Myocardial Amino Acid Transport in the Isolated Rabbit Right Ventricular Papillary Muscle

GENERAL CHARACTERISTICS AND EFFECTS OF PASSIVE STRETCH

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ABSTRACT

The amino acid analog, alpha-aminoisobutyric acid (AIB) has been used to study myocardial amino acid transport in the isolated rabbit right ventricular papillary muscle. Intracellular AIB accumulation is linear for 2 hours and reaches a plateau at 3 hours at an intracellular/extracellular concentration gradient of 3.4. Anoxia does not inhibit this process whereas simultaneous inhibition of aerobic and anaerobic metabolism inhibits AIB accumulation. Intracellular AIB accumulation follows Michaelis-Menten kinetics with a $K_m$ of $6.8 \times 10^{-9} \text{M}$ and a $V_{max}$ of $6.6 \mu\text{mol} \cdot \text{ml intracellular fluid}^{-1} \cdot \text{hour}^{-1}$. Sodium ion is obligatory for the transport process whereas no absolute potassium or calcium requirement exists. Ouabain, $10^{-5} \text{M}$, inhibits AIB uptake. Myograph techniques were utilized to determine the effect on AIB accumulation of subjecting unstimulated papillary muscles to various degrees of passive stretch. Muscles incubated at any tension above zero developed a greater intracellular/extracellular fluid AIB concentration ratio than muscles incubated at zero tension. These data suggest that increased myocardial wall tension may be a mechanical stimulus capable of inducing adaptive changes in myocardial metabolism.

ADDITIONAL KEY WORDS

hypertrophy tension ouabain cellular volume

The heart will hypertrophy when exposed to the stress of a sustained increase in mechanical work, thus suggesting that the mechanical activity of the heart partially regulates the cellular mechanisms which determine myocardial mass. Some of the biochemical characteristics of the hypertrophied heart have been described in the intact heart following banding of the aorta and pulmonary artery, or in an overloaded perfused heart preparation (1-11). While the loading of the myocardium is altered by these techniques, their complexity does not permit a definition of the specific mechanical stimuli which induce hypertrophy. In rats, prolonged isometric work in skeletal muscle appears to induce different biochemical changes when compared to isotonic work (12). This observation suggests that specific mechanical stresses may induce specific biochemical responses in striated muscle. With these considerations in mind, the isolated papillary muscle has been used for the present studies. In this preparation, mechanical variables can be precisely controlled and thus the specific mechanical stresses which induce specific alterations in myocardial metabolism can be defined. In rapidly growing tissues, including heart (13) and skeletal muscle (14), increased concentr-
tions of intracellular free amino acids are maintained (15-19). Furthermore, other studies suggest that the rate of protein synthesis in a cell may be regulated in part by the intracellular concentration of amino acids (13, 20, 21). Accordingly, the present studies were designed to define the effects of various mechanical stresses on myocardial transport of amino acids. The nonmetabolized amino acid analog, alpha-aminoisobutyric acid (AIB), has been utilized to study the transport process. Initially, the general characteristics of myocardial amino acid transport in the papillary-muscle preparation have been studied and the effects of the simple mechanical stress of passive stretch on the transport process determined.

Materials and Methods

**Materials**

Insulin was purchased from Fisher Chemical Company and resorcinol from Eastman Organic Chemicals. Ouabain octahydrate was obtained from Sigma Chemical Company and alpha-aminoisobutyric acid (AIB) "A" grade from Calbiochem; 3-14C-AIB was obtained from New England Nuclear Corp. A sample from each new lot of the isotope was chromatographed on thin-layer DEAE cellulose (butanol: acetic acid: water, 25:4:1) prior to use. The chromatography plates were sprayed with an acetone-ninhydrin layer. For these experiments the papillary muscles were dissected and mounted in a similar manner but left with a short tie hanging free in the bath. These muscles were held erect by the gas stream bubbling in the bath (Fig. 1B). All muscles used in the myograph experiments had a calculated cross-sectional area of less than 0.7 mm².

**Methods**

New Zealand white rabbits, weighing 2.0 to 2.5 kg, were fed a standard laboratory diet and water ad libitum until killed. Animals were anesthetized with intravenous pentobarbital (40 mg/kg) and their hearts rapidly removed. The right ventricular papillary muscles were removed and placed in an appropriate incubation vessel. For the determination of the metabolic dependencies of amino acid transport the muscles were incubated in 5-ml micro-Fernbach flasks in a Dubnoff apparatus at 37°C. Each flask contained 4 to 6 muscles in 4 ml of medium (see below) which was continuously aerated with a stream of 95:5 oxygen:carbon dioxide. For these experiments the papillary muscles were severed at the base, and as a consequence a free cut end was exposed during the incubation period. Muscles with a wet weight between 1.0 and 4.0 mg were used in these studies.

To study the effects of passive stretch on amino acid transport in the unstimulated muscle, papillary muscles were incubated in a myograph at zero tension or at fixed passive tensions ranging up to 8.0 g/mm². A button of ventricular myocardium was left attached to the base of the papillary muscle and the muscle base mounted in the myograph by a spring loaded pulley and attached to a preload (Fig. 1A). Tension on a given muscle was determined by the size of the preload and was held constant throughout the incubation period. Assuming a cylindrical shape and a density of 1.0 for the muscle, the passive tension exerted on each muscle was determined at the conclusion of an incubation period. The passive tension was calculated from the formula:

\[
\text{tension (g/mm²)} = \frac{\text{preload (g)} \times \text{muscle length (mm)}}{\text{muscle wet weight (mg)}}
\]

Muscles mounted at zero tension in the myograph were dissected and mounted in a similar manner but left with a short tie hanging free in the bath. These muscles were held erect by the gas stream bubbling in the bath (Fig. 1B). All muscles used in the myograph experiments had a calculated cross-sectional area of less than 0.7 mm².

For all experiments muscles were preincubated for 30 minutes in a Krebs-Ringer bicarbonate-glucose medium (Na+, 145 mM; K+, 4.7 mM; Ca²⁺, 2.5 mM; Mg²⁺, 1.2 mM; Cl⁻, 122 mM; SO₄²⁻, 1.2 mM; HCO₃⁻-CO₃⁻, 25 mM; phosphate, 1.2 mM; glucone, 10 mM). Following preincubation, the standard Krebs-Ringer medium was replaced with an identical solution that contained histone free, base stable insulin (7.5 mg/ml), and 3-14C-AIB (0.4 μmole/ml). At the end of the incubation period, the muscles were washed three times with 2-ml aliquots of distilled water, blotted and weighed to the nearest 0.01 mg on a Mettler H-20 T balance in preweighed aluminum boats. The muscles were rapidly placed in dry ice, lyophilized to constant weight, and weighed to obtain values for wet and dry weight as well as total tissue water. Insulin and 14C-AIB content of the muscle and the medium were determined (see below) and the insulin or extracellular fluid space (ECF) calculated. The intracellular concentration of 14C-AIB was calcu-
Schematic diagram of the myograph in which muscles were mounted at fixed passive tensions (A) or at zero tension (B). The Krebs-Ringer buffer was continually aerated as depicted and maintained at 37°C by circulating preheated water through the manifold.

The ratio of intracellular to extracellular \(^{14}\text{C}\)-AIB concentrations, i.e., the distribution ratio, was then calculated by the formula:

\[
\text{distribution ratio} = \frac{(\text{dpm}\text{-}^{14}\text{C}\text{-AIB})/\text{ml ICF}}{(\text{dpm}\text{-}^{14}\text{C}\text{-AIB})/\text{ml medium}}
\]

Inherent to the latter calculation is the assumption that the concentration of AIB in the extracellular fluid space and the medium are identical.

**Inulin and \(^{14}\text{C}\text{-AIB} Determinations.**—The lyophilized muscles were homogenized in 0.5 ml of 0.85 N NaOH and the basic homogenate hydrolyzed at 95°C for 15 minutes. The basic muscle hydrolysate was cooled to 0°C and neutralized with 8.5 N HCl. Proteins were precipitated by the addition of ice cold 25% trichloroacetic acid (TCA) to a final TCA concentration of 10%. After 1 hour at 0°C, the proteins were centrifuged and the supernatant fraction decanted. The protein pellet was washed twice with cold 10% TCA. The washes were combined with the original TCA extract and brought to a standard volume with 10% TCA. An aliquot of the extract was used for insulin determination with the resorcinol method (22).

Appropriate control experiments demonstrated the residual protein pellet to be free of \(^{14}\text{C}\text{-AIB} and insulin. In another control experiment, the TCA was removed from a pooled muscle extract by ether treatment. The residual aqueous fraction was chromatographed with thin-layer technique on DEAE cellulose, ECTEOLA, and silica gel in a butanol-acetic acid:water 25:4:10 solvent system. The ether extract was free of radioactivity and the \(^{14}\text{C} activity in the aqueous fraction cochromatographed with standard AIB in all three chromatographic systems. The \(^{14}\text{C} activity in the TCA extract was completely recoverable as AIB, thus indicating that AIB is in fact not metabolized by rabbit myocardium. A muscle blank was not routinely determined for the insulin assay since with the techniques utilized, the calculated insulin space for a control series of muscles incubated in an inulin-free medium ranged from 0 to 3%. Identical results in the insulin assay were obtained when commercial resorcinol and resorcinol that had been recrystallized in this laboratory were used and consequently the commercial product was used routinely (23).

Control experiments demonstrated the endog-
enous hexose content of the papillary muscle to be sufficiently high to invalidate inulin determinations by the resorcinol method (24), and consequently basic hydrolysis was utilized since this procedure destroys endogenous myocardial hexose stores. However, the basic hydrolysis procedure necessitated the preparation of a base stable inulin for use in the incubation medium. Commercial inulin was found to be contaminated with base labile saccharides (Fig. 2) (25), and to eliminate these contaminants, the commercial product was hydrolyzed in .85 N NaOH for 15 minutes at 100°C. This solution was then cooled at 0°C, neutralized with hydrochloric acid and exhaustively dialyzed against 0.001M Tris pH 8.0. The contents of the dialysis sac were precipitated by adding ethanol to a concentration of 70% v/v. The precipitate was collected, redissolved in warm water (60°C) and reprecipitated from ethanol. The precipitation step was repeated two or three times. This procedure had a 40 to 50% yield. The final product was a white, odorless powder which was stoichiometrically identical to commercial inulin in the resorcinol reaction (Fig. 2). However, in contrast to commercial inulin, the purified material was completely resistant to basic hydrolysis (Fig. 2) (24).

Radioactivity Determinations. 14C activity was determined in a Packard Tricarb Scintillation Spectrometer. Each liter of counting solution contained 700 ml toluene, 300 ml absolute ethanol, 4 g 2, 5-diphenylloxazole (PPO) and 100 mg 1, 4- bis [2-(5-phenyloxazolyl)] benzene (PPOPO). Factory calibrated 14C-toluene (New England Nuclear Corp.) was used to prepare quench standards. Absolute activity, disintegration per minute, was determined by the channels ratio method (26).

Statistical analysis was performed with Student's t-test (27).

Results

General Characteristics of AIB Accumulation in the Isolated Papillary Muscle.–In Figure 3, the mean distribution ratios developed by papillary muscle groups incubated in the Dubnoff apparatus under various conditions have been plotted as a function of time. Under control conditions (Krebs-Ringer buffer, 95:5-N2:CO2, 37°C) the transport ratio was 1.4 ± .17 at 1 hour, increased linearly through the second hour of incubation to a value of 3.2 ± .31 and leveled off at 3.4 ± .12 at 3 hours. The distribution ratio for muscles which were preincubated and incubated under anoxic conditions (Krebs-Ringer buffer, 95:5-N2:CO2, 37°C) were identical to those of the controls at 1, 2, and 3 hours. In contrast, intracellular accumulation of AIB was markedly inhibited when anaerobic metabolism was also blocked by the addition of iodoacetic acid (0.5 nM) to nitrogen-treated muscles. Control experiments demonstrated that 0.5, 1.0 and 2.0 mM iodoacetic acid alone did not affect intracellular AIB accumulation.
(Table 1). In contrast, when fluoride was used as a glycolytic inhibitor, it was not possible to discern a fluoride concentration at which AIB accumulation was inhibited under anaerobic conditions but proceeded normally under aerobic conditions. Fluoride 10^{-2}M inhibited AIB accumulation in both aerobic and anaerobic incubations (Table 2). Inhibition of intracellular AIB accumulation was noted when the muscles were incubated at 0°C or in a hypotonic medium that contained 90 mM rather than 145 mM sodium.

When the initial 30-minute preincubation was omitted, the transport ratio for muscles incubated for 1 hour under control conditions was considerably lower than that depicted in Figure 3. A plot of distribution ratio vs. time for these muscle groups demonstrated a definite lag phase. Similar data have been reported for AIB accumulation in liver slices (28).

Water Content-Extracellular Fluid Space.—As shown in Figure 4, the percent dry weight of muscles incubated under the various conditions described above remained stable at 23.5 to 25.1% (avg 24.5 ±.2%) at 1, 2, and 3 hours of incubation. These values are similar to those previously reported for rat solei (29), although a greater variability in the percent dry weight determinations was noted for the papillary muscles as compared to the solei. This may reflect evaporation from the surface of a muscle with a large surface-to-volume ratio and a small total weight since any delay in the determination of the muscle wet weight was found to result in a significant increase in the percent dry weight obtained.

The extracellular fluid space expressed as percent wet weight (ml inulin space

### Table 1

<table>
<thead>
<tr>
<th>Iodoacetate concentration</th>
<th>0 mM</th>
<th>0.5 mM</th>
<th>1.0 mM</th>
<th>2.5 mM</th>
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<tbody>
<tr>
<td>Distribution ratio</td>
<td>1.4 ± .20</td>
<td>1.3 ± .10</td>
<td>1.3 ± .20</td>
<td>1.3 ± .15</td>
</tr>
<tr>
<td>Extracellular fluid space (as percent wet weight)</td>
<td>27.8 ± 1.1</td>
<td>18.8 ± 1.5*</td>
<td>18.8 ± 2.0*</td>
<td>14.8 ± 0.8*</td>
</tr>
<tr>
<td>Percent dry weight</td>
<td>24.3 ± .65</td>
<td>20.3 ± .3</td>
<td>24.3 ± .5</td>
<td>25.0 ± .7</td>
</tr>
</tbody>
</table>

Each value represents the mean of 14 individual determinations ± 1 se. Preincubations were for 30 minutes under standard conditions as described in Methods. Incubation medium contained iodoacetate in the concentrations noted and incubations were for 1 hour under aerobic conditions.

*Value statistically different than the control value at the P < .01 level.
Effects of Fluoride under Aerobic and Anaerobic Conditions on Distribution Ratio, Extracellular Fluid Space and Percent Dry Weight

<table>
<thead>
<tr>
<th>Fluoride concentration</th>
<th>Distribution ratio</th>
<th>Extracellular fluid space</th>
<th>Percent dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.2 ± 0.1</td>
<td>24.0 ± 0.7</td>
<td>25.0 ± 0.3</td>
</tr>
<tr>
<td>10^{-3} M</td>
<td>1.2 ± 0.1</td>
<td>25.0 ± 0.2</td>
<td>24.8 ± 0.3</td>
</tr>
<tr>
<td>10^{-2} M</td>
<td>1.3 ± 0.2</td>
<td>24.8 ± 0.3</td>
<td>24.9 ± 0.2</td>
</tr>
<tr>
<td>10^{-1} M</td>
<td>1.3 ± 0.2</td>
<td>24.0 ± 0.3</td>
<td>25.0 ± 0.3</td>
</tr>
<tr>
<td>10 M</td>
<td>1.3 ± 0.2</td>
<td>24.0 ± 0.3</td>
<td>24.0 ± 0.3</td>
</tr>
</tbody>
</table>

Each value represents the mean of 15 to 20 individual determinations ± 1 SE. Preincubations were for 30 minutes in standard Krebs-Ringer buffer aerated with either an O_{2}/CO_{2} or N_{2}/CO_{2} atmosphere. The incubation medium contained fluoride in the concentrations noted and was aerated with the appropriate gas mixture. Incubations were for 1 hour.

*Value statistically different than the corresponding control value at the P < 0.01 level.

Percent dry weight and extracellular fluid space expressed as percent wet weight for papillary muscles incubated under various conditions plotted as a function of time. Each point represents the mean of 15 to 30 individual determinations ± 1 SE.
Intracellular concentrations of AIB developed by papillary muscles incubated for 1 hour under standard conditions plotted as a function of AIB concentration in the incubation medium. Each point represents the mean of at least 10 determinations ± 1 se. Definitions as for Figure 3.

X 10^3/mg wet weight X 100) for the control muscles and those incubated under anoxic conditions at 1 hour was 25.1 ± 0.71 and 25.7 ± 1.4%, respectively (Fig. 4). The inulin space did not increase progressively with time for these muscle groups and did not differ significantly from a value of 25% at 2 and 3 hours. Using 51C-inulin, Page (30) has reported an inulin space of 24% after 1 hour of incubation for the cat papillary muscle with a gradual increase to 30% at 3 hours. A 50% reduction in extracellular fluid space was noted at all times for muscle groups incubated under conditions which inhibit cellular metabolism such as cold (0°C), or the simultaneous inhibition of aerobic and anaerobic metabolism with nitrogen and iodoacetate or fluoride (Table 2). A smaller decrease in the extracellular fluid space was noted when iodoacetate was present under aerobic conditions (Table 1).

Similar to the control group, the penetration of inulin into the extracellular fluid space of muscles wherein metabolism was inhibited was maximal at 1 hour with identical values being obtained at 1, 2, and 3 hours of incubation. In contrast, the penetration of inulin into the extracellular fluid space of muscles incubated in a hypotonic medium containing 90 mM sodium followed a markedly different kinetic pattern. Under these conditions the inulin space was 12.5 ± 1.6% at 1 hour and increased linearly to a value of 20 ± 1.1% at 3 hours. The latter value is statistically different from the 3 hour value of 26.6 ± 5.1% found in the control group.

Effect of AIB Concentration—In Figure 5, intracellular concentrations of AIB generated by muscles incubated for 1 hour in media containing progressively increasing concentrations of AIB are plotted. The intracellular accumulation of AIB appeared to be mediated via a saturable process, since a linear relation between the intracellular fluid concentration of AIB and the AIB concentration in the medium was not found. The double reciprocal
plot of these data is shown in Figure 6 and the data fit the calculated regression line of 
\[ y = 1.02x + .151 \] with an \( r \) value of 0.93. Using the terminology of Michaelis-Menten enzyme kinetic theory, the calculated \( K_m \) and \( V_{max} \) are \( 6.8 \times 10^{-3} \) and \( 6.64 \) \( \mu \)moles \( \cdot \) ml intracellular fluid \( \cdot \) hour \(^{-1} \).

**Cation Requirements for Myocardial AIB Accumulation.**—In Figure 7 the effect of altering the sodium concentration of the medium on the distribution ratio, extracellular fluid space and percent dry weight are depicted. In these experiments the sodium concentration of the medium was reduced to 72.5 and 36.3 \( \text{mM} \) and replaced isoosmotically with choline chloride. Reducing the sodium concentration of the medium resulted in a progressive inhibition of intracellular AIB accumulation and a parallel decrease in the inulin space although percent dry weight remained constant. These data as regards AIB accumulation are similar to that reported in skeletal muscle and other tissues (31, 32).

The requirements of the transport process for potassium was evaluated by comparing data obtained from muscles incubated for 1 hour at standard conditions as described in Methods. Extracellular fluid space and dry weight are statistically identical in all groups. The distribution ratio at 0 \( \text{mM} \) \( K^+ \) is significantly less (\( P < .02 \)) than that for 4.7 and 9.4 \( \text{mM} \) \( K^+ \).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>0 mM</th>
<th>4.7 mM</th>
<th>9.4 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport ratio</td>
<td>1.6 ± .12</td>
<td>2.1 ± .10</td>
<td>2.2 ± .15</td>
</tr>
<tr>
<td>Extracellular fluid space</td>
<td>23.6 ± .91%</td>
<td>24.8 ± .83%</td>
<td>25.5 ± .54%</td>
</tr>
<tr>
<td>Percent dry weight</td>
<td>24.8 ± .53%</td>
<td>24.6 ± .51%</td>
<td>23.8 ± .30%</td>
</tr>
</tbody>
</table>

(\( n = 20 \))

(\( n = 31 \))

Each value represents the mean ± 1 SE with \( n \) equal to the number of individual determinations. Incubations were for 1 hour at standard conditions as described in Methods. Extracellular fluid space and dry weight are statistically identical in all groups. The distribution ratio at 0 \( \text{mM} \) \( K^+ \) is significantly less (\( P < .02 \)) than that for 4.7 and 9.4 \( \text{mM} \) \( K^+ \).
hour in normal Krebs-Ringer buffer ($K^+ = 4.7$ mM) to that for muscles incubated for 1 hour in media containing either no potassium ($K^+ = 0.0$ mM) or twice the normal concentration ($K^+ = 9.4$ mM) (Table 3). Since these manipulations of the potassium content of the medium resulted in less than a 2% alteration in the osmolarity of the medium, isosmolar substitutions with inert cation species were not made. Percent dry weight and extracellular fluid spaces were not affected by altering the potassium concentration of the medium. At zero millimolar potassium the transport ratio was $1.6 \pm .12$ compared to $2.1 \pm .16$ and $2.2 \pm .15$ for the muscles incubated at 4.7 and 9.4 mM potassium, respectively. The difference between the zero potassium group and the other two muscle groups is significant ($P < .02$) but such data are difficult to interpret since a small potassium loss from the high intracellular stores of this cation into the extracellular fluid compartment could result in a relatively normal extracellular potassium content, i.e., 0 mM potassium in the medium need not signify 0 mM potassium in the extracellular fluid space.

To evaluate the calcium requirements for myocardial AIB accumulation, experiments were performed comparing muscles incubated for 2 hours in media containing 0 mM, 2.5 mM, and 5.0 mM calcium (Table 4). Dry weight did not vary as a function of calcium concentration. The extracellular fluid space at 5.0 mM and 2.5 mM calcium was $26.7 \pm .75$ and $27.5 \pm .70$, respectively; whereas the extracellular fluid space for muscles incubated

<p>| TABLE 4 |
| Effects of Medium Calcium Concentration on Distribution Ratio, Extracellular Fluid Space and Percent Dry Weight |</p>
<table>
<thead>
<tr>
<th>Calcium Concentration (mM)</th>
<th>Distribution Ratio</th>
<th>Extracellular Fluid Space</th>
<th>Percent Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>4.5 ± .30</td>
<td>36.2 ± .84%</td>
<td>25.5 ± .71%</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>3.7 ± .46</td>
<td>26.7 ± .75%</td>
<td>24.5 ± .65%</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>3.8 ± .50</td>
<td>27.5 ± .70%</td>
<td>23.9 ± .59%</td>
</tr>
</tbody>
</table>

Each value represents the mean of 17 individual determinations ± 1 se. Incubations were for 2 hours at standard conditions as described in Methods. Distribution ratio and percent dry weight are statistically similar in all groups. The extracellular fluid space for the 0 mM calcium group is significantly greater than that for the 2.5 and 5.0 mM calcium groups ($P < .01$).
in the 0 mM calcium medium was 38.2 ± 0.8%. The latter value is significantly greater than those obtained at 2.5 and 5.0 mM calcium. The transport ratio attained by the muscles incubated in the 0.25, and 5.0 mM calcium media were 4.5 ± 0.4, 3.7 ± 0.5, and 3.8 ± 0.5, respectively. These values do not differ significantly.

Effects of Ouabain.—The effects of ouabain on the myocardial accumulation of AIB were determined by incubating muscles for 1 hour in Krebs-Ringer buffer containing ouabain between $10^{-6}$ to $10^{-4}$M. In Figure 8, the transport ratio, extracellular fluid space and percent dry weight for these muscle groups are plotted as a function of ouabain concentration. No effect on dry weight was found, although significant decreases in the intracellular accumulation of AIB and extracellular fluid space were found at $10^{-5}$ and $10^{-4}$M ouabain. These results closely parallel those observed in the presence of a lowered Na$^+$ concentration.

Effects of Passive Tension.—Myographic techniques were utilized to determine the effects of various degrees of stretch on AIB accumulation. Muscles were mounted in a myograph as described in methods, preloaded, preincubated in Krebs-Ringer buffer for 30 minutes, and then incubated at the given preload for 1 or 3 hours in Krebs-Ringer buffer containing insulin and $^{14}$C-AIB. At the end of the incubation period, percent dry weight,
Table 9: Distribution ratio (bottom panel), extracellular fluid space (middle panel) and percent dry weight (top panel) of muscles incubated for 1 hour (●) or three hours (▲) plotted as a function of the passive tension at which the muscles were incubated. Numbers in parentheses are number of muscles in each group with each value represented as the mean ± 1 SE. Definitions as for Figure 3.

After 1 hour, the distribution ratio attained by muscles incubated at tensions of 0.1 to 2.0, 2.1 to 4.0, and 4.1 to 6.0 g/mm² were 30 to 60% greater than values for the zero tension group (1.2 ± 0.11) but these differences were not significant (P < 0.1). At 3 hours, the distribution ratio increased linearly as a function of tension between zero and 2.1 to 4.0 g/mm², and leveled off at 4.1 to 6.0 g/mm². The distribution ratio of the zero tension group (2.3 ± 0.14) was significantly less than that of the other three groups. These data suggest that any degree of passive tension exerted over a 3-hour period can enhance AIB accumulation in the isolated papillary muscle as compared to muscles incubated at zero tension. It is worth noting however that in the 3-hour studies a unimodal distribution of distribution ratios was found in all muscle groups except those exposed to tensions of 0.1 to 2.0 g/mm². In this group of 16 muscles, the distribution was bimodal with nine attaining a mean ratio of 2.3 ± 0.15 and the remaining seven a mean of 6.0 ± 0.8. The value of 2.3 ± 0.15 is identical to the mean value noted for the zero tension muscles (2.3 ± 0.14) and the value 6.0 ± 0.8 is identical to the mean value (5.9 ± 0.8) attained by the muscles exposed to tensions of 2.0 to 4.0 g/mm². These data suggest the existence of a threshold tension between 0.1 and 2.0 g/mm² which...
must be exceeded before myocardial accumulation of AIB is affected by passive tension. Although the cross-sectional area of all muscles used in the myograph studies was less than 0.7 mm², the data in Figure 9 do not eliminate the possibility that the observed differences in distribution ratios were due to differences in cross-sectional area of the individual muscles rather than the tension to which these muscles were exposed. The data from the muscles incubated at tensions of 0 to 2.0 g/mm² serve as an internal control relative to this point, since as noted above the distribution ratios for this group of muscles segregated in a bimodal fashion with nine attaining low and seven high ratios. If the distribution ratios varied solely as a function of cross-sectional area, a plot of these variables for the muscles incubated at tensions of 0 to 2 g/mm² should demonstrate an inverse linear relation with the highest distribution ratios being noted at the lowest cross-sectional areas. No inverse relation was noted (Fig. 10) when the data were plotted. Furthermore, although the spread of distribution ratios was less in the other tension groups, plots of cross-sectional area versus distribution ratio for these muscle groups also failed to demonstrate an inverse relation and thus it seems unlikely that distribution ratios varied solely as a function of muscle cross-sectional area.

Although 1 hour of incubation did not reveal a statistical effect of tension on AIB accumulation (Fig. 9), this might have resulted from limitations of sensitivity in the assay. To investigate this possibility a group of muscles were mounted in the myograph at zero tension in the usual manner and a second group was mounted and subjected to passive tensions of 4.0 to 6.0 g/mm². To minimize biologic variability only rabbit hearts that contained two suitable papillary muscles were used for these experiments. In this manner a zero tension and a high tension muscle was obtained from each animal studied. All muscles were preincubated as usual for 30 minutes in Krebs-Ringer buffer. The medium was then changed and replaced with fresh Krebs-Ringer buffer that contained neither inulin nor 14C-AIB. Two and one half hours after this buffer change, the medium was replaced with Krebs-Ringer buffer containing inulin and 14C-AIB. After 40 minutes of exposure to this medium the experiment was terminated and distribution ratios determined. Routine cumulative 3-hour tension experiments wherein the zero tension and the high tension muscles were obtained from the same rabbit were performed in parallel to the 40-minute pulse-label experiments and these data are summarized in Table 5. The transport ratios attained by the zero tension and the high tension muscles in the pulse-label experiment were identical (1.5 ± .13), whereas a 50% increase in transport ratio was noted for the high tension muscles as compared to zero tension in the cumulative-label experiments (3.8 ± .25 vs. 2.5 ± .24, p < .001).

If passive stretch stimulated the rate of myocardial amino acid accumulation by a constant, albeit small percent throughout the entire 3-hour incubation, but the assay system was only sufficiently sensitive to detect a 3-hour cumulative effect, the data from the pulse-label experiment should approximate results obtained in the routine 0- to 1-hour incubations. Conversely, if a lag period exists

<table>
<thead>
<tr>
<th>Distribution ratio</th>
<th>Tension (g/mm²)</th>
<th>Pulse label (n = 12)</th>
<th>Cumulative label (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.5 ± .13</td>
<td>3.8 ± .25 &lt; .001</td>
</tr>
<tr>
<td></td>
<td>4 to 6</td>
<td>2.52 ± .24</td>
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</table>
and passive stretch stimulates amino acid accumulation only after the muscle has been stretched for 1 or 2 hours, the pulse-label experiment should demonstrate larger percent differences than the routine 0- to 1-hour incubation. The finding of identical distribution ratios for zero and high tension muscle groups in the pulse-label experiment suggests that passive stretch stimulates the rate of myocardial amino acid accumulation by a constant small factor from zero time and demonstrates definite limitations of the assay system for measuring relative AIB transport rates in different muscle groups.

Discussion

The results of the present study demonstrate the feasibility of using the isolated papillary muscle for studies of myocardial amino acid transport. Accumulation of AIB in the isolated papillary muscle appears to be an "active transport" process since the process is energy dependent (Fig. 3, Tables 1, 2) and since an intracellular/extracellular concentration gradient of greater than one can be generated. In resting cardiac muscle, glycolytic metabolism alone is sufficient to maintain this transport process as well as a normal extracellular fluid volume (Figs. 3, 4, Tables 1, 2). Under control and anoxic conditions, the intracellular accumulation of AIB was linear for 2 hours, reaching a plateau between 2 and 3 hours of incubation at a distribution ratio between 3.0 and 4.0. Identical data for the time course and the absolute values for AIB accumulation have been reported in isolated preparations of skeletal muscle (33). Similarly accumulation of AIB is linear for 2 hours in embryonic cardiac tissue, but in this rapidly growing tissue the equilibrium-distribution ratio attained at 3 hours is five times that found for adult rabbit cardiac muscle in the present study (34).

Certain features of the transport of amino acids appear to be similar in various animal cell systems (32, 35, 36). These common features include kinetic evidence compatible with a saturable carrier, and a dependence of amino acid transport upon the simultaneous transport of sodium ions. Evidence has been accumulated that the driving force for amino acid transport is not derived directly from an energy producing reaction but rather from the asymmetrical distribution of sodium (32, 35, 36) and possibly potassium (37) ions across the cell membrane. The step requiring energy in amino acid transport appears to be the ATP-dependent extrusion of intracellular sodium ions (32, 35, 36) which is mediated by an ATPase in the cell surface membrane which is Na⁺-K⁺ stimulated and ouabain inhibited (38).

In the present study, these features of amino acid transport were shown to exist in adult cardiac tissue. Transport is not linear with respect to substrate concentration and the kinetics of transport are amenable to analysis by the Michaelis-Menten equation. The values of \( V_{\text{max}} \) and \( K_m \) (6.64 μmoles • ml intracellular fluid⁻¹ • hour⁻¹ and 0.8 × 10⁻⁴ M) are similar to those obtained for AIB transport in rat diaphragm (\( V_{\text{max}} = 7.4 \) μmoles • ml intracellular fluid⁻¹ • hour⁻¹ \( K_m = 1.55 \times 10^{-3} \) M) (39). In contrast, the reported \( V_{\text{max}} \) of 26.3 μmoles • ml intracellular fluid⁻¹ • hour⁻¹ for AIB transport in chick embryo heart is four times that for adult rabbit heart and rat diaphragm whereas the \( K_m \) value is similar to that (\( K_m = 2.35 \times 10^{-3} \) M) of the adult preparations (34). Taken together, these data lend support for the existence of a saturable carrier for the transport of amino acids in heart muscle.

Support for the "sodium gradient" hypothesis (32) for amino acid transport has been gained from the demonstration of inhibition of myocardial amino acid transport by maneuvers that abolish the trans-sarcolemmal gradient of sodium. This gradient is reduced by lowering the extracellular concentration of sodium, by inhibiting directly the membrane Na⁺-K⁺ ATPase with ouabain (38), or by indirectly inhibiting this enzyme by depleting intracellular stores of ATP. In the rabbit papillary muscle, amino acid transport is reduced by: (1) lowering the concentration of sodium in the medium (Fig. 7); (2) incubating the tissue with ouabain (10⁻³ M) (Fig. 7, Table 3).

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or (3) inhibiting metabolism with iodoacetate and nitrogen, cold or fluoride (Fig. 3, Tables 1, 2). These observations suggest that the trans-sarcolemmal sodium ion concentration gradient is in fact a primary determinant of myocardial amino acid transport.

The osmotic properties of animal cells are such that the maintenance of normal cell volume requires the maintenance of normal sodium and potassium gradients across the cell membrane. A physiologic consequence of biochemical derangements which diminish transmembrane gradients of these cations is swelling of the cell (40). It is thus of note that whenever myocardial amino acid transport was inhibited (Figs. 3, 7, 8, Table 2), the extracellular fluid space decreased concomitantly. Since dry weight remained constant in these instances, a decrease in the extracellular fluid compartment indicates a transfer of water from extracellular to intracellular compartments, i.e., cellular swelling. These data lend indirect evidence for the sodium gradient hypothesis of amino acid transport and offer an explanation for the fact that anoxic muscles accumulate AIB normally (Fig. 3). In these muscles, the extracellular fluid volumes were normal throughout 3 hours of incubation and one must conclude that in resting cardiac muscle, glycolytic metabolism alone can provide sufficient energy to maintain normal transmembrane gradients of cations. The extracellular fluid space for muscles treated with iodoacetate under aerobic conditions was significantly less than control values, but not as low as noted in the muscles treated with the inhibitor under anoxic conditions (Table 1). Since percent dry weight did not vary, these results suggest that cellular swelling may occur in the presence of normal AIB accumulation and indicate a direct effect of iodoacetate on the penetration of inulin into the extracellular fluid space which is independent of the amino acid transport mechanism.

The mechanical factors which induce increased myocardial growth and cellular hypertrophy are poorly understood. While pressure loads in the intact heart are associated with hypertrophy (1-11), factors such as diastolic fiber length, systolic loading, velocity of contraction, or even coronary blood flow are all altered concomitantly.

In the present study, the papillary-muscle preparation has been used as a model to study the discrete mechanical factors which stimulate adaptive alterations in myocardial metabolism. The system has been shown to be metabolically competent and responsive to biochemical stimuli. As the initial approach, passive stretch of the nonstimulated muscle has been shown to be associated with increased intracellular accumulation of an amino acid analog. The fact that the inulin space is not altered by stretch suggests that the effect is not dependent on an enhanced rate of diffusion of the analog. Although the data in Figure 10 demonstrate that the higher distribution ratio noted at increased tension is not a function of different muscle cross-sectional areas between muscle groups, these data do not eliminate the possibility that the results are a function of lower sarcomere cross-sectional areas in a given muscle at higher tensions. Indeed, due to the constant volume requirement of any given muscle, the cross-sectional area of the entire muscle and the individual sarcomeres must decrease relative to its zero tension cross-sectional area as the muscle is progressively stretched. Thus the higher distribution ratios noted with increasing stretch may result from biophysical alterations in muscle structure (e.g., altered volume to surface ratios, unfolding of the endocardial envelope, etc.) rather than primary biochemical alterations in muscle metabolism (e.g., stimulation of the Na\(^+\)-K\(^+\) ATPase). However, these considerations notwithstanding, the experiments reported here demonstrate: (1) the feasibility of using the isolated papillary muscle as a model system in which to assay the ability of a specific mechanical stress to induce a specific biochemical adaptation in myocardial metabolism; and (2) the fact that with passive stretch the isolated papillary muscle develops an increased intracellular concentration of an amino acid analog as compared to unstretched controls.
It has been demonstrated that rapid muscle growth is associated with an increase in the intracellular concentrations of free amino acids (13, 14) as well as increased RNA synthesis and content, and increased rates of protein synthesis (1-11). Whether the increased intracellular concentration of amino acids determines the increased rate of protein synthesis or is merely a consequence of enhanced substrate requirements is presently unclear (13, 20, 21). Moreover, it is not known whether the present demonstration of an increased accumulation of amino acids is accompanied by any alterations in myocardial metabolism in general or increased synthesis of protein in particular. However, regardless of which mechanism holds, the present study demonstrates passive stretch to be a mechanical stress which stimulates the myocardium to maintain an increased intracellular concentration of an amino acid analog and consequently raises the possibility that passive stretch may be an adequate mechanical stimulus capable of inducing adaptive changes in myocardial metabolism, which subsequently initiate hypertrophy. Furthermore, although the passive tensions of 4 to 6 g/mm are clearly unphysiologic and probably alter mechanical function, these studies nevertheless support data obtained in other model systems which indicate that passive stretch may be a stimulus to muscle cell growth (41, 42).

References

Myocardial Amino Acid Transport in the Isolated Rabbit Right Ventricular Papillary Muscle: GENERAL CHARACTERISTICS AND EFFECTS OF PASSIVE STRETCH
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