SCIATECTOMIC STIMULATION OF MUSCLE
ARGINASE AND ITS IMPLICATIONS

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Denervated muscle is characterized by high turnover of proteins, hyperammonemia, degeneration and regeneration of muscle fibers (Shahzad, 1977; Goldspink 1978; Chetty et al, 1980). In view of these conditions an enzyme like arginase, which cleaves L-arginine to L-ornithine and urea was investigated mainly because, its detoxification role in ammoniotoxemia is well established and its role in extra hepatic tissues, where other urea cycle enzymes are absent, still remains to be elucidated.

Unilateral sciatic denervation was performed always on right side in healthy frogs, Rana hexadactyla as described by Dass and Swami (1972). One month after sciatectomy the animals were examined for marked atrophy in gastrocnemius muscle. After ensuring it the animals were sacrificed, the control and denervated muscles were excised with minimal loss of time from six animals and chilled to 0°C followed by immediate homogenization to 20% (wt/vol) in ice cold 0.1% (wt/vol) cetyltrimethylammonium bromide (CTB) in a glass homogenizer embedded in ice. The homogenate was centrifuged at 2000 x g for 15 min to eliminate the cell debris and the clear crude supernatant was employed as the enzyme source after dialysis against 0.1% CTB.

Arginase activity was assayed by the method of Campbell (1961) in a medium containing 100 m moles of glycine-sodium hydroxide buffer (pH 9.5), varied concentrations of L-arginine adjusted to pH 9.5 and 5 m moles of manganese chloride in a final volume of 0.7 ml. The reaction mixture was incubated at 37°C for 30 min by adding 0.3 ml of enzyme source and then stopped with 2 ml of 1.0 M perchloric acid. The urea formed in the aliquots was measured spectrophotometrically by diacetyl monoxime method. All the
enzyme velocities were corrected with non enzymatic hydrolytic velocities. The protein in the enzyme source was measured according to the procedure of Lowry et al (1951) using bovine serum albumin as the standard.

The results are summarized in the table 1.

The Vmax values of arginase in denervated muscle showed increased velocity of the enzyme indicating the increased rate of hydrolysis of L-arginine. The Km of arginase was found unaffected in denervated muscle as compared with the control muscle. Despite a reported 100% increase in L-arginine in denervated muscle, apparently no change in Km was witnessed in the present investigation. This fact suggests that the affinity between the substrate and enzyme molecule was not affected. The increase in Vmax could be due to substrate induced activation of the enzyme in denervated muscle. A similar induction of arginase by the addition of arginine in mammalian cell culture was reported by Klein (1960).

Table 1. Substrate dependent kinetic analysis of arginase activity in control and denervated amphibian gastrocnemius muscles.

(The Vmax values are per mg protein per hour. The Vmax and Km were derived from the Lineweaver Burke double reciprocal plots, using least squares as the best fit).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kinetic parameters</th>
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<tbody>
<tr>
<td></td>
<td>Vmax</td>
</tr>
<tr>
<td>Control muscle</td>
<td>$0.0540 \times 10^{-6}$ M</td>
</tr>
<tr>
<td>Denervated muscle</td>
<td>$0.0796 \times 10^{-6}$ M</td>
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<td>Percent change</td>
<td>47.4</td>
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The biochemical necessity of intensified arginase activity during denervation appears to be three dimensionally oriented. First, the increased ammonia could be fixed into urea cycle and the ammonia toxicity can be avoided. Secondly, during the process of elimination of ammonia toxicity through arginase activity, urea is produced and the presence of urea within the tissues has been reported to play a vital chemohomeostasis role, as is the case with marine elasmobranchs which are dependent at least on some