

Inhibition of arginase in sheep brain homogenates by some L-amino acids

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Summary. The effect of 16 L-amino acids on the activity levels of arginase in sheep brain homogenates was studied. The amino acids leucine, valine, lysine, and ornithine inhibited arginase activity significantly. The other amino acids tested did not show a significant influence on arginase activity. The inhibition was related to the carbon chain length of the amino acids.

Amino acids, free or as proteins, constitute over 40% of brain dry weight¹. Amino acid pools, and the protein composition of the brain, are also known to be influenced by factors like a high protein diet, or during pathological states²⁻⁶. Many of these amino acids are important in biosynthetic pathways. The existence in the brain of all necessary enzymes of the urea cycle⁷ is of unknown metabolic significance. It was observed that factors known to increase free amino acid levels influence rat hepatic arginase activity⁸⁻¹⁰. It was also shown that some of the L-amino acids inhibited arginase activity in sheep liver homogenates¹¹. Hence, an attempt was made to study the influence of L-amino acids on the activity level of arginase (L-arginine amido hydrolase EC.3.5.3.1) in sheep brain homogenates.

Material and methods. Sheep brains were collected from a local slaughter house immediately after the animals were killed, and brought to the laboratory packed with frozen mammalian Ringer's solution in a thermos flask. After washing in cold tris-buffer (pH 9.0) and blotting on filter paper, cortical slices were cut. Cortical slices were weighed and homogenized in 0.25 M sucrose to make a 10% (w/v) extract. Arginase activity was assayed by the method described by Sadasivudu and Indira¹². When amino acid solutions were tested for their effects on arginase activity they were preadjusted to the experimental pH. The protein content in the enzyme extract was determined by the biuret method as described by Gornall et al.¹³.

Results and discussion. All the enzyme assays were performed under conditions which measured initial velocities. Of the 16 L-amino acids used, only leucine, ornithine,

lysine and valine inhibited the arginase activity significantly (42–55%, table). Aspartic acid and histidine slightly stimulated the activity of the enzyme. The rest of the amino acids had an insignificant effect on arginase activity (table). The inhibition of arginase activity by ornithine, lysine, leucine and valine seems to be due to competitive inhibition. As evidence of this, it has been shown that these amino acids competitively inhibit arginase in bovine liver¹⁴, rat liver^{15,16} and sheep liver¹¹. The amino acid arginine, which is the substrate for the enzyme, has 6 carbon atoms. The amino acids, ornithine, lysine, leucine and valine are monocarboxylic acyclic amino acids with 5 or more carbon atoms. Hence, it is probable that carbon chain length is critical for effective competitive inhibition at the catalytic site of the enzyme molecule¹¹. Though it was reported that Proline competitively inhibited hepatic arginase activity^{11,15,16} in the present investigation the inhibition was not very significant. This may be due to arginase involvement in the synthesis of proline in the brain^{17,18}. Though glutamic acid and methionine contain 5 carbon atoms, the inhibition observed was insignificant. This may be due to the 2nd carboxylic group on the glutamate and to the sulphur atom in methionine, which make competitive inhibition at the active site less effective. Similar observations were made with hepatic arginase in sheep liver extracts¹¹.

Effect of L-amino acids on arginase activity in sheep brain homogenates

| Amino acid (2.5 µmoles) | Arginase activity (µmoles urea/mg protein/h)* | Percentage change | Significance |
|----------------------------|---|----------------------|--------------|
| None | 12.40 ± 0.38 | – | – |
| Glycine | 12.25 ± 0.25 | – 5 | N.S. |
| Alanine | 13.45 ± 0.41 | + 8 | N.S. |
| Aspartic acid | 16.15 ± 0.23 | + 30 | S.p < 0.05 |
| Glutamic acid | 10.00 ± 0.26 | – 20 | N.S. |
| Cystine | 11.10 ± 0.28 | – 12 | N.S. |
| Leucine | 5.65 ± 0.25 | – 55 | S.p < 0.01 |
| Proline | 9.95 ± 0.31 | – 20 | N.S. |
| Methionine | 11.95 ± 0.38 | – 9 | N.S. |
| Ornithine | 7.25 ± 0.22 | – 42 | S.p < 0.01 |
| Phenylalanine | 13.30 ± 0.38 | + 7 | N.S. |
| Valine | 6.25 ± 0.31 | – 49 | S.p < 0.01 |
| Histidine | 15.10 ± 0.26 | + 22 | S.p < 0.05 |
| Serine | 13.10 ± 0.18 | + 5 | N.S. |
| Tryptophan | 12.20 ± 0.31 | – 3 | N.S. |
| Tyrosine | 12.90 ± 0.34 | + 4 | N.S. |
| Lysine | 7.00 ± 0.21 | – 43 | S.p < 0.01 |

* Each value is the mean of 6 brains ± SD. N.S., Not significant; S., Significant.

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