GLUTAMATE DEHYDROGENASE AND GLUTAMINE SYNTHETASE IN BRAIN, LIVER AND KIDNEY OF BLUE ROCK PIGEON

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(Received December 15, 1992: Accepted June 25, 1993)

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Overall glutamate dehydrogenase (GDH) activity per mg protein in the liver, brain and kidney was found to be low. However, it does not appear so if considered on the basis of the total weight of the tissue concerned. The overall glutamine synthetase (GS) activity in liver is high and moderate in brain while kidney barely has any.

In our earlier work1 methods commonly employed for quantifying activities of the two ammonia metabolizing enzymes viz. glutamate dehydrogenase and glutamine synthetase were tried out in 2 and 10 day old chicken liver, kidney and brain. The results matched with those reported by earlier workers. We report here findings on enzyme activities in these tissues in an actively flying bird, the wild pigeon. The flight muscles and their neuronal regulatory centres comprising of sensory-motor arcs, associated interneurons in the spinal cord and brain, especially those in the cerebellum are expected to be used maximally especially during flight. The sympathetic centres making circulatory and respiratory adjustments from time to time are also bound to be involved in the process. In effect, much more ammonia is likely to be generated in a flying bird than in a non-flying one. Findings on glycogen body and appendage linked and non-linked parts of the spinal cord from adult pigeon are published elsewhere2.

MATERIALS AND METHODS

Fully grown, healthy, adult blue rock feral pigeons (Columba livia) of both sexes, weighing between 240 and 300 gms were used. The pigeons were sacrificed by exposing them to chloroform. Brain, liver and kidney were collected, blotted to free them from blood and stored at 4°C till they were processed further, the same day.
For homogenization, buffer used was phosphate-Triton for glutamate dehydrogenase (GDH), and Tris-Triton buffer for glutamine synthetase (GS). Homogenate was spun for 15 minutes at 11,000 g for GDH and for 10 minutes at 5,000 g for GS. Protein was estimated by the method of Lowry et al. Glutamate dehydrogenase (E.C.1.4.1.3) (GDH)-activity was measured as per spectrophotometric method of Lal and Clark. GDH was assayed in the direction of formation of glutamate using either NADH or NADPH at pH 7.0. The reaction mixture of 3 ml contained 100 mM Tris-buffer (pH 7.0), 162 mM ammonium acetate, 1 mM EDTA, 0.167 mM NADH or 0.3 mM NADPH, 1.7 mM ADP, 10 mM neutralized α-ketoglutaric acid and either 0.15 or 0.3 ml homogenate depending on the tissue concentration. The reaction was started by addition of NADH or NADPH. Enzyme activity was read against the respective substrate blank preparation at 340 nm at 29°C±1°C and expressed as µ moles of NADH converted to NAD or NADPH to NADP per minute per mg protein. Glutamine synthetase (E.C.6.3.1.2) (GS). The method adopted was that of Elliott with some modification.

The assay volume of 2.2 ml contained 80 µ moles of Tris-buffer (pH 7.6), 12 µ moles of ATP, 40 µ moles magnesium sulphate, 30 µ moles cysteine (pH 7.2), 100 µ moles hydroxylamine (pH 7.2), 200 µ moles sodium glutamate (pH 7.4) and 0.1 ml of homogenate. Samples were incubated at 39°C±1°C for 60 minutes. Reaction was stopped by adding 0.8 ml ferric chloride reagent. After removal of protein by centrifugation the sample was read at 540 nm against the respective substrate blank material treated in a similar manner. Enzyme activity is expressed as 1 µ mole glutamyl hydroxamic acid formed per mg protein per minute.

RESULTS

Highest NADH linked GDH activity was obtained in liver (Table 1) followed by brain and kidney. When NADH was substituted by NADPH (Table 1), there occurred a 35% reduction in enzyme activity in brain and 45% in liver and kidney. The overall GS activity is much less in all tissues. Highest activity was noted in liver followed by that of brain (Table 2). Kidney barely had any activity.

DISCUSSION

As explained in introduction, employing the methods described herein, the values of enzyme activities obtained in the tissues of 10 day old chick tallied with those reported by earlier workers. A comparison of enzyme activities in tissues of adult pigeon could therefore be made with those of 10 day old chick with an additional aim to compare metabolic pattern in an active flier with that of an active walker. Overall GDH activity per mg protein is much less in pigeon liver. It is almost half of what it is in 10 day old chick. In terms of total wet weight of the liver, however, the value becomes considerable. Values for brain are comparable with the ones in chicken. Those of the spinal cord are also similar while that of
Ammonia Metabolism in Pigeon Tissues

Table 1*— GDH activity in different tissues of pigeon. GDH activity is expressed as μ moles of NADH or NADPH oxidized to NAD or NADP/mg protein/minute.

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<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
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<tbody>
<tr>
<td>NADH</td>
<td>198.397</td>
<td>223.009</td>
<td>170.958</td>
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<tr>
<td>±13.772</td>
<td>±7.748</td>
<td>±12.071</td>
<td></td>
</tr>
<tr>
<td>NADPH</td>
<td>123.585</td>
<td>117.57</td>
<td>89.193</td>
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<tr>
<td>±0.576</td>
<td>±24.225</td>
<td>±16.571</td>
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Table 2*— Activity of glutamine synthetase is expressed as μ moles γ-glutamyl hydroxamate/mg protein/hour.

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
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<tbody>
<tr>
<td>GS</td>
<td>0.347</td>
<td>0.521</td>
<td>0.042</td>
</tr>
<tr>
<td>±0.046</td>
<td>±0.069</td>
<td>±0.007</td>
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*All values represent mean of 6 assays.

GB is also double. Thus it appears that despite the sumptuous activity GDH in liver, the main ammonia detoxifying organ lying outside the cerebro-spinal onolave, a good flier such as pigeon is likely to generate large quantities of ammonia and as a precautionary measure it is obliged to have within the enclaves a high activity of GDH in GB as well. The overall activity of GS in spinal cord and brain of pigeon is much less whereas that of liver is more as compared with the corresponding tissues of 10 day old chicken. Since the reaction is energetically uneconomical, Glycogen body of the adult bird also seems to depend more on GDH rather than GS and accordingly has less of it. During the period of growth, the end product glutamine is likely to be used for the formation of purines and pyrimidines and hence there is more activity in 10 day old chick. Since the GS reaction is energetically uneconomical, all adult tissues seem to have less of it.

ACKNOWLEDGEMENTS

K.N.S. is grateful to Govt. of India for a research fellowship. We thank Dr. P.M.
Ambedkar, Prof. and Head, Dept. of Zoology for research facilities and encouragement and Mr. Anoop Kumar for help rendered.

REFERENCES