Effect of Pyridoxal 5'-Phosphate on the Activity of Aspartate Aminotransferase Isoenzyme in Human Plasma After Physical Exercise

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Abstract: Untrained healthy male volunteers were subjected to the study of the effects of physical exercise (bicycle ergometer, 150 W for 30 min) upon the activities of aspartate aminotransferase (AST [GOT]) isoenzymes, soluble and mitochondrial (AST-s and AST-m) with and without reactivation by the co-enzyme pyridoxal 5'-phosphate (PALP) in plasma. Blood samples were drawn at the beginning and end of the exercise periods and then 20 min, and 1, 6, 24 and 48 hours later. There was a marked increase in AST-m activity with reactivation by PALP immediately after the exercise, whereas AST-s activity activated with PALP did not vary substantially. Inversely, a significant increase in AST-s activity with PALP added was observed at 48 hours after the exercise, but not in AST-m with PALP added. The changes in the effects of PALP on the activities of AST isoenzymes after physical exercise are discussed.

Keywords AST-m and AST-s isoenzymes—Holo- and apo-enzyme—Pyridoxal 5'-phosphate—Plasma—Exercise—Recovery pattern

INTRODUCTION

Aspartate aminotransferase (EC 2.6.1.1, AST, generally known as glutamic oxalacetic transaminase [GOT]) in mammalian tissues has two isoenzymes, one located in the cytoplasmic fraction of cells (soluble AST, AST-s) and the other in the mitochondrial fraction (mitochondrial AST, AST-m). Studies on the properties of these isoenzymes by Wada and Morino1) showed that they are different proteins with different kinetic and immunological properties. Since Hamfelt2) reported that the activity of this enzyme in sera from patients with various diseases was enhanced when supplemented in vitro with the co-enzyme pyridoxal 5'-phosphate (PALP), routine pre-incubation of sera with PALP prior to AST determination has been recommended by Moss3) and Jung et al.4) However, except for
two recent reports on hepatic\textsuperscript{5} and muscular\textsuperscript{6} diseases, activation of the two AST isoenzymes has been determined together and not separately. In our recent study\textsuperscript{7}, we found that the activities of both AST isoenzymes increase after physical exercise, and that the recovery pattern in AST-m activity is different from that in AST-s. The aim of the present study was to elucidate the effects of PALP on the AST isoenzyme activities in human plasma after physical exercise.

**MATERIALS AND METHODS**

1. **Subject**

Eleven untrained healthy male volunteers, aged 18–24 years, participated in the study. After fasting for about 12 hours, the subjects worked on a bicycle ergometer (Monark, Sweden) with a load of 150 W for 30 min. They were made to take a rest in supine position for 30 min prior to exercise and for 1 hour after exercise. Heparinized blood samples were withdrawn from the ante-cubital vein at the beginning and end of exercise session and then 20 min, and 1, 6, 24 and 48 hours later. During the sampling period they refrained from physical exercise and drinking alcohol. They also abstained from alcohol for a week before the start of the test. Heart rate and blood pressure were electrically recorded, simultaneously and continuously.

2. **AST isoenzyme activities with or without PALP added**

AST activity with and without the addition of PALP (Wako Pure Chemical Industries, Osaka, Japan) was measured by the C. F. Boehringer GOT-UV-Test (Mannheim, Yamanouchi, Japan) using a Hitachi model 624 spectrophotometer (Tokyo, Japan) after 10 min of pre-incubation at 37°C. The effect of PALP addition was investigated by a slight modification of the methods described by Moss\textsuperscript{3} and Kamei et al.\textsuperscript{5} The final PALP concentration was 100 μmoles/l. To measure AST-m activity, AST-s was immunologically removed from plasma using the test kits “Eiken” (Eiken Chemical Company, Tokyo, Japan)\textsuperscript{8}. Untreated plasma was employed to measure total AST (AST-t) activity. AST-s activity was calculated by subtracting AST-m activity from AST-t activity. Hematocrit was measured by a microcapillary tube technique.

3. **Statistic method**

Differences between pre- and post-exercise values were tested for significance using a paired t-test. Data in the text and tables were expressed as mean ±S.E.

**RESULTS**

1. **Heart rate, blood pressure and hematocrit levels**

As shown in Table 1, heart rate, systolic blood pressure and hematocrit levels
### Table 1. Heart rate, blood pressure and hematocrit levels before and after a 30 min of physical exercise session.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Just before exercise</th>
<th>Immediately after exercise</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 min</td>
<td>1 hr</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>68.6 ± 3.1</td>
<td>141.0 ± 4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.2 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>106.6 ± 3.4</td>
<td>141.3 ± 4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112.0 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>62.0 ± 3.1</td>
<td>57.8 ± 3.9</td>
<td>64.7 ± 3.2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.1 ± 0.6</td>
<td>46.1 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.9 ± 0.7</td>
</tr>
</tbody>
</table>

Number of cases=11.
Values are expressed as mean±S.E.
Significantly different from “before” value: <sup>a</sup>p<0.05, <sup>b</sup>p<0.001.

### Table 2. Effect of pyridoxal 5’-phosphate on the activities of aspartate aminotransferase isoenzymes in human plasma before and after a 30 min of physical exercise session. (Karmen units/ml)

<table>
<thead>
<tr>
<th>Aspartate aminotransferase isoenzyme</th>
<th>Just before exercise</th>
<th>Immediately after exercise</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 min</td>
<td>1 hr</td>
</tr>
<tr>
<td>AST-t</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without PALP added</td>
<td>15.1 ± 0.8</td>
<td>20.4 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.8 ± 1.2</td>
</tr>
<tr>
<td>With PALP added</td>
<td>20.5 ± 1.0</td>
<td>27.3 ± 1.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>24.8 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Increase in activity by PALP addition (%)</td>
<td>35.7 ± 1.8</td>
<td>34.0 ± 2.6</td>
<td>36.1 ± 2.1</td>
</tr>
<tr>
<td>AST-m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without PALP added</td>
<td>5.7 ± 0.2</td>
<td>7.4 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>With PALP added</td>
<td>9.1 ± 0.4</td>
<td>13.7 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.5 ± 0.9</td>
</tr>
<tr>
<td>Increase in activity by PALP addition (%)</td>
<td>60.7 ± 5.5</td>
<td>86.0 ± 5.2&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>78.6 ± 9.7</td>
</tr>
<tr>
<td>AST-s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without PALP added</td>
<td>9.5 ± 0.8</td>
<td>13.0 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.8 ± 1.3</td>
</tr>
<tr>
<td>With PALP added</td>
<td>11.4 ± 1.0</td>
<td>13.6 ± 2.3</td>
<td>14.3 ± 2.1</td>
</tr>
<tr>
<td>Increase in activity by PALP addition (%)</td>
<td>20.3 ± 3.5</td>
<td>-0.2 ± 5.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.7 ± 6.0</td>
</tr>
</tbody>
</table>

Number of cases=11.
Value are expressed as mean±S.E.
Significantly different from “before” value: <sup>a</sup>p<0.05, <sup>b</sup>p<0.02, <sup>c</sup>p<0.01, <sup>d</sup>p<0.001.
AST-t=total AST; AST-m=mitochondrial AST; AST-s=soluble AST.
showed marked increases immediately after exercise (p<0.001). During the recovery period, significant increases in heart rate and systolic blood pressure were still remained at 1 hour, and 20 min after the termination of exercise, respectively (p<0.05), whereas hematocrit returned to its pre-exercise level rapidly. Diastolic blood pressure did not vary substantially at any time after exercise.

2. **AST isoenzyme activities with or without PALP added**

As shown in Table 2, there were significant increases in plasma AST-t activities with and without reactivation by PALP immediately after exercise (p<0.001). Both the AST-t activities tended to increase during the 48-hour recovery period. On the other hand, no significant change was found for the relative activation rate of AST-t after exercise.

Similarly, AST-m activities with and without PALP added showed significant increases immediately after exercise (p<0.001 and p<0.01, respectively), but they nearly returned to their pre-exercise levels after 20 min of rest. A significant increase in the relative activation rate of AST-m was observed immediately after exercise (p<0.001), and it remained still unchanged after 20 min of rest (p<0.01).

A significant increase in AST-s activity without PALP added was observed immediately after exercise (p<0.02), but not in AST-s activity with PALP added. However, both the AST-s activities seemed to increase during the 48-hour recovery period. A significant decrease in the relative activation rate of AST-s was found immediately after exercise (p<0.01).

In the present study, the readings obtained for concentrations of enzymes were no doubt higher than those expected from the hemoconcentration.

**DISCUSSION**

In numerous investigations\(^9\)\(^-\)\(^\text{11}\), it has been reported that serum AST-t activity without PALP added is increased after physical exercise. Our previous study\(^7\) indicated that although both the AST isoenzyme activities measured without PALP added likewise showed significant increases immediately after physical exercise, the recovery pattern in AST-m activity is different from that in AST-s. Namely, the recovery in AST-s activity was delayed compared with that in AST-m.

The present study was undertaken to determine the effects of the co-enzyme PALP addition on plasma AST isoenzyme activities after physical exercise. Since a greater part of AST-t activity is attributed to the value of AST-s activity, the changes in AST-t values activated with PALP added showed the similar pattern as those in AST-s at any time except the changes immediately after exercise, as shown in Table 2. The turnover of AST-m isoenzyme in plasma seemed to be more rapid than that of AST-s after exercise, confirming our previous report\(^7\) as well as the study on the clearance from blood of intravenously injected AST
isoenzymes in dogs\textsuperscript{12,13}). Moss\textsuperscript{3}) reported that both holo- and apo-AST enzymes are cleared from the circulation at similar rates. In this study, AST-m and AST-s activities activated with PALP added likewise roughly paralleled the respective unactivated activities after exercise. It has been speculated that physical exercise brings about enzyme leakages from skeletal and cardiac muscles due to a general increase in the permeability of the cell membranes\textsuperscript{9,10,11,14,15}). Immediately after exercise, the increase in AST-m activity by PALP addition nearly doubled the unactivated activity, suggesting that half as much as the AST-m is in the inactive apo-enzyme form, whereas no such effect by PALP addition was found for AST-s. Rosalki and Bayoumi\textsuperscript{16}) reported that the normal serum enzyme, and the elevated enzyme activity of chronic conditions is nearly saturated by circulating PALP, however, that tissue AST may remain undersaturated. One may speculate that the increased AST-m apo-enzymes reflect the destruction of mitochondria in skeletal and cardiac muscle cells. During the recovery period, however, the increased AST-s activities, albeit decreased AST-m activities, might be, in part, due to hemolysis\textsuperscript{9}) or hypoxia, especially in the liver cells\textsuperscript{9,17}). It was reported that PALP has a greater affinity for AST-s than AST-m\textsuperscript{13}). Despite this fact, the reason for which the increase in AST-m activity following PALP addition was more than that in AST-s activity after exercise remains to be elucidated. However, one possible explanation may be that not a few AST-m isoenzymes exist as apo-enzyme form in plasma after exercise, and that, on the other hand, most of AST-s isoenzymes exist as holo-enzyme form. We are at present continuing our work along the line to clarify these physiological meanings.

References

