK group (J-2-N(II)). The values are mean ± S.E.

Discussion
AAC is an essential protein for myocardial energy metabolism. Several autoantibodies have been reported in dilated cardiomyopathy, and it has been suggested that autoantibodies to AAC may induce cardiac dysfunction associated with abnormal energy metabolism. It has also been reported that antibodies produced by immunizing animals can decrease the cytosolic ATP concentration and increase the mitochondrial ATP content.
The myocardial AAC content determined with EMA was significantly lower in cardiomyopathic hamsters than in control hamsters, and this trend was more prominent in the animals with more severe myocardial damage. These facts suggest that a decrease in the content or function of AAC may be important in the pathogenesis of cardiomyopathy.

We investigated more closely the decreased myocardial AAC content in cardiomyopathic hamsters by the molecular biological approach. RNA hybridization was performed by the dot-blot method after a Golden hamster cDNA probe was confirmed to hybridize specifically with AAC mRNA from each J-2-N hamster by Northern blotting. The highest AAC mRNA level was observed in the control hamsters followed by J-2-N hamsters with mild myocardial damage, J-2-N hamsters with severe myocardial damage, and Bio 14-6 cardiomyopathic hamsters.

These findings indicate that the AAC mRNA level was decreased in cardiomyopathic hamsters. However, analysis of the coding region of AAC cDNA by PCR-SSCP (single strand conformational polymorphism) revealed similar patterns suggestive of no point mutation or deletion of genes. According to the results of RNA dot-blot hybridization and Northern blotting, the mRNA level decreased in hamsters with severe cardiomyopathy. These results suggest that a decreased mRNA level of AAC is one of the mechanisms explaining the AAC abnormality in J-2-N cardiomyopathic hamsters.

References