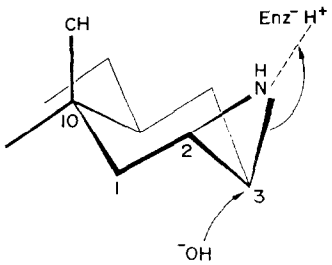

is inhibited with the former[13] might suggest that a single enzyme catalyzes both types of hydrolysis, we propose "aziridine hydrolase" tentatively for the name of the present enzyme.

Division of Organic Chemistry,
Tokyo College of Pharmacy,
Ueno-sakuragi, Taito-ku, Tokyo 110, Japan

TADASHI WATABE
KAZUE KIYONAGA
SHOJI HARA

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Effects of various hydrazines upon the metabolism of gamma aminobutyric acid (GABA)-1-^{14}C by rats

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STUDIES on the effects of simple hydrazines on GABA metabolism have been concerned mainly with the central nervous system. Administration of hydrazine to mice has been shown to cause a several fold increase of brain GABA levels, presumably because GABA transamination was more inhibited than was formation of GABA through decarboxylation of glutamic acid.[1–3] Monomethylhydrazine

is also an effective inhibitor of GABA transamination, but unsymmetrical dimethylhydrazine causes only moderate inhibition.[2] In addition, Medina[2] has presented evidence suggesting a relationship between hydrazine inhibition of GABA transaminase *in vivo* and interference with pyridoxal phosphate synthesis, possibly through the influence of the hydrazone of pyridoxal.

In spite of the reported low concentrations of GABA in non-central-nervous tissues,[4] and the fact that the pathway for GABA biosynthesis has until recently[5] been detected only in the central nervous system[6,7] there has been ample evidence that rats have substantial capability for catabolism of GABA outside the nervous system. Specific tissue studies have demonstrated substantial GABA transaminase activity in liver[8–10] and kidney.[9] Wilson *et al.*[11] found evidence that GABA-4-^{14}C entered the Krebs cycle, with conversion of 17 per cent of the label to $^{14}CO_2$. Bremer[12] in searching for potential precursors of carnitine, reported that 94 per cent of the radioactivity of a subcutaneous dose of GABA-1-^{14}C given to a rat emerged as $^{14}CO_2$. Horvath *et al.*[13] found rats to be capable of metabolizing more than 90 per cent of intraperitoneally administered GABA-1-^{14}C (0·8 μmoles to 1·2 m-moles/kg) to $^{14}CO_2$ over an 18-hr period. A conflicting report by Tsukada *et al.*[9] showed only 5 per cent conversion of GABA-1-^{14}C to $^{14}CO_2$ in 24 hr after oral administration to a mouse. Most of the remaining ^{14}C was recovered as GABA-1-^{14}C in the urine, and a small amount of radioactivity was found in the liver. The appearance of large amounts of unmetabolized GABA in urine has also been reported,[14] suggesting an ineffective metabolism of GABA.

Since passage of GABA from the blood to the brain does not readily occur,[14–16] the metabolism of systemically administered GABA described in the above reports and in our studies must be presumed to occur in non-central-nervous tissues.

The several examples of the apparent capability of non-nervous tissue for catabolism of GABA have not been sufficient to demonstrate a role for GABA outside the nervous system. It has recently been shown, however, that GABA exists in appreciable concentration in human kidney, diaphragm, lung and liver[17] and in rat kidney.[5] Most significant has been the identification of glutamic acid decarboxylase in substantial amounts in the renal cortex, liver and spleen of rats.[5] It is not likely that GABA serves a neuroinhibitory function in these tissues; instead the pathway is probably functional in oxidative metabolism.

We have observed the respective inhibitory effects of hydrazine, monomethylhydrazine (MMH) and 1,1-dimethylhydrazine (UDMH) upon the extensive catabolism of GABA-1-^{14}C in intact rats. Comparative experiments with β-alanine-1-^{14}C have been conducted to assess the specificity of whole-body metabolism of GABA, on the basis of studies by Roberts and Bregoff.[8] In the present studies, we have found that non-lethal doses of hydrazine strongly inhibit the extensive transamination of GABA-1-^{14}C and β-alanine-1-^{14}C in intact rats as determined by alterations in production of $^{14}CO_2$ from these compounds. Alkylhydrazines cause a much less pronounced change.

Instrumentation

The amount and time course of $^{14}CO_2$ production by animals metabolizing ^{14}C-labeled substrates was measured and recorded by a 4-unit radiorespirometric apparatus which has been previously described.[18,19]

A Spinco Model 120B automatic amino acid analyzer was employed to identify and measure amino acids in the urine, utilizing the procedure of Spackman *et al.*[20]

Chemicals and substrates

Gamma aminobutyric acid-1-^{14}C (GABA-1-^{14}C) was supplied by New England Nuclear Corporation with a specific activity of 3·27 mc/m-mole and was dissolved in water, at a concentration of approximately 10 μc/ml for experimental use. β-alanine-1-^{14}C was supplied by Atomic Associates, Inc., Valley Stream, New York (now a division of Baird Atomic Inc., Cambridge, Massachusetts) with a specific activity of 3·57 mc/m-mole. Unlabeled GABA and β-alanine supplied by the Sigma Chemical Company, St. Louis, Missouri, were used as carriers to provide the desired substrate dose/g body weight. Labeled and unlabeled substrate solutions were kept frozen until use.

Unsymmetrical dimethylhydrazine, anhydrous 98–99% (UDMH); hydrazine, anhydrous 95–100%; and monomethylhydrazine, b.p. 97–98° (MMH), were obtained from the Matheson Company, Inc., Matheson, Coleman and Bell Division, East Rutherford, New Jersey. These hydrazines were stored under nitrogen in the dark in glass ampoules. All solutions of hydrazines were prepared with distilled water immediately before each experiment. Each agent was used at an approximate LD_{10} dosage: Hydrazine, 1·0; MMH, 0·5; and UDMH, 1·5, m-moles/kg. Respective LD_{50} doses are 1·5, 0·6, 2·0 m-moles/kg. The intoxicants were administered 30–40 min before substrate.

Male Sprague–Dawley rats, obtained from Pacord Research, Inc., Beaverton, Oregon, were used at a weight of 240–250 g. They were maintained on Purina Lab. Chow and water, *ad lib.*

General procedure

Hydrazines were injected intraperitoneally 30–40 min before substrate administration.

Radioactivity of labeled substrates was standardized by liquid scintillation counting. A measured volume (usually 10 μl) of substrate solution was placed in 7 ml of ethanol–ethanolamine (2:1, v/v) and 10 ml toluene containing 0·3% (w/v) terphenyl and 0·003% (w/v) POPOP (1,4-bis-2(5-phenyl-oxazole)-1 benzene). The samples so prepared were counted in a liquid scintillation spectrometer (model 314 EX-2, Packard Instrument Company, La Grange, Illinois). A sufficient number of counts were collected to ensure that the relative standard deviation of the counting rate was no greater than 2 per cent. Counting efficiency was established by internal standardization.

Individual substrate solutions were prepared before experiment by adding the desired dose of unlabeled GABA or β-alanine to 0·1 ml (approximately 1 μc) of a solution of the radioactive compound. The solution was transferred with rinsings to a 1-ml syringe and injected into each rat intraperitoneally. The amount of radioactivity remaining in the syringe after injection was then measured in order to determine the actual amount injected. In experiments where urinary radioactivity was to be examined, approximately 3 μc of GABA-1-^{14}C were used.

The maximum dose of GABA was set at 4·0 m-moles/kg because higher doses cause visible distress; the lower doses used were 0·4 and 0·004 m-mole/kg. β-alanine caused apparent severe abdominal pain at a dose of 4·0 m-moles/kg and was employed only at 0·4 m-mole/kg for comparative purposes.

Urinary radioactivity was determined by liquid scintillation counting of 10 μl aliquots of the total volume of urine plus rinsings of each collection.

RESULTS

While each of the hydrazines examined depressed the metabolism of GABA in rats, hydrazine itself had the greatest and most persistent effect (Table 1, a, b and d). The maximum rate of $^{14}CO_2$ production from hydrazine-treated rats after administration of 0·004, 0·4 or 4·0 m-moles GABA-1-^{14}C/kg was less than 8 per cent of the maximum rate of appearance of $^{14}CO_2$ from non-intoxicated rats given the same amount of labeled GABA. At a dose of 4·0 m-moles GABA-1-^{14}C/kg, the observed low rate of $^{14}CO_2$ output by hydrazine-treated rats did not vary appreciably for 24 hr.

In separate experiments, urine amino acids were analyzed in samples collected from a group of four animals intoxicated with hydrazine and administered GABA-1-^{14}C. GABA was shown to be the only amino acid present in the urine in measurable quantities. Comparison of the specific activities of two dose levels of administered GABA with the ratio of urinary radioactivity to urinary GABA found for each animal (Table 2) indicates that GABA which was not metabolized by the hydrazine-treated animals appeared unchanged in the urine. In the two animals of this group which were administered 4·0 m-moles GABA-1-^{14}C/kg, $^{14}CO_2$ production continued for several hours after the appearance of radioactivity in the urine had virtually ceased (Table 3a), even though urinary recovery of ^{14}C in these

TABLE 1. EFFECTS OF MMH, UDMH AND HYDRAZINE UPON THE METABOLISM OF GABA-1-^{14}C AND AND β-ALANINE-1-^{14}C ADMINISTERED INTRAPERITONEALLY TO RATS

(a) 0·004 m-mole GABA/kg

Time after substrate (min)	Average percentage of administered radioactivity converted to $^{14}CO_2$ by rats intoxicated with			
	Control (3)*	MMH (2) (0·5 m-mole/kg)	UDMH (1·5 m-moles/kg)	Hydrazine (2) (1·0 m-mole/kg)
20	11	3	3	—
40	31 (27–35)†	16 (13–19)	12 (9–15)	—
60	45	28	27	1
80	52	38	38	—
120	59 (57–62)	50 (48–52)	51	2
160	62	56	57 (55–59)	—
200	65	59	61	3 (3–4)

* Number of animals observed.

† Figures in parentheses represent the range of values at selected points in the time course.

(b) 0·4 m-mole GABA/kg

Time	Average percentage administered radioactivity converted to $^{14}CO_2$ by rats intoxicated with			
	Control (2)	MMH (2)	UDMH (2)	Hydrazine (2)
20	12	4	5	1
40	37 (34–40)	10	14	—
60	51	21	30 (19–41)	—
80	58	32	39	2 (1–2)
120	66 (63–69)	47 (44–50)	49 (45–53)	—
160	70	52	54	—
200	72 (70–74)	54 (51–57)	57 (53–61)	5 (4–6)

(c) 0·4 m-mole β-alanine/kg

Time after substrate (min)	Control (3)*	MMH (2) (0·5 m-moles/kg)	UDMH (3) (1·5 m-moles/kg)	Hydrazine (2) (1·0 m-moles/kg)
20	16·8	3·5	8·8	1·5
40	44	9	23	2·9
60	61·3 (58–64·5)	17·7 (15–20·5)	37·3 (27·5–48·5)	4·9 (4·2–5·5)
80	69·3	34·2	49·8	6·2
120	75 (72–78)	49·2 (40·5–58)	65·5 (61–70·5)	9·6 (8·7–10·5)
160	76·6	58	70·5	12·2
200	78·3 (75–81)	63·2 (57·5–69)	72·8 (70–74·5)	14·5 (12·7–16·5)

(d) 4·0 m-moles GABA/kg

Time	Control (3)	MMH (4)	UDMH (2)	Hydrazine (5)
40	7	3	7	—
80	17	7	17	—
120	27	13	26	—
160	36	21	34	1
200	43 (38–50)	27 (22–23)	39 (37–42)	—
300	50	37	44	2 (1–3)
400	54 (50–58)	43 (38–44)	47 (45–50)	4 (2–6)

* Number of animals observed.

TABLE 2. SPECIFIC ACTIVITIES OF GABA-1-^{14}C ADMINISTERED TO HYDRAZINE-TREATED RATS AND SUBSEQUENTLY RECOVERED IN THE URINE

Animal	Amount of GABA-1-^{14}C administered (m-moles/kg)	Specific activity administered GABA (μc/μmole)	Specific activity urinary GABA (μc/μmole)
U-1	4·0	0·0027	0·0025
U-2	4·0	0·0027	0·0023
U-3	0·4	0·0266	0·028
U-4	0·4	0·0266	0·024

TABLE 3. RESPIRATORY AND URINARY RADIOACTIVITY FROM *hydrazine-treated* RATS (1 m-mole/kg) ADMINISTERED GABA-1-^{14}C INTRAPERITONEALLY

Hours after GABA-1-^{14}C	Percentage administered radioactivity, recovered as: Urinary GABA ^{14}C	Respiratory $^{14}CO_2$
(a) 4·0 m-mole GABA/kg		
4	44	3
8	55 (53–57)*	7 (7–7)
12	60	10
16	62	13
24	63	18
36	64	20 (20–20)
48	64 (63–66)	20
	Total recovery:	84 per cent
(b) 0·4 m-mole GABA/kg		
4	7	7
8	16	23
12	23	40
16	25	49
24	27 (25–29)*	56 (55–57)
36	30	59
48	30 (29–31)	60
	Total recovery:	90 per cent

* Figures in parentheses represent the range of values at selected points in the time course. Two animals were observed in each experiment.

animals was proportionally much higher than in animals which were given 0·4 m-moles GABA/kg (Table 3b). In this group of experiments, up to 90 per cent of the administered radioactivity was found either in the urine as GABA-^{14}C or as respiratory $^{14}CO_2$. No attempt was made to determine the distribution of the remainder.

The rate of GABA-1-^{14}C oxidation was sharply decreased by both methyl hydrazines during the first 4 hr, and then increased to only slightly less than the rate observed in control animals. This transient interference resulted in a moderate decrease in the total amount of GABA-1-^{14}C oxidized. The effect of alklyhydrazines on the metabolism of 0·004 and 0·4 m-mole GABA/kg was similar despite the 100-fold difference in substrate levels.

β-Alanine-1-^{14}C at a dose of 0·4 m-mole/kg intraperitoneally was converted to $^{14}CO_2$ at a slightly higher rate than was the molar equivalent dose of GABA-1-^{14}C (Table 1c). The effects of MMH and UDMH on oxidation of β-alanine-1-^{14}C also resemble those exerted on GABA-1-^{14}C conversion. Hydrazine was strongly inhibitory but allowed about three times as much conversion of β-alanine as of GABA. This inhibition was not increased by increasing the hydrazine dose to lethal levels.

DISCUSSION

It is unlikely that the effect of hydrazine upon GABA and β-alanine oxidation is related to any influence on Krebs cycle activity, since in similar experiments we have shown no impairment of glutamate or acetate oxidation by similar doses of hydrazine.* Both amino acids probably pass through the same initial metabolic sequence to enter the Krebs cycle at different points. Roberts and Bregoff[8] found that the GABA-α ketoglutarate transaminase system metabolizes β-alanine nearly as effectively as it does GABA, in both brain and liver. The product of β-alanine transamination is malonic semialdehyde,[21] which is probably decarboxylated to acetate.[22] The catabolism of β-alanine should therefore be rapid and has been demonstrated previously[22,23] as well as in the present work. The similarity between GABA and β-alanine in terms of catabolic rates, in either normal or intoxicated animals, also should be characteristic of a common pathway.

* F. N. Dost, D. J. Reed and C. H. Wang, unpublished observations.

The observed early but non-persistent depression of GABA and β-alanine oxidation by both alkylhydrazines is unexplained. It is doubtful that this similar effect by two compounds reflects change in the central nervous system, since Medina[2] has shown that the GABA transaminase activity of brain removed from MMH intoxicated animals is much more extensively inhibited than that of animals treated with UDMH.

The maximum rate of turnover of GABA by intact animals has yet to be measured, but during the initial 2 hr of catabolism of single doses of 4·0 m-moles GABA/kg (Table 1, D), the maximum rate of conversion of C-1 of GABA to CO_2 was about 15 per cent of the total dose per hour. This represents 0·6 m-mole of GABA or 2·4 mmoles CO_2/hr, a significant fraction of the roughly 10 m-moles CO_2/hr expected from rats of this size. Since non-nervous tissues have the capability to synthesize GABA, this degree of utilization suggests that the GABA pathway may be significant in the metabolism of tissues other than brain. The appearance of unmetabolized GABA-^{14}C in the urine of hydrazineized rats suggests that no alternative pathway to transamination and entry into the Krebs cycle exists for GABA.

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Science Research Institute,
Department of Biochemistry and Biophysics,
and Department of Chemistry,
Oregon State University, Corvallis, Ore. 97331, *U.S.A.*

F. N. Dost
D. J. Reed
C. H. Wang

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