TOPOGRAPHICAL DISTRIBUTION OF SHEEP BRAIN ARGINASE:
ITS RESPONSE TO SOME GUANIDINO COMPOUNDS

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INTRODUCTION

Despite several reports on the role of guanidines in the neurological symptoms of epilepsy, hyperargininemia and renal failure\(^1\)-\(^3\), many points are still unclear and a need for studies on the direct toxic effects of guanidines was indicated in the symposium on urea cycle diseases\(^2\). Though there is a clear metabolic interrelation between arginine and guanidines, the whole metabolic profile appears complicated in the mammalian brain due to a) the lack of a functional urea cycle b) the presence of complex regional differences and c) the unknown functional significance of arginase\(^6\). Hence, these metabolic uncertainties have prompted the authors to take up a study of the topographical distribution of mammalian brain arginase and its response to some guanidines, in vitro, in order to assess the direct neurotoxic nature of these compounds.

MATERIAL AND METHODS

Material

Brains were procured from healthy sheep of similar age after decapitation at the local slaughter house and transported in a dry beaker kept in a freezing mixture. The meninges were removed and brains were washed repeatedly with Krebs-Henseleit Ringer's solution. The different parts of the brain such as cerebellum, cerebrum and brain stem were separated and 10 % (w/v) homogenates were prepared in 0.1 % acetyltrimethylammonium bromide. The homogenates
were spun at 5000 x g for 20 min and the clear cell free extract was employed as the enzyme source to estimate arginase activity as described earlier\textsuperscript{5}. Unless otherwise specified, all these steps were carried out at 0-3 °C.

**Dose versus response study**

In addition to the reaction mixture, varied concentrations of guanidine hydrochloride (GHCl) (10, 20, 30, 50, 100, 150, 200 and 250 mM) and guanidinoacetic acid (GAA) (2.5, 5, 7.5, 12.5 and 25 mM) were added separately in vitro to study the dose dependent arginase response in different brain regions.

**Influence of GHCl and GAA on substrate dependency of arginase**

The influence of GHCl (20, 100 and 250 mM) and GAA (5 and 12.5 mM) on the substrate (L-arginine) dependency of arginase was tested, varying the substrate concentration from 10 to 80 mM. The $V_{\text{max}}$ and $K_{\text{m}}$ were determined using least squares as the best fit. The protein concentration in the enzyme source was estimated by the method of Lowry et al.\textsuperscript{6}.

**RESULTS AND DISCUSSION**

The results are summarised in tables 1-3.

The results clearly indicate that the neural arginase activity is variable topographically. The cerebellum registered the highest activity followed by cerebrum and brain stem. Though these regional differences follow the same pattern as that of rat brain arginase\textsuperscript{7}, it is difficult to interpret, as these results are attributable to the differential distribution of effectors modulating regionally the arginase activity.

The dose dependent studies with GHCl have shown a consistent inhibition of arginase activity with increasing concentrations of GHCl in all the regions of brain. With GAA, the response was different in the sense that with lower concentrations of GAA, the inhibition was more prominent and with increasing concentrations, the inhibitory influence was reduced in the cerebellum and cerebrum, while the brain stem arginase showed consistent inhibition.

Substrate dependent studies in the presence of GHCl (20, 100, and 250 mM) and GAA (5, and 12.5 mM) revealed a decreased $V_{\text{max}}$ with all concentrations of GHCl and GAA (except in 12.5 mM of GAA) suggesting that the hydrolysis of L-arginine by arginase is attenuated, perhaps by masking the enzyme active sites by these guanidines. Further, the increase in $K_{\text{m}}$ in all the regions (Table 3) suggests decreased affinity between E and S, thereby reducing the