Ammonia and IMP in different skeletal muscle fibers after exercise in rats

RONALD A. MEYER, GARY A. DUDLEY, AND RONALD L. TERJUNG
Department of Physiology, Upstate Medical Center, State University of New York,
Syracuse, New York 13210

Meyer, Ronald A., Gary A. Dudley, and Ronald L. Ter Jung. Ammonia and IMP in different skeletal muscle fibers after exercise in rats. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(6): 1037-1041, 1980. — Adenosine 5'-monophosphate (AMP) deamination, estimated from inosine 5'-monophosphate (IMP) accumulation, was studied in the different skeletal muscle fiber types of untrained rats anesthetized with ether immediately after 4 min of treadmill running at 45 or 60 m/min. The adenylosuccinate synthetase-inhibitor hadacidin was administered (200 mg/kg ip) before exercise to block IMP reamination and, therefore, to provide a better assessment of IMP formation. The increases in blood ammonia after exercise (2.5- and 5-fold, respectively) were highly correlated (r = 0.93) with the increases in blood lactate levels (6- and 11-fold). At both speeds, IMP increased in fast-twitch but not in slow-twitch (soleus) muscle. Of the fast muscles, the increase in IMP was greatest (up to 4 pmol/g wet wt) in the white vastus lateralis (fast twitch, glycolytic), intermediate in the plantaris (mixed fibers), and lowest in the red vastus lateralis (fast twitch, oxidative glycolytic). The increases in IMP were coincident with nearly equivalent decreases in ATP. Hadacidin treatment resulted in a greater IMP accumulation after exercise in both fast-twitch types but not in the soleus. The results indicate that fast twitch muscle fibers, particularly the fast twitch glycolytic fibers, are the source of the ammonia produced during strenuous exercise.

AMP deaminase; hadacidin; muscle fiber recruitment

It was recognized almost 50 yr ago that the ammonia that appears in the blood during exercise arises in muscle from the deamination of adenosine 5'-monophosphate (AMP) to inosine 5'-monophosphate (IMP) and ammonia, catalyzed by the enzyme AMP deaminase (18). Nevertheless, the functional significance of AMP deamination and ammonia production in muscle is only beginning to be understood (18). Ammonia production is greatest during intense exercise (8, 36) or tetanic muscle stimulation (20) when the rate of ATP utilization may exceed the rate of ATP resynthesis. This net ATP hydrolysis should lead to an adenosine 5'-diphosphate (ADP) and AMP accumulation. However, it has been suggested that under these conditions the removal of AMP by deamination functions to stabilize the relative ratios of ATP to ADP and AMP by favoring the production of ATP through the myokinase reaction (2ADP → AMP + ATP) (2, 8, 20). In addition, AMP deamination may contribute to the control of glycolysis during intense work, both by controlling the adenine nucleotide levels (32) and by supplying ammonium ion, an activator of phosphofructokinase (29).

We recently reported that AMP deamination occurs readily in rat fast-twitch glycolytic (FG) and fast-twitch oxidative-glycolytic (FOG) fibers, but not in slow-twitch oxidative (SO) fibers, during intense stimulation in situ (20). In that study (20), there was little difference in the extent of AMP deamination observed between FG and FOG fibers inasmuch as the intense tetanic contractile sequence was required from both fiber types simultaneously. This does not necessarily represent the situation found, for example, during treadmill running, since an ordered recruitment pattern of muscle fiber types is known to exist (3, 30). Furthermore, there may not be an equivalent AMP deamination in the FOG and FG fibers during less intense, more aerobic work conditions because the FOG fibers have a higher oxidative capacity than the FG fibers (4) and thus would be expected to provide a greater rate of ATP resynthesis. Therefore, the purpose of this study was to determine the extent of AMP deamination and ammonia production in the different fiber types of rat skeletal muscle during treadmill exercise. Inasmuch as IMP is produced within muscle fibers along with ammonia, but unlike ammonia does not diffuse from the fibers (20, 21), this was accomplished by determining the levels of IMP in the different fiber types after exercise. In addition, hadacidin (N-formyl-N-hydroxynicotinamide), which blocks adenine nucleotide synthesis by virtue of its inhibition of adenylosuccinate synthetase (17, 21), was used in this study to prevent the possible resynthesis of adenine nucleotides from IMP.

Methods

Animal care and exercise protocol. Adult male Sprague-Dawley rats weighing 363 ± 5 g (mean ± SE, n = 41) were housed 5 per cage in a temperature-controlled room (20-21°C) with a 12-h light/12-h dark cycle. Purina laboratory chow and water were provided ad libitum. During the week before the experiment, the animals were given two to three preliminary exercise bouts lasting less than 5 min in order to become acquainted with running on the treadmill (Quinton model 42-15); however, the animals were not endurance trained. Animals were assigned to one of three groups: resting controls, 45 m/min exercise, and 60 m/min exercise. Half of the animals in each of these groups were treated with sodium hadacidin (200 mg/kg, injected ip in 100 mg/ml solution

0161-7567/80/0000-0000$01.25 Copyright © 1980 the American Physiological Society
and distributed in 2 equal doses 45 and 15 min before exercise. We have shown that this dose of hadacidin inhibits the resynthesis of ATP from IMP in the fast-twitch muscle fiber types by more than 80% (21). Non-hadacidin control animals for each group were injected with equivalent volumes of 0.9% saline. In addition, a group of animals was exercised at 30 m/min, but muscle samples were not taken for assay. For each exercise group, running was carried out up a 15% incline and consisted of a 1-min warm-up at 17.5 m/min followed by a 4-min run at the indicated speed. The 4-min duration was established by the average maximal performance time of animals in the 60 m/min group (3.96 ± 0.35 min, n = 12). Hadacidin had no significant effect on this performance time. The resting control animals were placed on the stationary treadmill in lieu of exercise.

**Tissue sampling and assay.** Immediately after exercise, the animals were anesthetized with ether, the left hindlimb muscles were exposed, and the soleus, plantaris, white vastus lateralis, and red vastus lateralis muscle sections were excised and clamp-frozen in aluminum tongs precooled in liquid nitrogen and stored at −75°C. These muscles are predominantly composed of SO fibers, mixed fast-twitch fibers, FG fibers, and FOG fibers, respectively (1, 4, 30). Then, 1 ml blood samples were drawn from the abdominal aorta, deproteinized in 2 ml 3.5 N perchloric acid, neutralized, and used immediately to assay ammonia and lactate. From 3 to 5 min elapsed between termination of the exercise and completion of the sampling procedure for each rat.

Frozen muscles were pulverized in a mortar cooled with liquid nitrogen and extracted in cold alcoholic perchloric acid (19). Ammonia (15), lactate (13), IMP (12), ATP (16), phosphocreatine (16), and creatine (6) were assayed by standard enzymatic techniques, as done previously (20, 21). Assays were performed in duplicate under conditions where the values obtained were proportional to the amount of extract used. Metabolite recovery was greater than 90% for each assay system used. All enzymes, cofactors, and standards were obtained from Sigma Chemical, St. Louis, MO.

Because the water content of muscle changes with use (5, 20, 26), muscle metabolite contents were corrected to a constant water content, that of the resting muscle fiber type, using total creatine (26). Total creatine levels, expressed as μmol/g wet wt, decreased significantly after running in all muscles except the soleus (Table 1).

Where appropriate, statistical comparisons between groups were made by two-way analysis of variance (ANOVA) (hadacidin effects vs. running effects) or by Student's t test. An α = 0.05 level of confidence was used for these comparisons.

**RESULTS**

Both blood ammonia and lactate levels increased dramatically after running at speeds above 30 m/min (Fig. 1). Thus, as observed in human studies (9, 36), there was a high correlation between blood ammonia and lactate (r = 0.93, n = 41, P < 0.001). ANOVA of data from control and 45 and 60 m/min animals revealed no significant effect of hadacidin treatment on blood ammonia or lactate.

Metabolite contents of muscles from resting control and 45 and 60 m/min groups appear in Table 2. Muscle ammonia content increased in all fiber types after running, although the increases were greatest in the FG

<table>
<thead>
<tr>
<th>TABLE 1. Total creatine content of the different skeletal muscle sections at rest or after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle Type</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Plantaris, mixed*</td>
</tr>
<tr>
<td>Red vastus, FOG*</td>
</tr>
<tr>
<td>White vastus, FG†</td>
</tr>
<tr>
<td>Soleus, SO</td>
</tr>
</tbody>
</table>

Values are means ± SE, μmol/g wet wt; the number of animals in each group is given in parentheses. Data were obtained from animals at rest (nonrun) or after 4 min of exercise (run) at 45 or 60 m/min. Values given are combined data from hadacidin- and saline-treated animals inasmuch as no drug effect was found. FOG, fast-twitch oxidative-glycolytic fibers; FG, fast-twitch glycolytic fibers; SO, slow-twitch oxidative fibers. * Significant running effect (P < 0.01). † Significant running effect (P < 0.001).
vastus and plantaris. Just as in blood, hadacidin treatment had no significant effect on ammonia content in the muscles. Lactate also increased significantly in all muscle types with running. However, only in the FG vastus and plantaris muscles did the lactate levels equal or exceed the levels in the blood (Fig. 1), suggesting that the FG fibers were the major source of the increases in blood lactate (3). There was significantly greater lactate accumulation after running in the FG vastus and plantaris muscles of the hadacidin-treated rats compared with saline-treated controls.

There was a dramatic increase in IMP content in the FG vastus and plantaris muscles after running and a smaller but statistically significant increase in the FOG vastus section. Moreover, hadacidin treatment resulted in a significant increase in IMP (approx 1 µmol/g wet wt) after exercise in the FG vastus, plantaris, and FOG vastus muscle sections. As shown previously (20), these increases in IMP in the fast fiber types are reflected by nearly equivalent decreases in ATP content (Fig. 2). In contrast, there was no significant increase in IMP in the soleus muscle after running in any of the treatment groups.

**DISCUSSION**

The well-known increase in blood ammonia levels that occurs during heavy exercise in humans (23, 35, 36) was similarly shown to occur in exercising rats but only when the running speed was in excess of 30 m/min [i.e., greater than approximately 80% of maximal O2 uptake (27)]. During these heavy-to-intense work conditions when ammonia was readily produced, the ammonia levels in the three muscle fiber types were greater than the corresponding blood levels. This suggests that each fiber type could have contributed to the ammonia production. However, attributing ammonia production to a specific fiber type during exercise is complicated by the fact that muscle is also a major site of ammonia uptake whenever blood ammonia levels are increased (25). This has been explained by the fact that ammonia is a base (pK 9.3) and would be trapped as ammonium ion in tissues with a lower pH than plasma, since it is probable that only the nonprotonated form of ammonia is freely diffusible across the cell membrane (33). For example, even under resting conditions there would be a 2.5 times greater ammonia concentration in muscle than in plasma, assuming pH's of 7.0 and 7.4, respectively. It follows that an increase in blood ammonia, as occurred during running at 45 and 60 m/min, could result in an elevation in muscle ammonia levels. Therefore, our observation that amm-

**TABLE 2. Metabolite contents in the different skeletal muscle sections at rest or after exercise**

<table>
<thead>
<tr>
<th>Plantaris, mixed</th>
<th>Nonrun</th>
<th>Run, 45 m/min</th>
<th>Run, 60 m/min</th>
<th>Soleus, SO</th>
<th>Nonrun</th>
<th>Run, 45 m/min</th>
<th>Run, 60 m/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ammonia</strong></td>
<td>C 0.36 ± 0.04</td>
<td>1.61 ± 0.35</td>
<td>1.73 ± 0.13</td>
<td>C 0.65 ± 0.18</td>
<td>1.16 ± 0.09</td>
<td>1.35 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H 0.53 ± 0.13</td>
<td>1.29 ± 0.12</td>
<td>1.85 ± 0.09</td>
<td>H 0.64 ± 0.05</td>
<td>1.14 ± 0.08</td>
<td>1.24 ± 0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td>C 1.76 ± 0.29</td>
<td>11.7 ± 1.4</td>
<td>19.1 ± 2.1</td>
<td>C 1.71 ± 0.26</td>
<td>8.33 ± 1.06</td>
<td>12.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td><strong>IMP</strong></td>
<td>C 2.08 ± 0.37</td>
<td>13.8 ± 1.9</td>
<td>24.7 ± 0.7</td>
<td>H 1.22 ± 0.11</td>
<td>8.65 ± 1.16</td>
<td>14.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>C 0.11 ± 0.03</td>
<td>1.28 ± 0.17</td>
<td>1.18 ± 0.19</td>
<td>C 0.17 ± 0.06</td>
<td>0.12 ± 0.04</td>
<td>0.17 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H 0.13 ± 0.03</td>
<td>1.98 ± 0.25</td>
<td>2.30 ± 0.15</td>
<td>H 0.10 ± 0.01</td>
<td>0.14 ± 0.05</td>
<td>0.27 ± 0.06</td>
<td></td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>C 6.57 ± 0.14</td>
<td>5.01 ± 0.23</td>
<td>5.82 ± 0.27</td>
<td>C 4.82 ± 0.18</td>
<td>4.75 ± 0.10</td>
<td>4.18 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H 7.94 ± 0.23</td>
<td>6.00 ± 0.42</td>
<td>4.49 ± 0.18</td>
<td>H 4.73 ± 0.15</td>
<td>4.62 ± 0.23</td>
<td>4.22 ± 0.12</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>White Vastus, FG</th>
<th>Nonrun</th>
<th>Run, 45 m/min</th>
<th>Run, 60 m/min</th>
<th>Red Vastus, FOG</th>
<th>Nonrun</th>
<th>Run, 45 m/min</th>
<th>Run, 60 m/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ammonia</strong></td>
<td>C 0.23 ± 0.03</td>
<td>2.17 ± 0.22</td>
<td>2.44 ± 0.36</td>
<td>C 0.48 ± 0.10</td>
<td>1.21 ± 0.11</td>
<td>1.64 ± 0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H 0.42 ± 0.05</td>
<td>2.19 ± 0.26</td>
<td>3.36 ± 0.35</td>
<td>H 0.76 ± 0.08</td>
<td>1.31 ± 0.21</td>
<td>1.47 ± 0.08</td>
<td></td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td>C 2.80 ± 0.49</td>
<td>22.0 ± 3.3</td>
<td>33.2 ± 3.0</td>
<td>C 1.60 ± 0.28</td>
<td>6.21 ± 1.00</td>
<td>11.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td><strong>IMP</strong></td>
<td>C 3.56 ± 0.44</td>
<td>27.1 ± 2.9</td>
<td>45.0 ± 3.9</td>
<td>H 1.24 ± 0.13</td>
<td>7.01 ± 1.24</td>
<td>13.7 ± 0.5</td>
<td></td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>C 0.15 ± 0.06</td>
<td>2.46 ± 0.43</td>
<td>2.80 ± 0.68</td>
<td>C 0.07 ± 0.03</td>
<td>0.19 ± 0.06</td>
<td>0.43 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H 0.12 ± 0.03</td>
<td>3.20 ± 0.49</td>
<td>4.15 ± 0.29</td>
<td>H 0.14 ± 0.03</td>
<td>1.03 ± 0.14</td>
<td>1.39 ± 0.09</td>
<td></td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>C 7.75 ± 0.17</td>
<td>5.40 ± 0.40</td>
<td>4.95 ± 0.77</td>
<td>C 7.24 ± 0.16</td>
<td>7.12 ± 0.17</td>
<td>6.34 ± 0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H 7.85 ± 0.29</td>
<td>4.31 ± 0.39</td>
<td>3.69 ± 0.32</td>
<td>H 7.29 ± 0.14</td>
<td>5.99 ± 0.13</td>
<td>5.53 ± 0.17</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6 for each group), in µmol/g wet wt, corrected to a constant water content using total creatine. Data were obtained from animals at rest (nonrun) or after 4 min of exercise (run) at 45 or 60 m/min. SO, slow-twitch oxidative fibers; FG, fast-twitch oxidative glycolytic fibers; C, saline injected controls; H, hadacidin injected (200 mg/kg); IMP, inosine 5'-monophosphate. * Significant running effect (P < 0.05). † Significant hadacidin (P < 0.05). ‡ Significant interaction (P < 0.05).
nia content increased in all of the fiber types was to be expected and does not imply that all three fibers were producing ammonia. On the other hand, the IMP that is produced in a one-to-one stoichiometry with ammonia during intense muscle use does not diffuse from the fibers (20, 21) and, therefore, provides a better indicator of the site of ammonia production.

The present study confirms our earlier report (20) that AMP deaminase is readily activated during intense muscle use, resulting in an IMP accumulation in the FG and FOG fibers but not in the SO fibers of the soleus muscle. Although this striking contrast between these fast and slow muscle fibers remains unexplained, it suggests that a fundamental difference in adenylate metabolism exists between these muscle types. The results of the present study, however, seem at variance with our in situ work (20) because in the intact exercising animal AMP deaminase occurred to a greater extent in the FG than in the FOG fibers. This was evident even at the 45 m/min running speed where the FOG fibers are used extensively and possibly to a greater extent than the FG fibers (3, 30). The lesser extent of AMP deamination in the FOG fiber is probably related to the higher aerobic potential of these fibers (1) inasmuch as this could enable them to maintain a greater ATP resynthesis rate during work. An enhanced ATP supply would limit the extent of ADP and AMP formation and, thereby, AMP deaminase activity (7, 34). Even running at 60 m/min did not greatly enhance IMP formation in the FOG muscle section (Table 2). This suggests that even during supramaximal work efforts (27) the FOG fibers of the rat enjoy a richly aerobic energy environment. However, it is clear that if the intense muscle use is exaggerated, e.g., through tetanic stimulation in situ, AMP deaminase activation in the FOG fiber can be essentially equivalent to that found in the FG fiber (20). The sizable accumulation of IMP found in the FG muscle section after running at 45 m/min was even greater in the animals run at 60 m/min (Table 2). Although it is possible that this reflects a more intense use of each FG fiber in the muscle section, it is more likely due to a more complete recruitment of all of the FG fibers sectioned (30). Regardless of the exact basis, it may be concluded that during heavy exercise the fast-type muscle fibers, particularly the FG fibers, are the site of ammonia production.

Adenine nucleotides are resynthesized from IMP and aspartate by the sequential action of adenylosuccinate synthetase and adenylosuccinate lyase (18). Together, the formation and reamination of IMP comprise the purine nucleotide cycle, and it has been proposed that under certain conditions the entire cycle can operate concurrently (11, 18). If this were the case, then ammonia production could occur with little or no significant IMP accumulation within the muscle. Furthermore, with the present experimental design, some IMP reamination might have occurred during the 3- to 5-min period required to excise the muscles. In an attempt to ensure that the IMP measured in muscle after exercise provides a good estimate of its production, we used hadacidin, a specific inhibitor of adenylosuccinate synthetase (17, 21). The observation that hadacidin treatment resulted in greater IMP accumulations in the fast-twitch muscles, but not in the soleus (Table 2), supports the conclusion that AMP deamination occurred only in the fast fiber types during exercise. Because the use of hadacidin did not have any significant effect on either muscle or blood ammonia, it seems likely that the greater IMP accumulation is due to inhibition of IMP reamination and not to a greater AMP deamination. It cannot be determined from this experiment whether this IMP reamination occurred during the exercise or only during the following 3- to 5-min period. However, results from muscles stimulated in situ suggest that IMP reamination is largely confined to the recovery period after intense muscle work of short duration (20).

The precise physiological significance of AMP deamination in working muscle is not entirely clear, although several possibilities have been extensively discussed (18). The proposal that AMP removal by deamination serves to maintain the relative ratios of adenine nucleotides, or the energy charge (2), during periods of net ATP hydrolysis has received wide support (7, 8, 20, 26). This, of course, results in depletion of the total adenine nucleotide pool. In this study, exercise at 60 m/min resulted in conversion of about half of the ATP in the white vastus muscle to IMP. It is entirely possible that equally large ATP depletions occur in human muscle during intense exercise, although data obtained from mixed muscle sections generally show a smaller decline (14, 26, 31). Whether the recovery of muscle performance after exercise depends on the recovery of the total adenine nucleotide pool is uncertain. However, it is interesting to note in this context that adenylate resynthesis requires amino groups in the form of aspartate (18), and aspartate has been reported to hasten recovery after intense exercise (22).

AMP deamination may also contribute to the control of glycolysis during exercise both by producing ammonia, an activator of phosphofructokinase, and by controlling the levels of adenine nucleotides, which also regulate phosphofructokinase (29, 32). The high correlation between lactate and ammonia production in fast-twitch fibers might be considered consistent with this view. However, it is not clear whether AMP deamination is essential to the control of glycolysis or whether these processes are simply activated under similar conditions (20). One unexplained result of this study was the increase in lactate in the FG fibers after exercise in hadacidin-treated rats compared with the saline-injected controls. This may have been a nonspecific effect of the high drug dose used. Alternatively, inhibition of adenylate resynthesis might lead to enhanced glycolytic rates after exercise by lowering ATP, an inhibitor of phosphofructokinase (29), and by increasing IMP, an activator of phosphofructokinase (24).

In exercising humans, blood ammonia rises exponentially with work loads above approximately 60-70% of maximal oxygen uptake (30). However, the increase is dependent on the type of exercise and varies widely among individuals, even at the same relative work load (23, 35). An explanation of these responses can be offered if our observation that ammonia production is confined to fast-twitch fibers can be extrapolated to human muscle. Ammonia production should be minimal at low work loads because predominantly slow-twitch fibers are used (10). Ammonia production should become evident at
higher loads when more fast-twitch fibers are recruited (10). Finally, during intense exercise, which involves heavy recruitment of fast-twitch fibers (10), ammonia production should increase in proportion to adenine nucleotide depletion in these fibers (20). The variability in ammonia production among individuals, even during the same work task, may be related to the wide variability in fiber type composition among individuals (10), i.e., people with a large percentage of slow-twitch fibers might be expected to produce less ammonia during the effort. In any individual, ammonia levels might be expected to rise more during tasks that require heavy recruitment of the fast-twitch fibers within a muscle group. A similar explanation could account for the lack of serious disability in patients with muscle AMP deaminase deficiency (9, 28).

These patients would be expected to function normally during exercise intensities that do not require significant recruitment of fast-twitch fibers.

The skilful assistance of Mrs. Susan Viggiano is gratefully acknowledged. Hadacidin was a generous gift from Mr. W. B. Gall of Merck Sharp & Dohme Research Laboratories, Rahway, NJ.

This study was supported by National Institutes of Health Grant AM-21617.

R. L. Terjung is the recipient of National Institutes of Health Research Career Development Award AM-00681.

Address for reprint requests: R. L. Terjung, Dept. of Physiology, Upstate Medical Center, State University of New York, Syracuse, NY 13210.

Present address of R. A. Meyer: Dept. of Physiology, Harvard Medical School, Boston, MA 02115.

Received 26 November 1979; accepted in final form 11 July 1980.

REFERENCES


