ALTERED ACCUMULATION OF α-AMINO ISOBUTYRIC ACID BY MYOPATHIC HAMSTER HEART

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SUMMARY: *Transport of α-Amino isobutyric acid by normal and myopathic hamster heart is a saturable process which is independent of the Na^+ gradient and of metabolic energy. The Km for the myopathic carrier is about three times as large as that of normal heart. This finding supports a relationship between genetic myopathy and alteration of cellular membranes.*

Transport of amino acids by cardiac muscle has been investigated in considerable detail. In rat heart there are at least two transport systems and in chick-embryo heart cells four transport systems have been described, one of which is subject to regulation involving a repression-derepression mechanism by its substrate molecules (1,2). An inbred strain of polymyopathic Syrian hamsters (strain BIO 14.6) has come to be regarded as an experimentally-accessible model for muscular dystrophy and for chronic congestive heart failure (3). Since alterations in protein synthesis have been detected in myopathic hamster muscle (4,5) and since there is evidence relating myopathy to changes in processes associated with muscle membranes (6,7), we have examined cardiac muscle from polymyopathic and control animals animals with regard to amino acid transport.

METHODS: Six-month-old Syrian hamsters (strain BIO 14.6) were obtained from TELACO, Bar Harbor, Maine. After decapitation, the

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TABLE I

Water Spaces of Control and Myopathic Hamster Heart

	Total Tissue H_2O (ml/gram)	Intracellular H_2O (ml/gram)
Control	0.763	0.684
Myopathic	0.778	0.666

Total tissue water was determined by drying the tissue overnight at 100°C. Intracellular water was determined as the tissue water not penetrated by [^{3}H] inulin. Each value represents the mean of six separate determinations.

hearts were removed, dissected free of connective tissue, cut into slices approximately 1 mm thick and placed in cold physiological saline. The slices were incubated for two hours in 10 ml of oxygenated (95% O_2, 5% CO_2) Krebs-Ringer-bicarbonate buffer (pH 7.4) containing 0.3% D-glucose and an appropriate concentration of [^{14}C] L-α-amino isobutyric acid (AIB) obtained from New England Nuclear Corp. At the end of the incubation, the tissue was removed, blotted free of adherent fluid, weighed and extracted in 1.5 ml of 2.5% trichloroacetic acid. The extract was clarified by centrifugation at 20,000 g for 15 min. 0.75 ml of the supernatant fluid was added to 10 ml of scintillation solution containing 667 ml toluene, 333 ml Triton-X 100 and 4 grams Omnifluor (New England Nuclear) per liter. Samples were counted in a Nuclear Chicago Unilux liquid scintillation counter with efficiency being determined by the channels-ratio method. Uptake was expressed as the concentration of AIB in the tissue water unavailable to inulin.

RESULTS: The time course for uptake of AIB was found to be linear for 5 min and to reach a steady state between 90 and 120 min. Two

TABLE II

Effect of Inhibitors on AIB Accumulation by Normal and Myopathic Heart

Inhibitor	AIB Accumulation (μmoles/ml cell H_2O/2 hr) Normal	Myopathic
Control	0.463 ± .042	0.219 ± .016
0.5 mM Ouabain	0.382 ± .040	0.199 ± .011
1.0 mM 2,4-dinitrophenol	0.431 ± .039	0.208 ± .013

Accumulation was determined as described in the text. Each value represents the mean ± S.E. of six determinations.

hours was chosen as a measure of steady state AIB accumulation. It is seen in Table I that total tissue water and intracellular water were the same in hearts from control and myopathic hamsters. The transport of AIB in hamster heart is neither energy dependent nor Na^+-dependent as indicated by the data of Table II, since ouabain, which inhibits the membrane Na^+-K^+ ATPase, and 2,4-dinitrophenol, a potent uncoupler of oxidative phosphorylation, were without effect on the accumulation of AIB.

As can be seen in Figure 1, kinetic features of AIB accumulation are strikingly altered in the myopathic heart with substantial inhibition at the concentrations employed. While the V_{max} for transport is unchanged (40 μmoles/ml cell H_2O/2 hr), the K_m for the myopathic heart is about three times that of the control (K_m=15.2 mM and 5.4 mM respectively) indicating decreased affinity for AIB by the myopathic heart transport system.

DISCUSSION: The present communication reports decreased transport of α-amino isobutyric acid (AIB) by myopathic hamster heart. This decrease represents an alteration in the protein synthetic

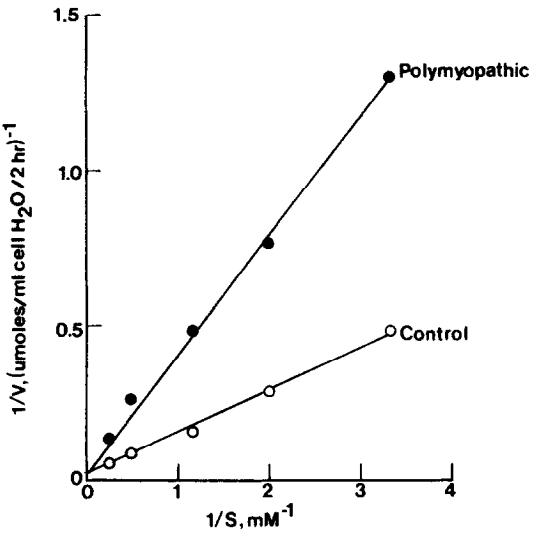

Figure 1. Lineweaver-Burk plots for AIB accumulation by normal and myopathic hamster heart. Experimental conditions were described in the text. Each data point is the mean of four experiments.

process distinct from that due to reported changes in the activity of ribosomes (8) or to limitation of intact tRNA molecules (4,5).

Since the relevant transport system is associated with the plasma membrane, inhibition of amino acid transport should be considered in light of evidence associating genetic myopathy with systemic membrane alteration. Thus, Sulakhi et al (6) have reported increased Na^+-K^+ ATPase in myopathic hamster muscle membranes and, in late stages of hamster polymyopathy, a defect in sarcosomal oxidative phosphorylation which is also membrane localized has been demonstrated (9-11).

Moreover, in genetic myopathy of mice there is evidence of alteration of membranes in tissues other than muscle. Proton permeability and related functions are changed in liver mitochondria isolated from animals with the disease (12) and a

dramatic alteration in the surface of erythrocytes from myopathic mice has been observed (13). Thus a change in the structure of the hamster cardiac cell membrane, perhaps the result of altered synthesis of membrane proteins, could easily explain the reduced affinity of the AIB carrier reported here.

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