Some Ionic Factors in Amino Acid Transport in Contracting Rat Diaphragm[1] (37572)

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(Introduced by M. B. Visscher)

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There is evidence for a relationship between muscle contractile activity and the active transmembrane movements of certain amino acids (1–4). In addition, there are reports which call attention to a possible connecting mechanism between active amino acid transport and the movement of certain inorganic ions (5–9). Previous studies in this laboratory (11, 12) have provided evidence of these relationships for the nonactive movement of nonmetabolizable sugars across cellular membranes in diaphragm muscle. A theoretical and experimental basis for delineating mechanisms of uptake of amino acids in isolated diaphragm has been presented by Akedo and Christensen (15). In this paper evidence is presented which shows an enhancement of the transmembrane flux of α-aminoisobutyric acid (AIB) and glycine in rat diaphragm muscle which is made to contract at various rates. Data are also presented demonstrating an influence of $[Na^+]$ and of ouabain on this process.

Materials and Methods. Isolated perfused diaphragm preparation (10, 11). The rat diaphragm was perfused by retrograde flow through the inferior vena cava, at a rate of 1–3 ml/min. Contractions were maintained throughout the experimental period at a rate of up to 10/sec by stimulation through silver clip electrodes attached to opposite sides of the rib cage. Stimuli of 20 V with a duration of 0.5 msec were generated by a Grass Model S-4 stimulator. This muscle preparation contracts vigorously for time periods of 1 hr or longer when glucose is present. The temperature of the diaphragm was maintained at 37 ± 1°.

Experimental procedures. The perfusing medium consisted of a modified aerated Krebs–Henseleit solution containing 250 mg % glucose. All muscle preparations were preperfused for 15 min to establish stable experimental conditions, then perfused for 30 or 60 min with the same solution containing various concentrations of the amino acids and tracer amounts of their respective labels (^{14}C). Ionic alterations were accomplished by replacing Na^+ with an equivalent amount of $choline^+$. Electrical stimulation of the muscle was started at the beginning of the 30 or 60 min perfusion period. After perfusion, the labeled amino acid was extracted from the right hemidiaphragm with water at 100° and counted in a scintillation counter. Total tissue water (79.5%) and extracellular space were determined. The latter volume was determined to be 16.8% of tissue wet weight after 30 min[3] of experimental perfusion and 18.6% after 60 min.

The data are presented as a distribution ratio of the calculated concentration of AIB in the intracellular water ($[amino\ acid]_I$) di-

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[3] In a previous paper (11) the 30 min ECS value was erroneously reported as a percentage of total tissue water, instead of the percentage of tissue wet weight.

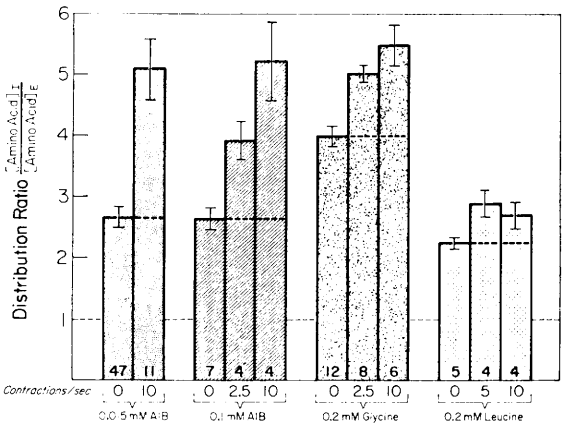

FIG. 1. Effects of various rates of muscle contractile activity upon the 60 min intracellular accumulation of AIB, glycine and leucine in the perfused rat diaphragm. The SEM and the number of experiments are shown for each bar. (- - -) Resting control values; (- - -) intracellular equilibration with the extracellular amino acid concentration.

vided by the concentration of AIB in the extracellular water ([amino acid]$_E$). The data were analyzed statistically and all p-values less than 0.05 were considered significant. We assume tentatively that there is active transport of an amino acid when the distribution ratio is greater than 1. The amino acid concentration in the extracellular water is assumed to be equal to that of the perfusion fluid.

Results. Figure 1 demonstrates the stimulatory effects of 60 min of muscular activity upon the cellular accumulation of AIB, glycine and leucine. Increasing the contraction rate caused a corresponding significant increase in the distribution ratios of AIB and glycine ($0.05 > p > 0.0005$). Under the same conditions, however, no significant change was found in the cellular accumulation of leucine ($p > 0.05$).

Figure 2 shows the results obtained with respect to 60 min of AIB transport as the sodium concentration in the perfusion medium was progressively lowered from the normal physiological level, 144 mEq/liter, to zero. In the presence of the normal sodium concentration (100%), muscle contracting at a rate of 1 or 10/sec exhibited a significant

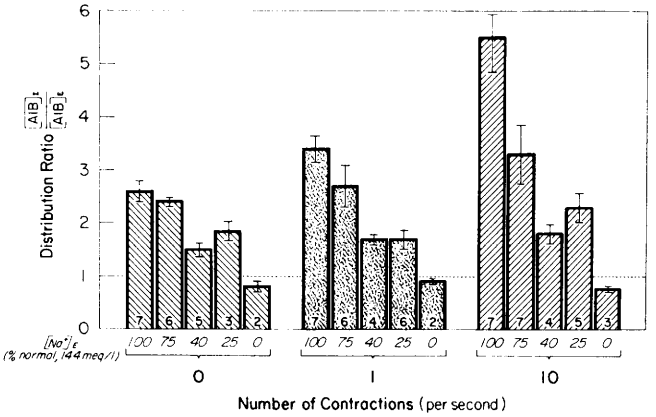

FIG. 2. Effects of extracellular [Na^+] on the 60 min intracellular penetration of AIB stimulated by muscle contractile activity in the perfused rat diaphragm (0.1 mM AIB). Symbols as in Fig. 1.

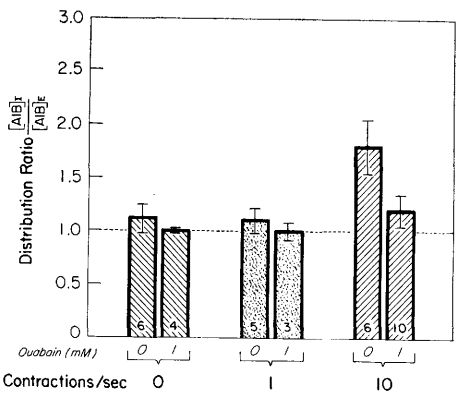

FIG. 3. Effects of 1.0 m*M* ouabain on the 30 min intracellular penetration of AIB stimulated by muscle contractile activity (0.1 m*M* AIB). Symbols as in Fig. 1.

enhancement of active AIB accumulation (1/sec: $p < 0.005$, 10/sec: $p < 0.0005$). Only in the cases where the full (100%) level of sodium was present was the increment in AIB accumulation related to muscle contraction clearly demonstrated. As the extracellular sodium was successively decreased, there was a decline in the degree of AIB accumulation in both resting and contracting muscle. At zero sodium muscle stimulation lost its ability to enhance transport of AIB.

To gain further insight into a possible role of a sodium pump on AIB transport, we perfused diaphragm muscles for 30 min with Krebs–Henseleit solution containing AIB (0.1 m*M*) and ouabain (1.0 m*M*). The results are shown in Fig. 3. In resting muscles or in muscles contracting at a rate of 1/sec no effect of ouabain was observed ($p > 0.05$). However, at a contraction rate of 10/sec, where there was a significant active accumulation of the amino acid under control conditions, the presence of ouabain in the perfusion fluid significantly decreased or eliminated active transport of AIB ($p < 0.05$).

Discussion. The information contained in Fig. 1 indicates a positive correlation between the degree of muscle activity and the level to which AIB and glycine are accumulated in diaphragm muscle. In view of the previously reported studies of Havivi and Wertheimer (13) and also Frederickson, Bihler and Dresel (14), one could interpret these data in terms of the hypothesis that the effects of muscle activity on transportive processes may be mediated indirectly by some chemical factor produced within the contracting muscle.

In addition, the results of these studies suggest that there are at least two different types of transport mechanisms for amino acids across striated muscle cell membranes. Specifically, in the rat diaphragm, leucine may be transported by a system(s) separate from that which transports AIB and glycine. In support of this proposition, Fig. 1 clearly demonstrates the lack of significant relationship between the accumulation of leucine and the rate of diaphragmatic contraction. This contrasts with the findings for AIB and glycine uptake.

Figure 2 shows that the augmentation of AIB accumulation associated with muscle contraction is sensitive to sodium depletion in the extracellular fluid. Apparently the active transport of AIB in both resting and contracting muscle is dependent upon the presence of some minimal level of sodium in the extracellular fluid. The depressing effect of ouabain (Fig. 3) and the dependence upon sodium concentration (Fig. 2) indicate that the augmented AIB transport in contracting muscle is intimately associated with the active transport of sodium.

Summary. Isolated, perfused rat diaphragm has been studied with respect to the influences of muscle contraction and of ionic conditions upon the active accumulation of α-aminoisobutyric acid, glycine and leucine. Muscle contractile activity increased the accumulation of AIB and glycine but not leucine. This finding provides evidence that at least two amino acid transporting systems exist in diaphragm muscle. The augmentation of AIB accumulation by muscle contraction is sensitive to sodium depletion in the extracellular fluid. Furthermore, augmented AIB transport in contracting muscle is closely related to the active transport of sodium.

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