Convulsant effect of L-glutamic acid-γ-hydrazide by simultaneous treatment with pyridoxal phosphate

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PREVIOUS work from this laboratory showed that the administration of GAH* to mice induced a marked increase in brain GABA concentration, which was attributed to an inhibitory effect of the hydrazide on GABA-T *in vivo*[1]. In this note, results are presented concerning the inhibition of GABA-T and GAD activities in brain tissue from GAH-treated mice (hemihydrate, A grade; California Corp. for Biochemical Research; 160 mg/kg body weight). GABA-T activity was estimated in brain homogenates by an adaptation of the procedures of Bessman *et al.*,[2] Baxter and Roberts,[3] and Tuena *et al.*[4] GAD activity was evaluated by measuring the increase in GABA according to the conditions established by Roberts and Frankel[5] and Rindi *et al.*,[6] with slight modification.[7] Fig. 1 shows that through a 36-hr period the inhibition of GABA-T corresponded to an increase of GABA levels. Significant GAD inhibition was observed (Fig. 1) at 6·5 hr, followed by a decline in a 24-hr

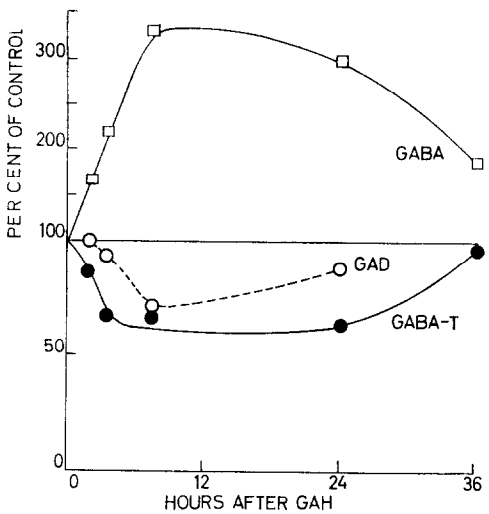

FIG. 1. Changes with time of GABA levels and GABA-T and GAD activities after injection of a single dose of GAH (160 mg/kg).

period. This finding distinguishes the effect of GAH *in vivo* from that of hydroxylamine and aminooxyacetic acid which, according to Baxter and Roberts,[8] have practically no inhibitory effect on GAD activity *in vivo*.

On the grounds that GAH is a carbonyl-trapping agent, the authors considered it of interest to investigate whether PyP could neutralize *in vivo* the effects of GAH on cerebral GABA levels and GABA-T activity. Unfasted adult mice (22–28 g, local strain) were injected intraperitoneally with PyP simultaneously (A grade, California Corp. for Biochemical Research; 50 mg/kg). The animals unexpectedly died in convulsion within a period of one-half to three-quarters of an hour. The brains from these mice as well as those from controls injected with saline, GAH, or PyP (decapitated simultaneously) were rapidly removed and immersed in liquid air for 1·5 to 2 min. The brains were weighed while frozen and homogenized in Potter-Elvehjem homogenizers with 15 vol of 80% ethyl alcohol. The methods for obtaining lipid and protein-free extracts for the analysis of the free amino acid content have been previously reported.[1,7] A striking decrease in the concentration of brain GABA and GAD activity of mice injected with GAH plus PyP was observed (Tables 1 and 2). Brain GABA in mice treated only with PyP also diminished significantly.

* The abbreviations used are: GAH, L-glutamic acid-γ-hydrazide; GABA, γ-aminobutyric acid; GABA-T, γ-aminobutyric acid-α-ketoglutaric acid transaminase; GAD, glutamic acid decarboxylase; PyP, pyridoxal phosphate.

The different distribution of GABA-T and GAD in brain tissue could be responsible for the opposite effects exhibited by GAH on the level of brain GABA in the absence or presence of PyP. According to the hypothesis of Roberts[9] the former enzyme is found in non-neural elements and the latter in neural elements. Therefore, in the absence of the injected cofactor, GABA-T is more

TABLE 1. FREE AMINO ACIDS OF BRAINS FROM CONTROL MICE AND MICE TREATED WITH GAH, PyP, OR GAH PLUS PyP*

Amino acid, mg in 100 g wet tissue	Group			
	Control	GAH	GAH + PyP	PyP
Aspartic acid	54·3 ± 3·34 (6)	63·9 ± 2·78 (6)	58·0 ± 2·50 (9)	56·0 ± 3·38 (6)
Glutamic acid	203·0 ± 5·10 (6)	202·0 ± 8·31 (6)	198·0 ± 8·89 (9)	196·0 ± 7·17 (6)
Glutamine	58·9 ± 1·82 (6)	54·0 ± 2·05 (6)	63·0 ± 3·44 (9)	58·0 ± 2·28 (6)
GABA	26·6 ± 1·17 (6)	27·6 ± 1·47 (6)	8·8 ± 0·66 (9)	16·7 ± 1·34 (6)
Alanine	2·3 ± 0·51 (6)	2·2 ± 0·36 (6)	4·2 ± 0·54 (9)	2·2 ± 0·54 (6)

* Values in both tables are mean ± standard error of the mean; number of animals in parentheses.

Statistical significance of results according to "t" test:

GABA	P	Alanine	P
26·6–16·7	<0·001	2·3–4·2	<0·05
26·6– 8·8	<0·001		

TABLE 2. GABA-T AND GAD ACTIVITIES OF BRAINS FROM CONTROL MICE AND MICE TREATED WITH PyP OR GAH PLUS PyP*

Activity	Group		
	GAH + PyP	PyP	Control
GABA-T (μmoles glutamic acid produced by 100 mg wet tissue)	2·77 ± 0·20 (7)	2·93 ± 0·25 (7)	2·73 ± 0·07 (7)
GAD (μmoles GABA produced by 100 mg wet tissue)	0·59 ± 0·09 (8)		2·33 ± 0·18 (8)

* Statistical significance of results according to "t" test: 0·59–2·33, P<0·001.

accessible to GAH than GAD, as inferred from the inhibition curves of both enzyme activities *in vivo* (Fig. 1). In the presence of injected PyP, GAH would be more accessible to neural elements and inhibit GAD activity more effectively, hence producing the lower GABA concentration and the appearance of convulsions.

There is evidence[10] that PyP increases membrane permeability to amino acids. This cofactor might also facilitate the entrance of GAH into the neurons through its combination with the hydrazide forming a di-substituted derivative. The permeation of this derivative into various areas of brain might be completely different from that of the γ-glutamyl hydrazide itself, as would the hydrolysis characteristics.

Preliminary experiments showed that simultaneous injections of GAH and deoxypyridoxine phosphate (A grade, California Corp. for Biochemical Research; 47 mg/kg) did not influence the changes induced by GAH on the free amino acid pattern of mouse brain within a 6·5-hr period.

The effects of several carbonyl-trapping agents *in vivo* and of some amino acid derivatives on cerebral amino acid concentrations are currently being studied in the presence or absence of PyP administration.

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REFERENCES

1. G. H. MASSIEU, R. TAPIA and B. G. ORTEGA, *Biochem. Pharmacol.* **11**, 976 (1962).
2. S. P. BESSMAN, J. ROSSEN and E. LAYNE, *J. biol. Chem.* **201**, 385 (1953).
3. C. F. BAXTER and E. ROBERTS, *J. biol. Chem.* **233**, 1135 (1958).
4. M. TUENA, G. H. MASSIEU, B. G. ORTEGA and H. PASANTES, *An. Inst. Biol. (Méx.)* **32**, 21 (1962).
5. E. ROBERTS and S. FRANKEL, *J. biol. Chem.* **190**, 505 (1951).
6. G. RINDI, V. PERRI and V. VENTURA, *Nature (Lond.)* **183**, 1126 (1959).
7. G. H. MASSIEU, B. G. ORTEGA, A. SYRQUIN and M. TUENA, *J. Neurochem.* **9**, 143 (1962).
8. C. F. BAXTER and E. ROBERTS, *J. biol. Chem.* **236**, 3287 (1961).
9. E. ROBERTS, *Acta neurol. scand. Suppl.* 1, **38**, 19 (1962).
10. H. N. CHRISTENSEN, in *Advances in Protein Chemistry*, C. B. ANFINSEN, JR. *et al.*, Eds., p. 239. Academic Press, New York (1960).

3′, 5′-AMP-induced hyperglycemia in intact rats and in the isolated perfused rat liver*

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PREVIOUS reports have shown that the hyperglycemic response to epinephrine is mediated by a cyclic nucleotide, 3′,5′-AMP,[1] which acts as a cofactor for dephosphophosphorylase kinase, the enzyme that catalyzes the formation of the active form of phosphorylase.[2] Several papers recently have appeared in which 3′,5′-AMP has been shown to exert a physiological response in organs both *in situ* and when completely isolated. Hilton *et al.*[3] have shown that infusions of either ACTH or 3′,5′-AMP into the adrenal artery of the dog result in elevation of hydrocortisone concentration in the adrenal vein. Orloff and Handler[4] demonstrated a vasopressin-like effect by painting the serosal surface of the toad bladder with 3′,5′-AMP.

Unpublished data have shown that infusion of epinephrine into the portal cannula of the isolated artificially perfused rat liver causes hyperglycemia, glycogenolysis, and activation of phosphorylase.[5] Perske *et al.*[6] and Niemeyer *et al.*[7] in studies employing the intact rat, were unable to demonstrate epinephrine-induced phosphorylase activation. On the other hand, intravenous injections of 3′,5′-AMP (4 mg/kg) into dogs showed slight increases in blood glucose concentration.[2]

This communication is concerned with the effect of intraperitoneal administration of 3′,5′-AMP into the intact rat and the effect of infusion of the cyclic nucleotide into the isolated perfused liver preparation.

METHODS

The liver perfusion method used is a modification by Miller† of the method reported by Miller *et al.*[8] and further modified for these studies by Northrop and Parks.[5] By the use of this technique it is possible to determine blood glucose concentrations immediately before and after passage of blood through the liver and to remove representative samples of liver tissue at selected times before and after infusion of an agent such as 3′,5′-AMP. Thus rapid changes in the concentration of metabolites and the activities of various enzymes can be determined in the tissue. Blood glucose concentrations

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† J. A. Miller, personal communication.