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Amino acids were assayed in muscles from rats exposed to 5 days of hypogravity and 12 h of gravity (F) for 5 days of suspension with (R) or without (H) 12 h of loading. In these groups, lower aspartate was common only to the soleus (SOL) relative to control muscles, the smallest difference being in group R. This difference in aspartate for F and H, but not for R, correlated with lower malate suggesting diminution of citric acid cycle intermediates. The R:SOL value was increased over the H SOL. Therefore despite 12 h of loading, the F SOL was more comparable to the 4 SOL. The role of stress in preventing recovery of the F SOL was apparent from the ratios of glutamine/glutamate. Synthesis of glutamine is enhanced by glucocorticoids and is reflected by an increased ratio. In 5 of the 6 F muscles studied, this ratio was greater than in controls. In contrast, the ratio in all R muscles was similar to controls and showed recovery from the values in H muscles. Hence the post-flight treatment of F rats may have produced additional stress. Despite this stress, in some respects the SOL responses to hypogravity were similar to its responses to unloading by suspension.

Previous work from this laboratory has shown that certain amino acids undergo marked changes in the soleus muscle in response to unloading by tail- cast hindlimb suspension (1-5). For instance, fresh tissue tyrosine and its in vitro release from the muscle are increased, suggesting a more negative protein balance. Tissue levels of aspartate are markedly lower as is the utilization of this amino acid by isolated soleus of suspended rats. Finally, the production of glutamine by this muscle is slower and this difference is reflected in lower ratios of glutamine/glutamate both in fresh and incubated muscle. The slower synthesis of glutamine by the unloaded soleus is apparently due to limited availability of ammonia owing to reduced flux of the purine nucleotide cycle. Despite this slower production of glutamine, the unloaded soleus has a greater total capacity for synthesizing glutamine apparently due to induction of muscle glutamine synthetase by greater circulating glucocorticoids. In light of these several changes in muscle amino acids in response to decreased use through unloading, it seemed important to make similar measurements in muscles subjected to unloading by hypogravity.

METHODS

Several groups of animals were studied. One group of 6 rats was flown on the SL-3 mission for 7 days and 12 h and then placed in a cold room (3°C) and pieces of muscle for homogenization in cold perchloric acid (0.2 N). Glutamine synthetase activity was measured in these muscles with methods similar to those used for other muscles (3,4). In these experiments, the supernatant solution was removed and neutralized to pH 6.5-7.5 with KOH. Fluorometric analysis of glutamine, glutamate, aspartate and malate were completed within 3 days of sample preparation.

RESULTS AND DISCUSSION

Concentrations of glutamine, glutamate, aspartate (as asparagine) and alanine were compared in hindlimb muscles of SL-3 and ground control rats. Alanine was lower in the soleus of flown rats (Table 1) but not of suspended animals (Table 2), with no response in other muscles except a slight increase in the unloaded plantaris (Table 2). With recovery, alanine in the soleus was elevated. Since we found no differences in alanine metabolism by isolated muscle (3,4), changes in muscle alanine are probably due to altered body use of this amino acid leading to varied plasma levels.

Glutamine and Glutamate. Generally, both glutamine and glutamate were lower in the muscles of flown rats, except for no difference of glutamine in the tibialis (Table 1). Unloading by suspension also generally lowered glutamine but not glutamate (Table 2). Since hindlimbs of flown rats were weighing bearing for 12 h, we tested the effect of loading on suspended rats. Glutamine remained unchanged in all muscles except in the soleus where it increased (Table 3). Glutamate was unchanged in all muscles. These results suggest that lower muscle glutamine may reflect a general response to unloading while lower muscle glutamate in the flown rats was probably a function of their overall treatment.

Glutamine Synthesis. Ratios of glutamine/glutamate provide an indication of muscle glutamine synthesis. With unloading by tail-cast suspension, the ratio falls in soleus and plantaris in accord with decreased in vitro synthesis. In contrast, synthesis appears increased in the extensor digitorum longus, probably as a result of glucocorticoid effects on glutamine synthetase. These results agree with our previous findings. When activity of the unloaded soleus is restored, the ratio rises (Table 3) since now sufficient ammonia is probably available. These results help to interpret the elevated ratios of glutamine/glutamate in muscles of flown rats (Table 1). Most likely, the stress of flight on SL-3 and...
Table 1. AMINO ACIDS IN MUSCLES FROM SL-3 RATS

Percent Difference from Ground Controls

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Gln</th>
<th>Glu</th>
<th>Gin</th>
<th>Asp</th>
<th>Mal</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>-30</td>
<td>+58</td>
<td>-52</td>
<td>+70</td>
<td>+40</td>
<td>NS</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>-26</td>
<td>-47</td>
<td>+45</td>
<td>+45</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Plantaris</td>
<td>-26</td>
<td>+30</td>
<td>-76</td>
<td>-34</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Extensor</td>
<td>+11</td>
<td>+38</td>
<td>-20</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Significance of difference: aP<0.005; bP<0.001; cP<0.005; dP<0.001
NS = not a significant difference

Table 2. AMINO ACIDS IN SUSPENDED RATS

Percent Difference from Weight Bearing

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Gln</th>
<th>Glu</th>
<th>Gin</th>
<th>Asp</th>
<th>Mal</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>-10</td>
<td>+91</td>
<td>+116</td>
<td>+74</td>
<td>+120</td>
<td></td>
</tr>
</tbody>
</table>

Significance of difference: aP<0.005; bP<0.001; cP<0.005; dP<0.001
NS = not a significant difference

Table 3. AMINO ACIDS IN SOLEUS FOLLOWING RECOVERY

Percent Difference from Unloaded

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Gln</th>
<th>Glu</th>
<th>Gin</th>
<th>Asp</th>
<th>Mal</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>+103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Differences were significant at P<0.005

Concluding Remarks. Despite the 12 h of exposure to normal gravity following 7 days in space, amino acids in muscles of SL-3 rats showed some similarities to those of suspended rats. Since aspartate recovered in unloaded soleus but apparently not in soleus of flown rats, it is conceivable that the additional stress to these animals of landing and transcontinental flight, may have preserved their catabolic state to some extent. However, the ability to synthesize glutamine may have returned to near normal (i.e., ammonia is produced) while the capacity to synthesize glutamine is clearly increased presumably due to glucocorticoid (i.e., response to stress) effects on the synthetase. Although it was unfortunate that the flown animals were not sacrificed within 1 to 2 h, the data still support the possibility that the suspension model may mimic the effects of weightlessness (see also this issue, 5).

ACKNOWLEDGEMENTS

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REFERENCES


