DAILY RHYTHMS IN PHOSPHORYLASE ACTIVITY OF THE FRESHWATER FIELD CRAB, OZIOTELPHUSA SENEX SENEX (FABRICIUS)

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ABSTRACT

Muscle and hepatopancreatic phosphorylase (active and total) of a freshwater field crab exhibited maximal activity at 0 hr and 0800 hr while the minimal activity was found at 1200 hr of a 24-hr day.

INTRODUCTION

RHYTHMICITY has been known to occur in several enzyme systems of arachnids and insects. Although there are a few studies on the rhythmicity of chromatographic potency of the neurosecretory system and colour changes, such studies on different enzyme systems appear to be very scarce in crustaceans. Laboratory observations revealed that the freshwater field crab, O. senex senex exhibits enhanced locomotor activity in the morning (immediately after sunrise) and at midnight implying that there could be a parallelism in metabolic parameters also. Hence an attempt was made in the present investigation to see whether or not the muscle and hepatopancreatic phosphorylase—the enzyme responsible for glycogen breakdown—exhibits rhythmic variations in synchrony with the observed enhanced locomotor activity.

MATERIALS AND METHODS

Crabs, collected from local freshwater fields were maintained in the laboratory in large glass troughs

Table 1

Daily rhythms in claw muscle and hepatopancreatic phosphorylase activity (μmoles Pi/mg protein/hr) in O. senex senex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1600</th>
<th>2000</th>
<th>0000</th>
<th>0400</th>
<th>0800</th>
<th>1200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylase 'a'</td>
<td>8.67</td>
<td>11.05</td>
<td>17.40</td>
<td>9.05</td>
<td>15.41</td>
<td>6.85</td>
</tr>
<tr>
<td>± 1.51</td>
<td>± 1.29</td>
<td>± 1.69</td>
<td>± 1.48</td>
<td>± 2.76</td>
<td>± 0.38</td>
<td></td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylase 'a + b'</td>
<td>19.55</td>
<td>22.07</td>
<td>43.17</td>
<td>23.88</td>
<td>38.57</td>
<td>15.45</td>
</tr>
<tr>
<td>± 2.78</td>
<td>± 1.28</td>
<td>± 4.38</td>
<td>± 1.66</td>
<td>± 3.84</td>
<td>± 1.72</td>
<td></td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Claw muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylase 'a'</td>
<td>4.15</td>
<td>5.08</td>
<td>12.16</td>
<td>4.95</td>
<td>9.36</td>
<td>3.18</td>
</tr>
<tr>
<td>± 0.63</td>
<td>± 0.47</td>
<td>± 1.33</td>
<td>± 0.54</td>
<td>± 0.87</td>
<td>± 0.28</td>
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</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Phosphorylase 'a + b'</td>
<td>10.41</td>
<td>14.63</td>
<td>34.63</td>
<td>18.72</td>
<td>27.53</td>
<td>7.84</td>
</tr>
<tr>
<td>± 1.25</td>
<td>± 2.12</td>
<td>± 3.78</td>
<td>± 2.08</td>
<td>± 3.24</td>
<td>± 0.94</td>
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<tr>
<td>P&lt;0.001</td>
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</table>

Determinations are mean ± S.D. of six individual observations.
with daily change of water for about a week and were fed on sheep meat ad libitum. After a brief adaptive period, claw muscles (CLM) and hepatopancreas (HP) were isolated at regular intervals by dissecting crabs of similar size and either sex in a room maintained at 5°C. A 2% (W/V) homogenate was prepared in an aqueous medium containing 0.0037 M EDTA (pH 6.5) and 0.1 M NaF (pH 6.5) in the ratio of 1:1 buffered with cysteine (0.03 M)—sodium-B-glycerophosphate (0.015 M) (3:7) buffer (pH 7.4) as recommended by Guillory and Moomaerts. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used as the enzymatic source. Activities of phosphorylase 'a' (active) and 'a + b' (total) were estimated in the absence and presence of AMP, respectively, following the method of Cori et al. The inorganic phosphate was estimated by the method of Fiske and Subbarow, protein by the method of Lowry et al. and calcium according to Dall. To ascertain whether or not the trend is similar on all the days, the experiment was repeated for three consecutive days. Statistical significance of the data was assessed by students 't' test.

RESULTS AND DISCUSSION

Data given in table 1 show the presence of a daily (24 hr) rhythm in active (a) as well as total (a + b) phosphorylase activity of CLM and HP. Maximal activity was recorded at 0 hr and 0800 hr while the lowest activity was found at 1200 hr. Differences between maximal and minimal phosphorylase activity levels of both CLM and HP were found to be statistically significant. Even though total (a + b) phosphorylase is more than its active (a) counterpart in both CLM and HP, there seems to be a parallelism in the rise and fall of the activity patterns throughout the course of a 24 hr period. It can also be seen from table 1 that HP phosphorylase activity is higher than CLM phosphorylase. Hepatopancreatic glycogen is a labile store of energy and its breakdown accounts for most of the blood glucose. It was also reported that the blood glucose level of crab is maximal at 0 hr and 0800 hr and has a minimum at 1200 hr while glycogen exhibited a reverse trend. The maxima of hepatopancreatic phosphorylase activity reported in the present investigation could explain these periodic rises of blood glucose levels.

Results presented in table 2 clearly indicate that there exists a daily rhythm in CLM and HP calcium levels. Since the presence of calcium activates phosphorylase b-kinase which converts inactive phosphorylase into the active form whereas its absence blocks the conversion, it is likely that the variations

<table>
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<th>Tissue</th>
<th>0800</th>
<th>1200</th>
<th>0000</th>
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</thead>
<tbody>
<tr>
<td>Hepatopancreas (HP)</td>
<td>10.65</td>
<td>6.80</td>
<td>12.36</td>
</tr>
<tr>
<td></td>
<td>± 0.97</td>
<td>± 0.53</td>
<td>± 1.42</td>
</tr>
<tr>
<td></td>
<td><em>P &lt; 0.001</em></td>
<td><em>P &lt; 0.001</em></td>
<td><em>P &lt; 0.001</em></td>
</tr>
<tr>
<td>Claw muscle (CLM)</td>
<td>13.54</td>
<td>9.85</td>
<td>16.89</td>
</tr>
<tr>
<td></td>
<td>± 1.57</td>
<td>± 0.82</td>
<td>± 1.63</td>
</tr>
<tr>
<td></td>
<td><em>P &lt; 0.001</em></td>
<td><em>P &lt; 0.001</em></td>
<td><em>P &lt; 0.001</em></td>
</tr>
</tbody>
</table>

Values (mM/kg) are mean ± S.D. of six individual observations.

in calcium levels are largely responsible for variations in phosphorylase activity. The increased phosphorylase activity ultimately produces large amounts of glucose which might be oxidised to meet the energy requirements of the animal. Based on the foregoing observations it may be said that the variations in phosphorylase and calcium levels of CLM and HP are in perfect correlation with the enhanced locomotor activity of the animal at 0 hr and 0800 hr of a 24-hr day.

ACKNOWLEDGEMENT

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11 October 1982; Revised 3 February 1983


ANNOUNCEMENTS

XV INTERNATIONAL CONGRESS OF GENETICS

The XV International Congress of Genetics of will be held during 12-21 December, 1983.

For the 34 Symposia Sessions leading Geneticists from all over the world have accepted to present their contributions. In the 34 Symposia, topics like Recombinant DNA technology, Gene Sequencing and synthesis, Somatic Cell Genetics, Clinical Genetics Environmental Mutagenesis, Genetic Engineering, Biotechnology, Newer approaches to Plant/Animal Genetics and Breeding, Biofermentation and Genetics of Nitrogen Fixation will be discussed. The major emphasis in the programme of the Congress will be on recent exciting developments, particularly in the field of recombinant DNA-based technologists and their applications in Agriculture, Medicine and Industry.

In deference to the wishes of many Scientists and to ensure wider participation, the deadlines for receipt of Abstracts and payment of Registration Fee are being extended as follows: Receipt of Abstracts—July 1, 1983; Receipt of Registration Fee—September 1, 1983.

Further details may be had from Prof. V. L. Chopra, Secretary General, Division of Genetics, 129 NRL Building, Indian Agricultural Research Institute, New Delhi 110 012.

JAWAHARLAL NEHRU CHAIR OF THE CAMBRIDGE UNIVERSITY, U.K.

Prof. C.N.R. Rao, F.K.S., Chairman of the Solid State and Structural Chemistry Unit and Material Research Laboratory, Indian Institute of Science, Bangalore, has been invited by the University of Cambridge, England to occupy the first Jawaharlal Nehru Visiting Professorship of the University. The Professorship was instituted by the Cambridge University last year.

Prof. Rao is the President of the Current Science Association; a Fellow, Vice-President and Editor of Publications of the Indian Academy of Sciences. Bangalore. Prof. Rao is likely to leave for Cambridge during November.