Studies on Esterases Levels in Various Tissues of *Uromastix Hardwickii* during Activity and Hibernation

FARIDA FARZANA and S. N. HASNAIN

Department of Biochemistry, University of Karachi, Karachi-32, Pakistan

(Z. Naturforsch. 27 b, 977—980 [1972]; received May 5, 1972)

Esterases, acetylcholinesterase, cholinesterase, lizard, hibernation

Esterases, acetylcholinesterase and cholinesterase activities were determined in various tissues of *Uromastix hardwickii* during hibernation and activity.

1. The protein concentration was significantly reduced during hibernation.
2. The esterase activity in all the tissues was found to be significantly reduced during hibernation.
3. Acetylcholinesterase activity was significantly increased in all the tissues.
4. There was no significant difference in the cholinesterase activity during the two periods.
5. The specific activities of all the enzymes were found to be significantly increased during hibernation.

Esterases and cholinesterases because of their wide and overlapping substrate specificities have been extensively studied and well characterised in the blood and tissues of higher vertebrates. CLEMENT and HUNTER resolved and partly characterised more than ten different esterases by their substrate and inhibitor specificities in mouse tissues. Cholinesterase is present in nearly all tissues but mainly in blood plasma, heart, muscle, intestine, skin, adrenal glands and liver where it is probably produced. These enzymes are thought to play an important role in the physiology of the nervous system in destroying the acetylcholine immediately after the transmission of the nerve impulse. The biological role of cholinesterase and its natural substrates is however still not clear. The present study was undertaken with a view to investigate the levels of esterases in various tissues of *Uromastix hardwickii* and to study the changes in enzyme levels during hibernation.

Materials and Methods

The present study was carried out on *Uromastix hardwickii* which hibernates during colder months (November to April) and resumes normal activity in the spring. This study was carried out during both periods. The activities of esterase, acetylcholinesterase and cholinesterase were determined in six different tissues including brain, muscle, liver, stomach, small and large intestines.

10% homogenates of these tissues were prepared in 0.59% cooled saline in an electrical homogeniser except for brain which was homogenised manually. The homogenates were filtered through fine cloth to remove fine connective tissues. The filtered homogenates were kept in ice bath till the activity was measured.

Esterase activity was determined by the modified method of BERGMANN et al. with *p*-Nitrophenyl acetate as substrate. The enzyme activity was expressed in terms of micromoles of *p*-Nitrophenol released per ml of enzyme solution due to hydrolysis after one minute of incubation at 37 °C. The method used by DE LA HUERGA et al. for the estimation of acetylcholinesterase and cholinesterase activities has been employed with some modifications in the present studies. The substrate (acetylcholine or benzoylcholine) was hydrolysed by the enzyme to choline and the corresponding acid and the amount of leftover substrate was determined by means of a colour reaction with hydroxamic acid. One unit of acetylcholinesterase (or cholinesterase) activity corresponds to the hydrolysis of one micromole of acetylcholine (or benzoylcholine) per ml of the homogenate per hour at 37 °C.

Protein concentration in each tissue was determined by LOWRY et al. method. The blue colour developed with Folin phenol reagent was estimated at 600 nm. The specific activities of all these enzymes were then calculated in terms of units of activity per mg of protein.

Results

Protein concentration in each of the tissues during hibernating and active periods in terms of mg of protein per 100 mg wet wt of the tissue is shown in Table I. The protein concentration in the hibernating period varied from 5.10 in large intestine to 14.27 mg per 100 mg tissue in liver, whereas the concentration during the active period varied between 7.26 in large intestine and 19 mg per
100 mg tissue in liver. The protein concentration was found to be significantly higher in all the tissues during active period than in the hibernating period.

Table II indicates the activities of esterase in different tissues during hibernation and activity in terms of micromoles of p-Nitrophenol released per min/100 mg wet wt of the tissue. The esterase activity during hibernation varied between 28.35 units in muscle and 52.6 units in liver, while the activity during active period varied from 39.1 units in muscle to 53.5 units in liver. The esterase activity was found to be lower in all tissues during hibernation than in activity.

Table III indicates the activity of acetylcholinesterase in different tissues during active and hibernating periods in terms of micromoles of acetylcholine hydrolysed by 1 ml of 10% homogenate (100 mg tissue) in 1 hr. The acetylcholinesterase activity during hibernation varied from 6.62 in liver to 8.27 in brain, whereas the activity during active period varied between 4.26 in liver and 7.4 in brain. The activity was found to be significantly higher in all tissues during hibernation than in the active period.

Activity of cholinesterase in various tissues during hibernation and activity in terms of micromoles of benzoylcholine hydrolysed by 1 ml of homogenate (100 mg tissue) in 1 hr. is shown in Table IV. The activity of cholinesterase during hibernation varied from 3.3 in brain to 3.76 in large intestine whereas the activity in active period varied between 3.3 in muscle and 3.62 in small intestine. There was no significant difference in cholinesterase activity during the two periods.

Results of esterase, acetylcholinesterase and cholinesterase specific activities in various tissues during activity and hibernation are shown in Tables.

### Table I. Protein concentration in various tissues during hibernation and activity.

<table>
<thead>
<tr>
<th>Period</th>
<th>Brain</th>
<th>Muscle</th>
<th>Liver</th>
<th>Stomach</th>
<th>Small Int</th>
<th>Large Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibernation</td>
<td>42.7* ± 2.0</td>
<td>28.35 ± 0.24</td>
<td>52.60 ± 0.11</td>
<td>30.20 ± 0.38</td>
<td>38.15 ± 0.03</td>
<td>31.10 ± 0.05</td>
</tr>
<tr>
<td>Active</td>
<td>44.50 ± 0.35</td>
<td>39.10 ± 0.07</td>
<td>53.50 ± 0.23</td>
<td>43.75 ± 0.37</td>
<td>50.20 ± 0.45</td>
<td>41.70 ± 0.22</td>
</tr>
</tbody>
</table>

* Mean ± S. E. of 10 observations. Results are expressed as mg protein/100 mg wet wt of tissue.

### Table II. Esterase activity in various tissues during hibernation and activity.

<table>
<thead>
<tr>
<th>Period</th>
<th>Brain</th>
<th>Muscle</th>
<th>Liver</th>
<th>Stomach</th>
<th>Small Int</th>
<th>Large Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibernation</td>
<td>8.88* ± 0.60</td>
<td>12.09 ± 0.07</td>
<td>14.27 ± 0.03</td>
<td>8.14 ± 0.72</td>
<td>6.34 ± 0.36</td>
<td>5.10 ± 0.36</td>
</tr>
<tr>
<td>Active</td>
<td>12.57 ± 0.35</td>
<td>17.72 ± 0.07</td>
<td>19.00 ± 0.23</td>
<td>13.52 ± 0.37</td>
<td>11.72 ± 0.45</td>
<td>7.26 ± 0.22</td>
</tr>
</tbody>
</table>

* Mean ± S. E. of 10 observations. The values represent esterase activity in terms of μmoles of p-Nitrophenol released per ml of enzyme solution per min.

### Table III. Acetylcholinesterase activity in various tissues during hibernation and activity.

<table>
<thead>
<tr>
<th>Period</th>
<th>Brain</th>
<th>Muscle</th>
<th>Liver</th>
<th>Stomach</th>
<th>Small Int</th>
<th>Large Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibernation</td>
<td>8.27* ± 0.40</td>
<td>7.74 ± 0.24</td>
<td>6.62 ± 0.20</td>
<td>7.56 ± 0.37</td>
<td>8.26 ± 0.22</td>
<td>7.48 ± 0.29</td>
</tr>
<tr>
<td>Active</td>
<td>7.40 ± 0.27</td>
<td>5.06 ± 0.24</td>
<td>4.26 ± 0.20</td>
<td>4.98 ± 0.22</td>
<td>6.16 ± 0.29</td>
<td>4.62 ± 0.29</td>
</tr>
</tbody>
</table>

* Mean ± S. E. of 10 observations. The values represent acetylcholinesterase activity in terms of μmoles of acetylcholine hydrolysed by 1 ml of the homogenate in 1 hr.

### Table IV. Cholinesterase activity in various tissues during hibernation and activity.

<table>
<thead>
<tr>
<th>Period</th>
<th>Brain</th>
<th>Muscle</th>
<th>Liver</th>
<th>Stomach</th>
<th>Small Int</th>
<th>Large Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibernation</td>
<td>3.30* ± 0.38</td>
<td>3.76 ± 0.42</td>
<td>3.37 ± 0.38</td>
<td>3.48 ± 0.37</td>
<td>3.20 ± 0.45</td>
<td>3.10 ± 0.22</td>
</tr>
<tr>
<td>Active</td>
<td>3.30 ± 0.30</td>
<td>3.37 ± 0.30</td>
<td>3.37 ± 0.30</td>
<td>3.48 ± 0.30</td>
<td>3.20 ± 0.45</td>
<td>3.10 ± 0.22</td>
</tr>
</tbody>
</table>

* Mean ± S. E. of 10 observations. The values represent acetylcholinesterase activity in terms of μmoles of acetylcholine hydrolysed by 1 ml of the homogenate in 1 hr.
V, VI and VII respectively. The specific activity of esterase during hibernation varied between 2.53 in muscle to 6.64 in small intestine whereas the activity during active period varied from 2.17 in muscle to 5.73 in large intestine. The specific activities of esterase were found to be significantly higher during hibernation than in the active period in brain, liver and small intestine. There was however no significant increase in muscle, stomach and large intestine. Acetylcholinesterase specific activities varied from 0.48 in liver to 1.61 in large intestine during hibernation. The specific activities were higher in the digestive tract than in brain, muscle and liver. The specific activities during active period varied between 0.28 in liver and 0.64 in large intestine. The specific activities were significantly higher in all tissues during hibernation than activity. During hibernation cholinesterase activity varied between 0.23 in liver and 0.78 in large intestine while during the active period the values were found to be be-
between 0.17 in liver and 0.46 in large intestine. The specific activities were significantly higher in all tissues during hibernating than in the active period.

Discussion

The protein concentration was found to be significantly lower in all the tissues during hibernation. This drop in protein concentration lies between 25% in liver and 46% in the small intestine. This decrease may be due to the fact that this animal lives under the surface of the earth during winter season and has to depend upon the endogenous non-carbohydrate substances to meet the energy requirements.

During both the periods highest esterase levels were observed in the liver, whereas the lowest levels were found in the muscle homogenate. Similar results have been reported in the various tissues of rat and dog by Huggins and Moulton. Higher esterase levels in the liver contribute to the significance of this organ in the metabolism of lipids. Esterase levels were found to be significantly higher in muscle, liver, stomach, small intestine and large intestine during the active period. The fall in esterase level in these tissues during hibernation may be attributed to (1) the fact that the overall metabolic activity of the animal is reduced and (2) the disappearance of the fat pads in winter. About 90% of the fat pads in Uromastix hardwickii has been reported to be composed of esterified fatty acids which are the natural substrates of these enzymes. Khalil and Masseih have reported that the two fat bodies in the abdominal region of Varanus griseus and Uromastix aegyptia are prominent in the spring and summer but disappear in winter.

As compared to esterase activity the activity of acetylcholinesterase in all the tissues was significantly higher during the hibernating period. Shellhammer has also reported higher acetylcholinesterase activity in the brain of Mus musculus and Reitherodontomys megatolis in winter than in spring and summer. The catecholamine content of the kidney tissue has been found to be elevated during hibernation. Higher acetylcholinesterase levels during hibernation may be regarded as a regulatory mechanism for the adaptation to the external environments.

No significant differences were observed in the cholinesterase activities of various tissues within and outside the hibernating and active group of animals.

Specific activities of esterase in all the tissues except muscle and large intestine were found to be significantly higher during hibernation. The specific activities of acetylcholinesterase and cholinesterase were significantly increased in all the tissues during hibernation. Higher specific activities are due to significant reduction in protein concentration in these tissues during hibernation.