Effects of disuse and denervation on amino acid transport by skeletal muscle

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GOLDBERG, A. L., AND H. M. GOODMAN. Effects of disuse and denervation on amino acid transport by skeletal muscle. Am. J. Physiol. 216(5): 1116-1119. 1969.—The rate of α-aminoisobutyric acid (AIB) accumulation by the soleus and plantaris muscles decreased within 3 hr after section of the sciatic nerve. This decrease preceded any loss of muscle weight. A similar but reversible reduction in uptake occurred after anesthetization of the sciatic nerve. Section of the spinal cord also decreased AIB uptake by the paralyzed muscles, but did not affect AIB uptake by the diaphragm muscle, which continued to function normally. It was concluded that the inhibition of AIB transport is related to disuse of muscle. At longer periods after denervation, AIB accumulation increased. This secondary rise in uptake occurred earlier in the plantaris than in the soleus, and earlier in normal rats than in hypophysectomized rats. The onset of increased AIB uptake in the denervated muscle correlated with the onset of spontaneous fibrillations, but this increased accumulation of amino acids did not alter the rate of atrophy of the muscles. It was concluded that the amino acid uptake by muscle is influenced by the amount of muscular activity.

MATERIALS AND METHODS

The uptake of AIB-14C and inulin-3H by muscles of the hindlimb of hypophysectomized and normal rats was measured as described in the preceding article (9). Hypophysectomized rats were employed to permit study of the effects of disuse and denervation independently of body growth (7). The animals were anesthetized with ether during all surgical procedures. To denervate the soleus and plantaris, a 1-cm incision was made vertically immediately below the hip. The sciatic nerve was then sectioned as it emerges from beneath the gluteus maximus. To produce reversible paralysis of the soleus, a cotton pellet saturated with 3 % Xylocaine (Astin Pharmaceuticals, Worcester, Mass.) was placed on the intact nerve. In both instances, a sham operation was performed on the opposite limb; the sciatic nerve was exposed but left intact. The hindlimb muscles were also paralyzed by sectioning the spinal cord at L4 as described previously (7). This procedure also left the sciatic nerve intact.

RESULTS

Within 3 hr after denervation, AIB uptake by soleus muscles of hypophysectomized rats was markedly reduced (Fig. 1), although no change in muscle weight was evident at this time. Decrease in muscle dry weight was first seen 24 hr after section of the sciatic nerve. The dry weight of the denervated soleus averaged 4 ± 1.4 % (P < 0.05) less than that of the contralateral control. The decrease in dry weight of the denervated plantaris averaged 3.6 ± 1.1 % (P < 0.05). This rapid decrease in AIB accumulation cannot be explained by a change in the extracellular space of the denervated muscle, as...
AMINO ACID TRANSPORT IN DENERVATED MUSCLE

FIG. 1. AIB uptake in the soleus muscle of hypophysectomized rats following section of the sciatic nerve. Measurements were made after 1-hr exposure to AIB; mean concentrations of AIB/total muscle water (intracellular plus extracellular) are shown for 5 animals. A significant decrease in accumulation occurred at each time ($P < 0.01$).

FIG. 2. AIB uptake by denervated muscles 1 day after section of the sciatic nerve. Each point is the mean ± SEM of 5 observations. Animals were injected with AIB-$^{14}$C 24 hr after nerve section, and AIB/total muscle water measured at various times thereafter.

measured with inulin-$^{3}$H. Four hours after nerve section, the extracellular space of the denervated muscle averaged $23 ± 2\%$ of the muscle water, whereas that of the control was $20 ± 1\%$. Further measurements of AIB accumulation after longer exposures to AIB provide additional evidence that denervation reduced the rate of transport (Fig. 1). Four hours after denervation, AIB uptake by planaris muscles was also reduced, although this change was less marked because of the low basal rate of AIB accumulation in this muscle (6, 8). When AIB accumulation by the denervated planaris was compared with that of controls 7 hr after administration of AIB, a marked diminution became evident (Fig. 2).

Experiments were then undertaken to determine if decreased uptake of AIB resulted from the muscle's inactivity or from loss of some "trophic" factor normally released from the nerve. Spinal cord section, which paralyzes the muscles without injuring the motor neurons, reduced AIB accumulation by various muscles of the hindlimb (Table 1). The data in Table 1 were obtained 4 hr after injection of AIB and 20 hr after spinal section at L4. No such change occurred in the diaphragm, which continued to function normally. Similarly local anesthesia of the sciatic nerve with 3% Xylocaine reduced the uptake of AIB by the soleus within 3 hr (Table 2). The effects of anesthesia were reversible and, with the return of normal function, the ability of the soleus to concentrate AIB returned to control levels.

Additional studies were carried out on muscles at longer times after denervation, when spontaneous fibrillations occur (unpublished observations). Although AIB uptake was decreased for the first few days after denervation, increased accumulation was found in the soleus muscle 4 weeks after denervation (Fig. 3). Increased uptake of AIB appeared even sooner after nerve section in the planaris muscle. The secondary rise was not a result of nerve regeneration, and did not reflect any change in the rate of muscle atrophy (Fig. 4). In normal rats, the secondary increase in the accumulation of AIB occurred much sooner after denervation than in hypophysectomized rats. The increase in amino acid transport after denervation correlates well with the onset of denervation fibrillations (unpublished data). In general, when fibrillations were found, denervated muscles accumulated AIB to a greater extent than controls; when electrically inactive, denervated muscles concentrated amino acids less avidly than controls.

TABLE 1. $AIB-^{14}$C uptake 20 hr after spinal section

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Distribution Ratios, 4 hr after Injection of AIB</th>
<th>State of muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control animals</td>
<td>Spinal animals</td>
</tr>
<tr>
<td>Soleus</td>
<td>3.4±.24</td>
<td>1.5±.06*</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>2.1±.13</td>
<td>1.4±.12*</td>
</tr>
<tr>
<td>Plantaris</td>
<td>1.7±.09</td>
<td>1.3±.15t</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>1.4±.06</td>
<td>1.0±.17t</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>2.4±.19</td>
<td>3.1±.40</td>
</tr>
</tbody>
</table>

Values are means ±SEM. Each value represents the mean of 7 rats measured 4 hr after the injection of AIB. *$P < 0.01$. t$P < 0.05$.

TABLE 2. Effects of anesthesia of motor neurons on AIB accumulation by soleus muscle

<table>
<thead>
<tr>
<th>Time After Anesthesia</th>
<th>Control limb</th>
<th>Anesthetized limb</th>
<th>Increment</th>
<th>State of muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>1.64±.15</td>
<td>1.85±.12</td>
<td>-.21±.15</td>
<td>Paralyzed</td>
</tr>
<tr>
<td>24 hr</td>
<td>1.06±.06</td>
<td>1.62±.18</td>
<td>.56±.14</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Values are means ±SEM. Each value represents the mean of distribution ratios measured in 6 rats 1 hr after injection of AIB. *$P < .01$. **$P < 0.01$. **$P < 0.001$. 

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HYPOPHYSECTOMIZED
SOLEUS

Denervated
Control

DISTRIBUTION RATIO

2.0
3.0
1.0

1.0
2.0
3.0

TIME AFTER DENERVATION

3 Days 12 Days 28 Days

NORMAl
SOLEUS

PLANTARIS

FIG. 3. AIB accumulation by the soleus and plantaris muscles of hypophysectomized rats at various times after nerve section. These results were obtained 1 hr after injection of AIB. In the hypophysectomized rats, the plantaris had increased ability to concentrate AIB 12 and 28 days after denervation (P < 0.01). At 28 days after denervation, accumulation by the soleus was increased. Although the denervated soleus at 12 days and the plantaris at 3 days usually showed decreased AIB uptake, occasional animals showed the opposite behavior. In normal rats, accumulation of AIB by the plantaris was increased 3 days after nerve section (P < 0.01) although it was reduced 1 day after denervation.

FIG. 4. Rate of atrophy of plantaris and soleus in hypophysectomized rats following section of the sciatic nerve, and its relationship to changes in AIB uptake. (Distribution ratio of denervated muscle/distribution ratio of contralateral control.) Each value represents the mean ± SEM of 5 observations.

DISCUSSION

The experiments described above and those reported in the preceding article (9) indicated that changes in muscular activity cause parallel changes in the accumulation of amino acids. When muscular activity decreased, AIB transport also decreased; when muscular activity increased following either tenotomy of synergistic mus- 

FIG. 4. Rate of atrophy of plantaris and soleus in hypophysectomized rats following section of the sciatic nerve, and its relationship to changes in AIB uptake. (Distribution ratio of denervated muscle/distribution ratio of contralateral control.) Each value represents the mean ± SEM of 5 observations.

cles or the onset of spontaneous fibrillations (unpublished observations), AIB accumulation increased. The physiological significance of such changes in transport and their relationship to hypertrophy and atrophy, however, are not clear. Reduced ability to accumulate amino acids may be associated with atrophic events, just as increased uptake appears important in compensatory growth (9). This view is attractive because of the possibility that work-induced hypertrophy and disuse atrophy are closely related processes, possibly even the exact opposite of one another. It is of interest that cortisone (14), which can induce muscle atrophy, also causes a rapid decrease in AIB accumulation, whereas insulin (12) and growth hormone (13), both anabolic to muscle, increase AIB transport.

The observations on denervated muscles after the onset of fibrillations, however, argue against a general correlation between AIB uptake and growth, since denervated fibrillating muscles continued to lose weight at a constant rate, even though AIB uptake was increased (Fig. 4). In addition, the secondary rise in AIB transport occurred sooner after denervation in normal than in hypophysectomized rats, although the rates of atrophy in the two groups of animals did not differ significantly (10) (Fig. 4). Gutmann and others (10, 11), however, have argued that denervation and disuse atrophy of muscle are separate processes, and that, unlike simple disuse, denervation constitutes a pathological state for muscle, resulting from loss of trophic influences normally released by the motor neuron. The present findings on denervated muscle therefore do not necessarily contradict the notion that in normal muscle amino acid transport is correlated with rate of growth. For example, it is possible that the normal coupling between amino acid entry and utilization is altered after denervation.
Further studies of the relationship between amino acid transport and protein synthesis following denervation and disuse are necessary if we are to understand the mechanisms underlying muscle atrophy. Interpretation of data on protein synthesis during atrophy will be complicated by the finding that rates of amino acid transport also vary. Since changes in transport affect the entry of labeled amino acids into intracellular pools, they can influence the amount of labeled precursors incorporated into proteins independently of the rate of protein synthesis. To constitute a valid measure of protein synthetic rates, measurements of the amino acid incorporation must be corrected for differences in the specific activities of intracellular pools. For example, the biphasic variations in AIB uptake (Fig. 3) could lead to increased or decreased incorporation of labeled amino acids in denervated muscle even in the absence of any change in protein synthesis. Previous investigations have failed to consider this complication, which may account for the conflicting findings on protein synthesis in denervated muscles.

The present finding and the recent report of Bombara and Bergamini (2) that AIB transport changes in a biphasic manner after denervation may provide an explanation for other apparently contradictory observations in the literature (2–5). For example, in the rabbit and rat, Dreyfus (5) found decreased transport of AIB by the gastrocnemius one day after denervation, whereas Diehl and Jones (4) reported increased uptake by this muscle in the rat 7–21 days after nerve section. Both of these observations, however, are consistent with the present findings and would be anticipated on the basis of Fig. 3.

Denervation fibrillations may have important metabolic consequences in muscle (unpublished data). This suggestion was first offered by Langley and Kato (15), who, along with others (16), proposed that such increased spontaneous activity might even be responsible for denervation atrophy. Since the loss of muscle weight preceded the onset of fibrillations, and since the rate of muscle atrophy was not affected by the onset of fibrillations and the associated increase in amino acid transport, this argument is untenable. Nevertheless, fibrillations distinguish the effects of denervation from those of disuse and thus complicate the study of mechanisms responsible for atrophy.

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