DIFFERENTIAL INDUCTION OF CYTOPLASMIC GUANINE DEAMINASE ISOZYMES UNDER GUANINE STRESS IN RAT LIVER AND BRAIN

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Summary

Guanine stress induced preferential increase in one of the two isozymes of guanine deaminase in the 15,000 x g supernatant from rat liver. In brain also induction resulted in a preferential increase in one of the components of the supernatant enzyme.

Introduction

When isozymic forms of an enzyme occur in tissues, a means of determining the metabolic significance of the different forms is by study of the pattern of induced activity. In most of the studies conducted, the multiple forms had different subcellular location (1,2). In induction of transaminase, it has been claimed that the specific leucine transaminase, but not the unspecific branched chain amino acid transaminase, increased in liver (3). In all the above cases, the induction was not due to a specific substrate. Earlier experiments from this department revealed that in both liver (4) and brain (5) of the albino rat the 15,000 x g supernatant fraction contained isozymes of guanine deaminase differing in reaction rate response to increase in substrate concentration and in modulation by GTP and allantoin. In the present study, the differences in the relative content

of the two forms of enzyme each in brain and liver supernatant were determined under conditions of guanine stress.

Materials and Methods

animal groups

Fortyeight adult male albino rats were divided into 4 groups. A group with 16 served as the control, while a second group with 12 received saline injections daily. The third group, also with 12 animals, received injections of guanine daily. The fourth (8 animals) received daily injections of guanine for the first 8 days, followed by daily injections of a mixture of guanine and ethionine during the subsequent period. All injections were by the intraperitoneal route. Guanine and ethionine were administered in doses respectively of 120 mg. and 70 mg. per Kg. initial body weight per day, as suspension in physiological saline. The animals were all fed the standard laboratory ration.

plan of slaughter

At the commencement of the experiment, 4 animals from the first group were sacrificed: additional animals of the group, 4 each at a time, were slaughtered along with the experimental animals. At the end of 8, 16 and 24 days, 4 animals each from the second and third groups were sacrificed. Animals of the fourth group were sacrificed in batches of 4 at the end of 16 and 24 days.

cell fractionation

The excised and cleaned liver and whole brain from the animals of a group were pooled separately, weighed, minced

fine and 4 g. sample each used in the preparation of 20 % (w/v) homogenate in 0.25 M sucrose, employing a Potter-Elvehjem homogenizer. After sedimenting and washing the nuclear fraction at 700 x g and the mitochondrial fraction at 15,000 x g, the supernatant was collected.

enzyme fractionation

Liver supernatant fraction was fractionated with $(NH_4)_2SO_4$ and after dialysis, centrifugal clarification and adjustment of phosphate concentration and pH, loaded on DEAE-cellulose column. Other details were as reported (4). The processing of the supernatant fraction of brain was essentially as reported by Sitaramayya and Krishnan (5).

enzyme assay

Guanine deaminase activity was determined by the spectrophotometric method reported earlier (4).

Results and Discussion

The enzyme activity in the supernatant of liver was increased 45 % in the guanine treated animals sacrificed at the end of 8 days and this level was maintained in the animals at the end of the 16 and 24 days. In brain there was only a slight increase in activity in the supernatant fraction at the end of 8 days; the activity at the end of 16 days was 35 % higher and at the end of 24 days 47 %. The results of chromatographic fractionation of the crude liver and brain enzyme precipitated with ammonium sulfate are given in Figures 1 and 2 the various bars representing the % recoveries of the activity loaded on column eluted as isozyme A and B.

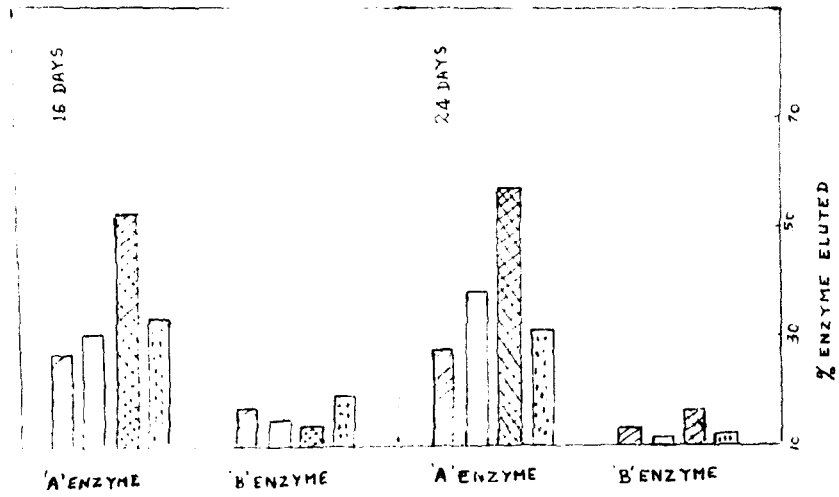

Fig. 1. Per cent recovery of supernatant guanine deaminase activity in isozymes A and B in the liver of the adult rat. The 15,000 x g supernatant was fractionated with $(NH_4)_2SO_4$ and chromatographed on DEAE-cellulose column. The bars represent the % recovery of enzyme activity in the two isozyme fractions, in the control and the saline-administered animals and in those administered guanine and guanine plus ethionine.

Normal control
Saline control
Guanine injected
Guanine plus ethionine injected

The content of enzyme A in relation to B in liver increased markedly in the guanine-administered animals at the end of 16 and 24 days. The brain tissue also showed a change, but it was the B component which increased in relation to the A component. Judging on the basis of the preferential increase in response to guanine administration, the more metabolically active form of guanine deaminase isozyme

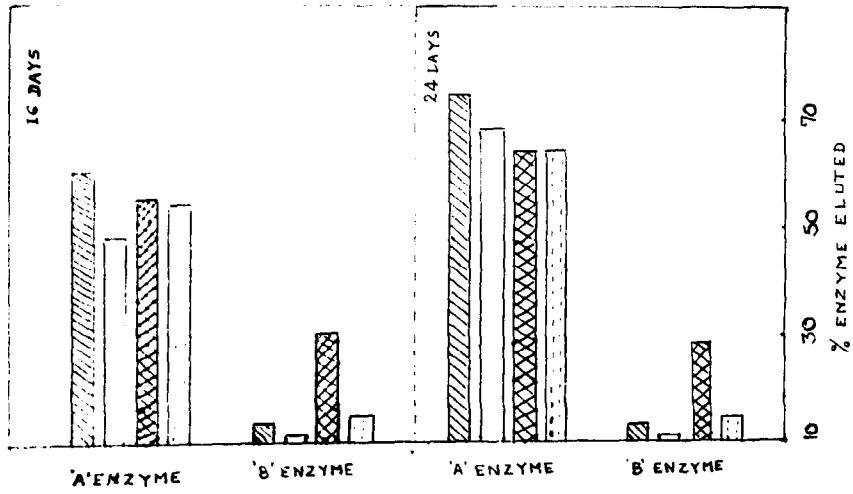

Fig. 2. Per cent recovery of supernatant guanine deaminase activity in isozyme A and B in the whole brain of the adult rat. The 15,000 x g supernatant was fractionated with $(NH_4)_2SO_4$ and chromatographed on DEAE-cellulose column. The bars represent the % recovery of enzyme activity in the two isozyme fractions, in the control and the saline-administered animals and in those administered guanine and guanine plus ethionine.

▨ Normal control ☐ Saline control

▩ Guanine injected ▣ Guanine plus ethionine injected

appeared to be the component subject to allantoin inhibition, both in liver and brain. Ethionine suppressed the relative increase occurring in the A fraction in liver and B in brain, suggesting induction by *de novo* synthesis of these proteins.

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