Induction of Guanine Deaminase and Its Inhibitor in Rodent Liver and Brain

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1. Guanine deaminase activities in homogenates and supernatant fractions of liver and brain of rat and mouse were elevated by administration of guanine to the animals. The maximum induction in mouse tissues occurred within 24h and in rat tissues within 48h.
2. Mitochondria of rat (but not mouse) liver and brain contain an inhibitor of supernatant guanine deaminase, and this was also increased by guanine treatment.
3. Administration of ethionine, cycloheximide or actinomycin D prevented the guanine-dependent increase in deaminase activity and also the increase in mitochondrial inhibitory activity; chloramphenicol suppressed only the latter.

Earlier investigations from this laboratory showed regulatory behaviour for guanine deaminase in the liver and brain of rats and brain of mice (Sree Kumar *et al.*, 1972, 1973), and the inhibition of the guanine deaminase activity in the 15000g supernatant fraction of rat brain and liver (Kumar *et al.*, 1967), but not of mouse tissues (Sree Kumar *et al.*, 1972), by a protein component of the mitochondrial fraction. The present investigations are concerned with the effects of treating rats and mice with guanine on (a) guanine deaminase activity as analysed in homogenates, (b) enzyme and inhibitor activities as analysed in subcellular fractions and (c) the effect of dosage with ethionine, cycloheximide, chloramphenicol or actinomycin D on enzyme and inhibitor analysed in subcellular fractions. A preliminary account of some aspects of the induction of guanine deaminase has been published (Sree Kumar *et al.*, 1970).

Materials and Methods

Animals

Albino mice and rats, originally purchased from the Industrial Toxicology Research Centre, Lucknow, U.P., India, were bred in the departmental colony. Adult male mice and rats weighing respectively 30-35g and 180-200g were used in the experiments. All injections (0.5ml each, in 0.9% NaCl) were intraperitoneal and at intervals of 24h. The experimental animals received guanine (E. Merck A.-G., Darmstadt, Germany) in doses of 120mg/kg body wt. and the controls 0.9% NaCl. Other animals received ethionine (Sigma Chemical Co., St. Louis, Mo., U.S.A.), cycloheximide (Sigma Chemical Co.), chloramphenicol [as succinate, Parke-Davis (India)

Ltd., Bombay, India], or actinomycin D (C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany), in doses respectively of 70, 1, 500 and 0.5mg/kg body wt., together with the guanine. In experiments designed to test the time taken for maximum response to guanine, groups of animals were killed after 24, 48 and 96h, the animals having received one, two and four injections respectively (i.e. death followed 24h after the last injection). In subsequent experiments the homogenates from tissues of mice and rats which received one and two injections respectively (killed at the end of 24 and 48h) were fractionated and the activities of enzyme and inhibitor determined in the subcellular fractions.

Subcellular fractionation

The preparation of homogenates and their separation into subcellular fractions were by the method of Sree Kumar *et al.* (1972), with the differences that the 'heavy' and 'light' mitochondrial fractions were sedimented together from the post-nuclear (700g, 10min) supernatant at 15000g for 60min, the particulate fractions were washed four times instead of three and the volumes of medium used for washing were increased 6-fold for the nuclear fraction and 3-fold for the mitochondrial fraction. Under these modified conditions of isolation, the nuclear and mitochondrial fractions of liver and brain were devoid of guanine deaminase activity, both in the rat and the mouse.

Enzyme assay

For determination of guanine deaminase activity in homogenates or subcellular fractions, the assay system (b) described by Sree Kumar *et al.* (1972)

was used. To avoid any interference by xanthine oxidase 10 μ M-allopurinol, a specific and potent inhibitor of xanthine oxidase (Iwata *et al.*, 1969), was incorporated into all assays except those for mouse liver. The low guanine deaminase activity in mouse liver necessitated the use of large samples of homogenates and subcellular fractions in the assay system, with the result that the blank absorption was high and supplementation with allopurinol would have rendered the assay insensitive by increasing the absorption.

Inhibitor assay

To test the inhibitory activity of the mitochondrial fractions towards the supernatant enzyme, the two fractions were mixed in the same proportions as in the unfractionated homogenate and the mixture was analysed for residual enzyme activity. Under these conditions about 25–35% inhibition was caused, and the measured 'units' of inhibition (see below) were independent of the enzyme concentration. Allopurinol was not used because of the high absorption.

Test for 'latent' inhibitor activity

To test for 'latent' inhibitory activity in mitochondrial fractions, the pooled mitochondrial material of each group was frozen overnight at -18°C , thawed, ground in a VirTis homogenizer and Triton X-100 was added to give 1% (v/v). The mixtures were left in the cold for 1 h and then centrifuged for 1 h at 15000g. The supernatants (which contained about 50% of the protein in the mitochondrial fraction) were analysed for inhibitory activity; the residues were devoid of activity.

Protein determination

The protein content of homogenates and subcellular fractions was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma, crystallized and freeze-dried) as standard.

Expression of results

One unit of guanine deaminase deaminated 1 μ mol of guanine at 30°C in 15 min under the assay conditions and a unit of inhibitor caused 50% inhibition of 1 unit of enzyme activity under the above conditions of enzyme assay. Enzyme activity and 'overt' inhibitor activity were calculated as units/g equivalent of fresh tissue and as units/mg of protein in the fraction. The protein content of liver or brain were not significantly altered as a result of administration of guanine or guanine plus an inhibitor of protein synthesis. The 'latent' activity of the mitochondrial inhibitor was obtained by subtracting

the 'overt' inhibitory activity in the mitochondrial fraction from the total activity of the extract of the Triton-treated fraction. The activity was calculated as units/g equivalent of tissue and as units/mg of protein in the mitochondrial fraction from which the extract for activity determination was obtained.

The results, except when the pooled mitochondrial fractions were used for determination of latent inhibitor activity, were subjected to statistical evaluation and the data presented as the mean $\pm$ S.D. of the observations. The statistical significance of the data calculated on the basis of protein was evaluated by Student's *t* test.

Results

Influence of treatment with guanine and inhibitors of protein synthesis on enzyme activities: analyses of homogenates

Mice. Administration of a single dose of guanine resulted in about 65% increase ($P < 0.001$) in the activity of guanine deaminase in liver at the end of 24 h; in brain there was a 40% ($P < 0.001$) rise. On treatment for longer periods, the enzyme activity in brain returned to the control value in animals killed at the end of 96 h, but that in liver declined only slightly and remained 45–50% higher ($P < 0.001$) than in controls. Administration of ethionine together with the guanine blocked the increases in activity of guanine deaminase in both liver and brain.

Rats. A single dose of guanine elevated the activity of enzyme by 30% ($P < 0.05$) in liver and 16% ($P < 0.05$) in brain. At the end of 48 h, after two doses of guanine, there were 50% and 40% rises ($P < 0.001$) in enzyme activity in liver and brain respectively. Thereafter there was a decline in both tissues, but the values were still higher than in the controls. As in mice, administration of ethionine prevented the increase in enzyme activity in both liver and brain.

Influence of guanine treatment on guanine deaminase and inhibitory activity studied in subcellular fractions

Studies on subcellular fractions were conducted with the tissue obtained from mice and rats treated respectively for 24 and 48 h, since the maximum response to guanine occurred during this period.

Guanine deaminase. The results obtained for enzyme activity in homogenates and the corresponding 15000g supernatant fractions are presented in Table 1.

Inhibitory activity associated with mitochondria. The mitochondrial fraction from mouse tissues did not contain 'overt' or 'latent' inhibitory activity towards the supernatant guanine deaminase.

In rat liver, the 'overt' inhibitory activity of the mitochondrial fraction in guanine-treated animals was increased by 24% ($P < 0.001$) and 28% ($P < 0.001$),

Table 1. *Influence of guanine treatment and the effect of ethionine as studied in subcellular fractions*

Groups of mice and rats receiving 0.9% NaCl, guanine, or guanine plus ethionine, at 24h intervals were killed at the end of 24 and 48h, the mouse groups having received one injection each and the rats two each. The subcellular fractionation and enzyme assay were as reported in the text. Six animals were used in each group, and the results are presented as the mean values $\pm$ S.D. The nuclear and mitochondrial fractions were devoid of enzyme activity. Values in parentheses are activities/mg of protein. H, homogenate; S, 15000g supernatant.

Treatment	Guanine deaminase activity (units/g of tissue)					
	Mice			Rats		
	Liver		Brain	Liver		Brain
0.9% NaCl	H 1.86 $\pm$ 0.021 (0.013 $\pm$ 0.001)	S 1.79 $\pm$ 0.017 (0.033 $\pm$ 0.005)	H 5.85 $\pm$ 0.037 (0.113 $\pm$ 0.028)	H 12.16 $\pm$ 1.15 (0.051 $\pm$ 0.004)	S 14.28 $\pm$ 1.3 (0.265 $\pm$ 0.014)	H 9.57 $\pm$ 0.022 (0.104 $\pm$ 0.010)
Guanine	3.07 $\pm$ 0.042 (0.021 $\pm$ 0.002)	3.07 $\pm$ 0.042 (0.053 $\pm$ 0.010)	8.66 $\pm$ 0.07 (0.156 $\pm$ 0.014)	18.5 $\pm$ 1.8 (0.067 $\pm$ 0.006)	21.5 $\pm$ 1.85 (0.389 $\pm$ 0.018)	13.25 $\pm$ 0.082 (0.145 $\pm$ 0.009)
Guanine + ethionine	1.96 $\pm$ 0.05 (0.014 $\pm$ 0.002)	2.08 $\pm$ 0.032 (0.032 $\pm$ 0.004)	5.92 $\pm$ 0.03 (0.116 $\pm$ 0.007)	12.5 $\pm$ 1.0 (0.053 $\pm$ 0.006)	14.83 $\pm$ 1.05 (0.273 $\pm$ 0.047)	9.71 $\pm$ 0.125 (0.105 $\pm$ 0.018)
						S 11.20 $\pm$ 1.74 (0.320 $\pm$ 0.057)
						15.3 $\pm$ 1.3 (0.467 $\pm$ 0.066)
						11.0 $\pm$ 1.0 (0.330 $\pm$ 0.030)

calculated on the basis of protein and tissue weight respectively. The brain mitochondrial fraction, however, showed increases of 21% ($P<0.01$) and 29% ($P<0.01$) respectively. In animals which received ethionine together with guanine, the 'overt' mitochondrial inhibitory activity in liver and brain was the same as in animals receiving 0.9% NaCl only. The 'latent' inhibitory activities in liver and brain mitochondrial fractions from animals receiving guanine or guanine plus ethionine were nearly the same as in the corresponding fractions from control animals.

Effect of cycloheximide, chloramphenicol and actinomycin D on guanine deaminase and inhibitor activity in vivo in rat liver

Like ethionine, cycloheximide given with guanine abolished the guanine-dependent increase in deaminase activity of liver homogenates or supernatant fraction and also prevented the increase in 'overt' mitochondrial inhibitory power. Chloramphenicol only slightly decreased the guanine-induced increase in deaminase activity, but did abolish the increase in inhibitory power.

To test whether RNA synthesis was essential for the increase in enzyme activity caused by guanine treatment, rats were given guanine plus actinomycin D and killed after 48h, as were appropriate control animals. The administration of actinomycin D prevented the increase in enzyme activity, and also caused the disappearance of all 'overt' mitochondrial inhibitory activity. However, the 'latent' inhibitory activity was more than doubled by actinomycin D treatment, although the total inhibitory activity ('latent' plus 'overt') was decreased by about 40%.

Ethionine, cycloheximide, chloramphenicol and actinomycin D, added directly to the assay systems, were all without effect on guanine deaminase or the mitochondrial inhibitory activity.

Discussion

Previous reports from this laboratory (Sree Kumar *et al.*, 1973) had established that guanine deaminases in rat liver and brain, and also mouse brain, showed regulatory properties. The present data show that the deaminase activity in liver and brain of rats and mice is increased by administration of guanine *in vivo*. The results with inhibitor suggest that this increase is dependent on RNA and protein synthesis.

In rats, but not mice, the 'overt' inhibitory activity in the mitochondrial fraction of both liver and brain was also increased by guanine treatment, suggesting a possible regulatory role for the inhibitor. This

increase was also blocked by the inhibitors of protein synthesis tested. Chloramphenicol, which did not prevent the increase in deaminase activity, did block the increase in inhibitory activity, suggesting that mitochondrial protein synthesis might be involved in this case.

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