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FREE AMINO ACIDS AND GLUTAMATE DECARBOXYLASE ACTIVITY IN BRAIN OF MICE DURING DRUG-INDUCED CONVULSIONS

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Abstract—Previous administration of L-glutamic acid-γ-hydrazide (GAH) to mice did not protect them against the convulsant action of thiosemicarbazide, methionine sulfoximine, insulin, or pentylenetetrazol (Metrazol). The concentrations of some free amino acids were measured in the brains from these mice at the moment of convulsions.

The changes in free amino acid concentrations produced by the convulsant agents used were in general similar whether GAH had been administered or not. In the first case, however, the changes were exerted on the altered pattern of amino acids obtained by the previous GAH treatment. In all cases when GAH and the convulsant agent were injected and convulsions were produced, a two- to four-fold increase of γ-aminobutyric acid (GABA) concentration was found in brain. In other experiments, it was found that GAH administration increased both free and bound GABA concentrations.

When a single convulsant dose of GAH was injected, brain glutamate decarboxylase activity progressively decreased with time. The maximal glutamate decarboxylase inhibition was observed at the onset of convulsions; at this moment GABA levels were increased. γ-Aminobutyric aminotransferase activity was diminished more intensely before the onset than at the occurrence of the convulsive state.

Similarly, after the injection of a convulsant dose of amino-oxyacetic acid, glutamate decarboxylase activity was decreased at the onset of convulsions, whereas γ-aminobutyric transferase activity was totally inhibited; GABA levels were significantly increased.

Anticonvulsant doses of amino-oxyacetic acid protected mice against convulsions induced by the simultaneous administration of GAH and pyridoxal phosphate. Glutamate decarboxylase activity was found equally diminished in protected and in non-protected mice in comparison with control animals.

It is concluded that the inhibition of glutamate decarboxylase activity, independently of the total concentration of GABA in brain, may be a factor involved in the production of some types of convulsions, and probably the anticonvulsant action of amino-oxyactic acid is not related to its effect on GABA metabolism in brain.

PREVIOUS work has shown that L-glutamic acid-γ-hydrazide (GAH*) treatment induces in mice a five- to six-fold increase in brain GABA concentration.[1] This effect is due to the inhibition of aminobutyrate aminotransferase (ABAT) activity.[2] Some other carbonyl-trapping agents, like hydroxylamine and amino-oxyacetic acid (AOA), exhibit similar effects and are powerful anticonvulsants: hydroxylamine is effective

* The abbreviations used in this work are: GAH, L-glutamic acid-γ-hydrazide; GABA, γ-aminobutyric acid; ABAT, aminobutyrate aminotransferase (EC 2.6.1.19); GAD, glutamate decarboxylase (EC 4.1.1.15); TSC, thiosemicarbazide; MS, methionine sulfoximine; AOA, amino-oxyacetic acid; PALP, pyridoxal phosphate.

against convulsions induced by electroshock[3] or pentylenetetrazol (Metrazol),[4] and AOA is effective against convulsions induced by thiosemicarbazide and methionine sulfoximine[5] and Metrazol.[6] On the other hand, studies have shown that the elevation of GABA levels does not necessarily imply a rise in the convulsions threshold[7,8] and, conversely, some substances that do not significantly affect GABA levels are powerful anticonvulsants.[9]

In view of these contradictory data, it was considered of interest to investigate whether GAH, which has a relatively low toxicity, behaves as an anticonvulsant resembling hydroxylamine and AOA. Also, it was considered interesting to study to what extent the changes induced by GAH on the brain free GABA and other amino acids were modified by the action of convulsants which by themselves modify the brain free amino acid content, such as TSC or MS. Furthermore, since Elliott[10,11] has suggested that it is not free but a bound GABA that is involved in brain excitability, the effect of GAH on the concentrations of free and bound GABA was also studied.

In other experiments, GAD and ABAT activities were measured in brains of mice treated with convulsant doses of GAH and AOA, in order to investigate the possible relationship of these enzymes to convulsions, independently of the total concentration of GABA in brain.[12] GAD activity was also measured in animals protected against convulsions by AOA.

TABLE 1. DOSES AND TIMES OF ADMINISTRATION OF DRUGS USED

Group	GAH dose (mg/kg)	Convulsant agent (c.a.) dose (mg/kg)	Time between GAH administration and c.a. administration	Time between c.a. administration and sacrifice of animals*
I	160	TSC (20)	5·0–5·5 hr	1·0–1·5 hr
II	20	TSC (20)	0	(amino acids not studied)
III	80/day for 5 days	TSC (20)	6·5 hr after the 5th injection	1·5–2·0
IV	160	MS (100)	15 min	24 hr
V	160	Insulin†	5·5 hr	0·75–1·0 hr
VI	40	Metrazol (75)	1·0 hr	(amino acids not studied)
VII	160/day for 6 days	None‡		mice sacrificed 6·5 hr after 6th injection of GAH

* Control animals were sacrificed at times when the maximal convulsant effect of the corresponding c.a. occurred.
† Dose of insulin, 20 units/kg.
‡ Some of these animals died in convulsions within 5–7 days after the begining of GAH treatment see Results).

MATERIALS AND METHODS

Adult nonfasted mice from a local strain, weighing 23–28 g, were used. GAH (Calbiochem, A grade), MS (Calbiochem), insulin (Lilly, regular insulin) and Metrazol (Parke Davis) were obtained from commercial sources; AOA was synthesized by Dr. Guillermo Carvajal as the hemihydrochloride. The animals were randomized in groups of 24 or 36, and each group was divided into four subgroups according to the intraperitoneal injection of substances, as follows: (1) GAH and saline solution; (2) saline solution and convulsant agent; (3) GAH and the convulsant agent, and (4) two injections of saline solution (controls). Different doses and times of administration were used, as indicated in Table 1. In all cases the same number of animals of each subgroup was handled simultaneously throughout the amino acid determination.

When animals were in terminal convulsions they were decapitated and their brains rapidly extracted and frozen in liquid air. Control mice were handled similarly. The brains were homogenized in a Potter-Elvehjem homogenizer in 80% ethyl alcohol; the homogenates were treated according to Awapara's method[13] to obtain aqueous extracts free of proteins and lipids. The free amino acids present in the extracts were separated by bidimensional paper chromatogaphy[2] and measured by the colorimetric method of Naftalin.[14] By this procedure the recoveries of the amino acids measured are approximatively 95 per cent for GABA, glutamic acid, and aspartic and 90 per cent for glutamine, as has been pointed out previously.[9,15] In animals of Group V (Table 1), glycemia was measured according to the Nelson–Somogyi method[16] in a sample of blood obtained at the moment of decapitation.

GAD activity was estimated by measuring the CO_2 production by the conventional manometric technique in a Warburg apparatus, at 37° in an atmosphere of nitrogen (see Table 9 for incubating conditions). ABAT activity was measured by determining glutamic acid production in 1-hr incubation at 37°, pH 8·2, as previously described.[2,9]

Free and bound GABA were determined in the mouse brains 6·5 hr after i.p. GAH administration (160 mg/kg). For the extraction of free and bound GABA, a modification of the method of Lovell and Elliott[17] was followed: (1) Two brains were pooled and homogenized in cold 0·9% NaCl solution (4 vol) in a Potter–Elvehjem homogenizer. (2) The homogenates were centrifuged (15 min) at 15,000 *g* at 0–4°. (3) Six volumes of absolute ethyl alcohol were added to the supernatant fraction containing free GABA; the volume of the supernatant was measured in order to correct the error given by the volume of fluid retained in the residue, which was fairly constant in all determinations. (4) Nine volumes of 80% ethyl alcohol were added to the residue; this procedure liberates bound GABA. (5) Both the supernatant and the residual fractions were treated according to Awapara's technique[13] for the separation of free amino acids. Unidimensional chromatography in 80% phenol was used for the separation and subsequent quantitative determination of GABA.[2] In all the experiments total GABA was determined simultaneously.

RESULTS

Amino acid levels in brain

The results are summarized in Tables 2 through 6. GAH-treated mice showed the previously reported increase in the concentrations of GABA and alanine and the decrease in glutamine levels.[1] TSC administration induced a significant decrease of aspartic acid and GABA concentrations and tended to increase those of glutamine and alanine. In animals treated with GAH and TSC the effect of both substances on alanine levels was additive, whereas the effect on GABA and glutamine was antagonistic. In this group of mice the brain concentration of GABA was higher than in control animals (Table 2).

A daily GAH dose of 80 mg/kg for 5 days (Group III) induced an approximately 100 per cent increase of GABA and alanine levels without any apparent sign of toxicity. The administration of TSC to these animals did not influence the concentration of their brain free amino acids, except for alanine, which increased 50 per cent (Table 3).

When GAH treatment was prolonged for 6 days by a daily dose of 160 mg/kg (Group VII), GABA levels were increased to values 100 per cent higher than those found 6·5 hr after a single dose (160 mg/kg) of the drug.[1] Aspartic acid concentration

TABLE 2. FREE AMINO ACIDS IN BRAIN OF CONTROL MICE AND OF MICE TREATED WITH GAH, TSC, AND GAH + TSC* (GROUP I)

Treatment	Aspartic acid	Glutamic acid (mg/100 g)	Glutamine	GABA	Alanine
Control	56·2 ± 3·51 (9)	190·0 ± 8·21 (9)	58·3 ± 2·78 (9)	27·8 ± 1·44 (9)	2·26 ± 0·24 (9)
GAH	54·8 ± 2·54 (9)	182·0 ± 4·41 (9)	33·1 ± 2·17 (9)	114·5 ± 4·29 (9)	12·0 ± 0·71 (9)
TSC	46·8 ± 4·60 (9)	176·0 ± 7·68 (9)	68·6 ± 3·12 (9)	23·1 ± 2·42 (9)	4·95 ± 0·38 (9)
GAH + TSC	45·7 ± 2·56 (9)	170·0 ± 5·30 (9)	44·4 ± 2·96 (9)	91·2 ± 2·30 (9)	14·4 ± 1·14 (9)

* The values are in mg/100 g; mean ± standard error of the mean; number of animals in parentheses; for Tables 2–7.

Statistical significance according to *t* test:

Aspartic acid	P	Glutamine	P	GABA	P	Alanine	P
56·2 vs 46·8	<0·02	58·3 vs 33·1	<0·001	27·8 vs 114·5	<0·001	2·26 vs 12·0	<0·001
56·2 vs 45·7	<0·02	58·3 vs 68·6	<0·05	27·8 vs 23·1	<0·05	2·26 vs 4·95	<0·001
		58·3 vs 44·4	<0·01	27·8 vs 91·2	<0·001	2·26 vs 14·4	<0·001

was decreased by the prolonged treatment, whereas glutamine levels remained constant (Table 4). Some mice in this group died in convulsions within 5–7 days. Their brains were not used for the amino acid analyses.

Mice treated with methionine (100 mg/kg) showed a remarkable decrease in aspartic acid, glutamic acid, GABA and glutamine brain levels. When MS was injected to mice previously given GAH, glutamine concentration was slightly lower; a higher level of aspartic acid was observed in mice treated with MS alone. In the

TABLE 3. FREE AMINO ACIDS IN BRAIN OF CONTROL MICE TREATED WITH GAH FOR FIVE DAYS, TSC AND GAH (5 DAYS) + TSC (GROUP III)

Treatment	Aspartic acid	Glutamic acid	Glutamine	GABA	Alanine
		(mg/100 g)			
Control	56·1 ± 4·20 (5)	241·8 ± 7·00 (5)	81·0 ± 8·25 (5)	42·8 ± 3·00 (5)	3·63 ± 0·48 (5)
GAH	66·2 ± 2·09 (7)	236·0 ± 6·80 (7)	82·3 ± 3·56 (7)	79·6 ± 7·82 (7)	8·63 ± 1·02 (7)
TSC	60·3 ± 3·35 (6)	256·0 ± 8·80 (6)	90·1 ± 6·00 (6)	22·9 ± 2·89 (5)	5·34 ± 0·44 (6)
GAH + TSC	59·1 ± 6·32 (6)	228·0 ± 9·90 (6)	92·4 ± 6·09 (6)	70·4 ± 11·1 (5)	12·5 ± 1·61 (6)

Statistical significance according to *t* test:

GABA	P	Alanine	P
42·8 vs 79·6	<0·01	3·63 vs 8·63	<0·01
42·8 vs 22·9	<0·01	3·63 vs 5·34	<0·05
42·8 vs 70·4	<0·1	3·63 vs 12·5	<0·001

TABLE 4. FREE AMINO ACIDS IN BRAIN OF CONTROL MICE AND OF MICE TREATED WITH GAH (160 mg/kg/day) FOR 6 DAYS (GROUP VII)

Subgroup	Aspartic acid	Glutamic acid	Glutamine	GABA
		(mg/100 g)		
Control	72·9 ± 3·76 (4)	276·0 ± 11·4 (5)	74·7 ± 2·07 (5)	44·9 ± 3·55 (5)
Treated	53·9 ± 0·74 (4)	249·0 ± 9·55 (4)	82·6 ± 3·74 (4)	219·2 ± 12·8 (4)

Statistical significance according to *t* test:

Aspartic acid	P	GABA	P
72·9–53·9	<0·01	44·9–219·2	<0·001

former animals GABA levels were higher than those observed after GAH treatment alone (Table 5). MS at lower doses (30 mg/kg) did not apparently affect the amino acid content of mouse brain.

Insulin-treated mice showed a significant decrease in the concentrations of glutamic acid and alanine of brain. In these animals the increase of aspartic acid and the decrease of GABA levels were less marked than those observed in earlier work.[18] When the hormone was administered after GAH injection, GABA, glutamine, and aspartic acid levels were found to be essentially the same as in the animals treated

TABLE 5. FREE AMINO ACIDS IN BRAIN OF CONTROL MICE AND OF MICE TREATED WITH GAH, MS, AND GAH + MS (GROUP IV)

Treatment	Aspartic acid	Glutamic acid	Glutamine	GABA	Alanine
	(mg/100 g)				
Control	46·4 ± 2·16 (9)	174·0 ± 6·40 (9)	50·0 ± 2·89 (9)	24·5 ± 2·32 (9)	2·20 ± 0·29 (9)
GAH	45·0 ± 2·74 (8)	170·0 ± 6·70 (8)	55·6 ± 2·58 (8)	88·0 ± 8·20 (8)	14·4 ± 2·30 (8)
MS	19·6 ± 1·17 (7)	115·0 ± 2·24 (7)	17·7 ± 0·60 (7)	16·5 ± 1·90 (7)	2·50 ± 0·32 (7)
GAH + MS	37·4 ± 1·68 (8)	119·0 ± 1·00 (8)	13·5 ± 2·32 (8)	110·0 ± 12·7 (8)	8·60 ± 0·99 (8)

Statistical significance according to t test:

Aspartic acid	P	Glutamic acid	P	Glutamine	P	GABA	P	Alanine	P
46·4 vs 19·6	<0·001	174·0 vs 115·0	<0·001	50·0 vs 17·7	<0·001	24·5 vs 88·0	<0·001	2·20 vs 14·4	<0·001
46·4 vs 37·4	<0·001	174·0 vs 119·0	<0·001	50·0 vs 13·5	<0·001	24·5 vs 16·5	<0·05	2·20 vs 8·60	<0·001
						24·5 vs 110·0	<0·001		

TABLE 6. GLYCEMIA* AND FREE AMINO ACIDS† IN BRAIN OF CONTROL MICE AND OF MICE TREATED WITH GAH, INSULIN AND GAH + INSULIN (GROUP V)

Treatment	Glycemia	Aspartic acid	Glutamic acid	Glutamine	GABA	Alanine
Control	105·9 ± 5·7 (14)	49·0 ± 3·29 (9)	186·0 ± 7·11 (9)	54·9 ± 4·22 (9)	22·0 ± 1·29 (9)	1·34 ± 0·26 (9)
GAH	86·1 ± 6·8 (14)	49·3 ± 2·88 (9)	162·0 ± 7·83 (9)	32·1 ± 2·72 (9)	86·0 ± 5·51 (9)	9·60 ± 0·94 (9)
Insulin	12·4 ± 4·4 (6)	56·8 ± 6·43 (9)	149·6 ± 9·20 (9)	50·0 ± 4·92 (9)	19·4 ± 1·25 (9)	Trace (9)
GAH + insulin	3·3 ± 0·8 (6)	56·2 ± 3·39 (9)	144·0 ± 6·78 (9)	38·5 ± 1·91 (9)	76·0 ± 5·04 (9)	5·73 ± 0·50 (9)

* mg/100 ml.

† The figures are mg/100 g; mean ± standard error of the mean. Number of animals in parentheses.

Statistical significance according to *t* test:

Glycemia	P	Glutamic acid	P	Glutamine	P	GABA	P	Alanine	P
105·9 vs 86·1	<0·05	186·0 vs 149·6	<0·01	54·9 vs 32·1	<0·001	22·0 vs 86·0	<0·001	1·34 vs 9·6	<0·001
105·9 vs 12·4	<0·001	186·0 vs 144·0	<0·001	54·9 vs 38·5	<0·01	22·0 vs 19·4	<0·5	1·34 vs 5·7	<0·001
105·9 vs 3·3	<0·001					22·0 vs 76·0	<0·001		

exclusively with GAH. However, it should be noted that a significant decrease of alanine and glutamic acid concentrations was induced by insulin both in control and in GAH-treated mice (Table 6).

The hypoglycemia induced by insulin in GAH-treated mice was practically of the same order as that induced in control animals. GAH administration per se produced a slight but significant (−18 per cent, $P < 0.05$) hypoglycemia after 6·5 hr (Table 6).

Effect of GAH on free and bound GABA levels in brain

The data summarized in Table 7 indicate that the increase in total GABA levels induced by GAH was due to an increase of both forms of this amino acid. Within

TABLE 7. FREE AND BOUND GABA IN BRAIN OF CONTROL MICE AND OF MICE TREATED WITH GAH (160 mg/kg) AND SACRIFICED 6·5 hr AFTER TREATMENT

Subgroup	Free GABA	Bound GABA	Total GABA	Free GABA / Bound GABA
Control	23·6 ± 3·07 (6)	20·5 ± 2·13 (6)	42·0 ± 5·51 (6)	1·15
Treated	63·9 ± 5·69 (6)	43·7 ± 5·28 (6)	118·5 ± 12·7 (6)	1·46

Statistical significance of results according to t test:

Free GABA	P	Bound GABA	P	Total GABA	P
23·6–63·9	<0·001	20·5–43·7	<0·01	42·0–118·5	<0·001

TABLE 8. EFFECT OF PREVIOUS TREATMENT WITH GAH ON CONVULSIONS INDUCED BY TSC, MS, INSULIN AND METRAZOL

Group*	Treatment	Time to maximal seizures	No. of dead mice / No. of treated mice
I†	TSC	55–107 min	8/9
	GAH + TSC	69–97 min	6/9
II‡	TSC	27–73 min	8/8
	GAH + TSC	42–125 min	8/8
III	TSC	78–92 min	4/6
	GAH + TSC	64–108 min	7/7
IV	MS	10–22 hr	2/9
	GAH + MS	14–22 hr	4/9
V	Insulin	48–111 min	5/9
	GAH + insulin	34–69 min	7/9
VI	Metrazol	1–12 min	4/5
	GAH + Metrazol	2–18 min	4/5

* Groups as indicated in Table 1.

† Other experiments in which mice were injected with TSC 0·5–0·75 hr after GAH administration showed similar results.

‡ GAH administration at a dose of 40 mg/kg showed no anticonvulsant effect against TSC.

experimental error, the sum of free and bound GABA equalled the quantity of total GABA in control and treated mice. However, the free/bound GABA ratio was higher in treated than in control mice.

Convulsant action of TSC, MS, Metrazol, and insulin in GAH-treated mice

As can be seen in Table 8, the previous administration of GAH, including the daily treatment for 5 days, failed to protect mice against convulsions induced by any of the

drugs employed, at the times and doses indicated in Table 1. However, a group of mice treated with a low dose of GAH (20 mg/kg, Group II) showed a significant delay in the occurrence of TSC-induced fatal seizures ($P < 0{\cdot}01$).

GAD and ABAT activities in brain of mice treated with convulsant doses of GAH and AOA

The fact that some animals treated daily with GAH (160 mg/kg) for 5–7 days died in convulsions and that high single doses of GAH induced convulsions,[1] led us to a further study. Mice were injected with 2 g GAH/kg and the brain GAD and ABAT activities were measured at various times between GAH administration and the onset of the convulsive state. The results in Fig. 1 show that GAD activity is progressively inhibited with time, the maximal inhibition percentage corresponding to the

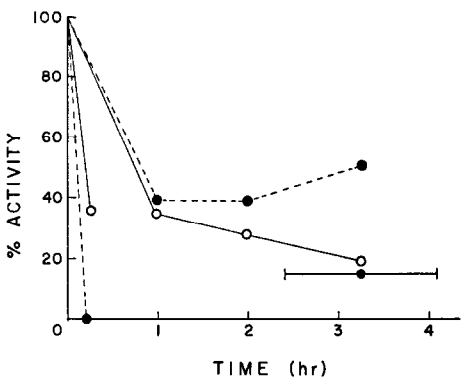

FIG. 1. Changes with time of GAD (○——○) and ABAT (●——●) activities after a single convulsant dose of AOA (400 mg/kg, data shown about 14 min) or GAH (2 g/kg, all other data). Each point represents the mean value of at least four determinations. The horizontal bracket indicates the death time in convulsions ± the standard deviation after GAH treatment (17 animals). All animals treated with AOA died in convulsions within 10–20 min (14 animals).

moment of convulsion; the maximal inhibition was partially reversed by the addition of PALP to the incubation mixture (Table 9). GABA levels at this time were greatly increased (Table 9). On the other hand, ABAT maximal inhibition was obtained 1 hr after GAH treatment (Fig. 1).

Other groups of mice were injected with convulsant doses of AOA (400 mg/kg).[19] In these experiments the brain GABA concentration and GAD and ABAT activities were determined at the moment of convulsion, since the animals died within the first 20 min after AOA administration. Both enzymes were notably affected in these conditions, especially ABAT, which was totally inhibited; GABA levels were increased by 40 per cent (Table 9 and Fig. 1).

GAD activity in brain of mice protected by AOA against convulsions

In these experiments, AOA at the anticonvulsant dose of 50 mg/kg[5] was tested against the convulsant action of the simultaneous administration of GAH and PALP; the latter substances notably inhibit GAD activity.[20] GABA concentration and GAD

TABLE 9. GABA CONCENTRATION AND GAD AND ABAT ACTIVITIES IN BRAIN OF CONTROL MICE AND OF MICE SACRIFICED AT THE MOMENT OF CONVULSIONS AFTER TREATMENT WITH CONVULSANT DOSES OF GAH AND AOA*

Treatment	GABA†	GAD‡		ABAT§
		Without PALP	With PALP	
Control	32·2 ± 1·54 (4)	114·5 ± 6·47 (4)	230·3 ± 5·31 (3)	2·67 ± 0·27 (5)
GAH (2 g/kg)	183·7 ± 10·8 (4)	20·6 ± 1·42 (4)	90·0 ± 6·00 (3)	1·32 ± 0·12 (6)
Control	24·0 ± 0·84 (5)	117·0 ± 3·79 (4)	232·6 ± 12·0 (3)	3·45 ± 0·27 (5)
AOA (400 mg/kg)	33·8 ± 0·96 (7)	42·6 ± 4·97 (4)	181·2 ± 9·20 (4)	0 (6)

* See Fig. 1 for time to convulsions after treatment. The figures are means ± standard error of the mean. Number of determinations in parentheses.

† mg/100 g wet tissue.

‡ μl CO_2/hr. Final concentrations in the incubation mixture: 0·05 M phosphate buffer, pH 6·3; 0·033 M glutamic acid neutralized to pH 6·3 with NaOH; 240 mg of cerebral tissue homogenized in double-distilled water (1:4 w/v); when PALP was added, its final concentration was $1{\cdot}26 \times 10^{-4}$ M.

§ Micromoles glutamic acid produced by 100 mg wet tissue in 1-hr incubation at pH 8·2.

Statistical significance of results according to *t* test:

GABA	P	GAD	P	ABAT	P
32·2–183·7	<0·001	114·5–20·6	<0·001	2·67–1·32	<0·001
24·0–33·8	<0·001	230·3–90·0	<0·001		
		117·1–42·6	<0·001		
		232·6–181·2	<0·02		

TABLE 10. ANTICONVULSANT EFFECT OF AOA (50 mg/kg) AGAINST CONVULSIONS INDUCED BY GAH (80 mg/kg) PLUS PALP (50 mg/kg) TREATMENT, AND GABA CONCENTRATION IN BRAIN OF CONTROL, PROTECTED, AND NONPROTECTED MICE

Treatment	Mean time to death in convulsions	No. of dead mice / No. of treated mice	GABA*
Control			27·8 ± 2·60 (3)
GAH + PALP	38·8 ± 2·99 min†	9/9	10·6 ± 0·25 (3)
AOA + GAH + PALP‡	§	0/12§	183·1 ± 7·80 (6)

* mg/100 g wet tissue. Mean ± standard error of the mean. Number of determinations in parentheses.

† Mean ± standard deviation.

‡ AOA was injected 90 min before the simultaneous administration of GAH and PALP.

§ Maximum time studied: 100 min.

Statistical significance of results according to *t* test:

GABA	P
27·8–10·6	<0·01
27·8–183·1	<0·001

activity in the brain of these animals were measured. It was found that AOA protected the mice against this type of convulsion when GABA concentration was six times increased (Table 10), but GAD activity was found equally diminished in both the protected and the unprotected animals, whereas the administration of AOA alone did not affect it (Fig. 2).

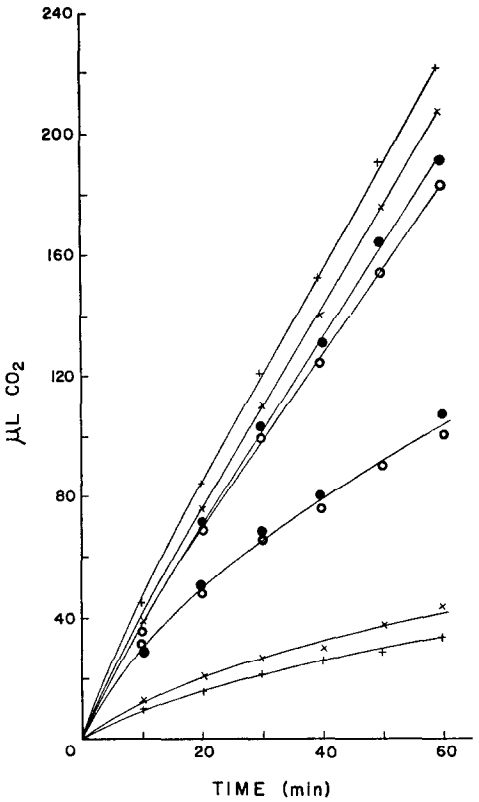

FIG. 2. GAD activity in brain of control mice (○) and of mice treated with AOA (●), GAH plus PALP (×), and AOA plus GAH plus PALP (+), in absence (lower curves) and in presence (upper curves) of PALP in the incubation mixture. For doses, incubating conditions, and other data, see Table 9.

DISCUSSION

Free amino acids in brain

The changes induced by TSC, MS, and insulin in general agree with those reported by Killam *et al.*,[21,22] Peters and Tower,[23] Cravioto *et al.*,[24] Dawson,[25] and Massieu *et al.*[18] However, the increase of glutamine concentration and the decrease of aspartic acid concentration produced by TSC administration have not been previously reported; the rise in alanine levels had been found by Dante Roa *et al.*[6] in cerebral tissue of TSC-treated cats.

The fact that TSC antagonized some of the effects of GAH, such as the influence of the latter drug on glutamine and GABA levels, could indicate that both compounds act in the same metabolic locus. The additive action of the two drugs in increasing alanine levels may be equally explained.

The remarkably diminished concentration of glutamine and glutamic acid found in the brains of MS-treated mice is in agreement with the data from brain slices reported by Tower.[26] In this respect, Speck[27] and Gershenovich *et al.*[28] have reported an inhibitory action of MS on glutamine synthetase activity. This result can be invoked to explain the effect *in vivo* of this antimetabolite on the glutamine level of brain. However, it is difficult to offer an explanation for the effects of MS in lowering the brain concentrations of GABA and of glutamic and aspartic acids.

In GAH-treated mice the effect of MS on glutamine and glutamic acid levels was practically the same as that observed after the administration of MS alone, which is not surprising in view of the lack of effect of GAH alone on the levels of such amino acids 24 hr after GAH administration. However, in the case of aspartic acid and GABA, it is difficult to explain the results obtained; regarding the former amino acid, GAH apparently blocked the effect of MS, whereas the level of the latter was higher than that observed with GAH alone (Table 4).

During insulin hypoglycemia, free glutamic acid is utilized as oxidizable substrate;[18,24,25,29] thus it could be expected that its oxidation by the Krebs cycle would be diminished if the pathways required for its introduction into the cycle were blocked. Since GAD and ABAT activities are diminished after GAH administration,[2] it can be assumed that the "GABA pathway" is partially blocked. In GAH-treated animals given insulin, a 10 per cent decrease of the glutamic acid level ($P < 0{\cdot}05$) was obtained, in comparison with animals treated only with GAH; in mice treated with insulin alone, the decrease of glutamic acid concentration, in comparison with control values, was 20 per cent ($P < 0{\cdot}01$) (Table 5). This observation could mean that under such a blockade of the GABA pathway there is a significant interference with the utilization of glutamic acid in brain. These data can be interpreted as supporting the idea that such a pathway is quantitatively important regarding the oxidative metabolism of glutamic acid, as some authors have claimed.[12]

Effect of GAH on free and bound GABA

The fact that both free and bound GABA concentrations are increased by GAH treatment and that this drug does not act as an anticonvulsant suggests that the decrease of brain excitability is not specifically related to the level of either form of the amino acid.

Convulsant action in relation to GAD and ABAT activities

The failure of GAH to protect mice against convulsions induced by insulin hypoglycemia was expected, since these symptoms are rather secondary to the drastic alteration produced by the hormone on the energetic metabolism in brain tissue. As discussed above, GAH pretreatment increased the action of insulin by interfering with the utilization of glutamic acid as oxidizable substrate.

The lack of anticonvulsant effect of GAH against TSC agrees with the findings of Baxter and Roberts[7] with hydroxylamine. This drug also augments GABA levels by inhibition of ABAT activity *in vivo*[30] and does not protect against TSC convulsions. However, AOA, another carbonyl-trapping agent which also increases GABA level,[19] exhibits a powerful anticonvulsant effect against TSC.[5] Moreover, hydroxylamine and AOA show anticonvulsant effects against Metrazol,[4,6] and AOA is also effective against MS convulsions,[5] whereas GAH is not.

In order to explain these differences between GAH action and AOA or hydroxylamine effects, it was thought that the reported inhibitory effect *in vivo* of GAH on GAD activity[2,20] could be an important one, since neither hydroxylamine nor AOA shows it at anticonvulsant doses.[30] Furthermore, relatively low doses of GAH are convulsant when PALP is simultaneously administered, at a time when GABA concentration and GAD activity are diminished.[20] The results obtained with convulsant doses of GAH or AOA, which show inhibition on GAD activity at the onset of convulsions, in spite of the simultaneous increase in GABA levels (Fig. 1 and Table 9), indicate that the inhibition of GAD activity is an important factor in the production of certain types of convulsions, the inhibition of ABAT activity accounting for the increase in GABA levels. The fact that 2,4-diaminobutyric acid resembles convulsant doses of GAH or AOA in relation to its convulsant action and its effect on GABA levels and GAD activity at the onset of convulsions,[31] and the fact that the seizures induced by hydroxamic anthranilic acid occur at the moment of maximal inhibition of GAD activity,[32] seem to support such a hypothesis.

In addition to GAD inhibition, the decrease of ABAT activity could be important in the production of convulsions. Figure 1 shows that in AOA-treated animals convulsions occur at 64 per cent inhibition of GAD activity simultaneously with a 100 per cent inhibition of ABAT activity, whereas within the same range of inhibition of GAD activity and at a 60 per cent inhibition of ABAT, GAH-treated mice did not show convulsions. In this case convulsions occurred when GAD activity had been largely inhibited. Apparently, a critical alteration in the balance between the activities of these particular enzymes of GABA metabolism, which can be attained either by the inhibition of GAD alone[20] or by inhibition of both, is one important factor in the production of some types of convulsive states. This interpretation could explain the lack of convulsions in some reported cases in which GAD activity is about 60 per cent inhibited,[33] but no figures are reported on ABAT activity.

Having postulated that a large inhibition of GAD activity is an important factor involved in the production of convulsions, it would seem logical to postulate that substances with anticonvulsant action against agents that inhibit GAD activity would act by preventing this inhibition. This was tested by the simultaneous administration of GAH and PALP, which apparently act as convulsants primarily by inhibiting GAD activity,[20] and AOA as anticonvulsant (at a dose of 50 mg/kg). The results, however, showed that GAD activity was equally diminished in protected and nonprotected animals (Fig. 2) although a great increase of GABA levels was observed (Table 10).

Thus, on the basis of these and other available data obtained by analyzing total homogenates of brain tissue, it can be concluded that the *convulsant* effect of some substances could be due, at least in part, to the inhibition of GAD activity independently of the total brain GABA concentration, and that the *anticonvulsant* effect of AOA and possibly other substances[34] is due to a yet unknown mechanism, different from that involving GABA metabolism.

In order to accept or to discard this hypothesis, it is necessary to await further and more detailed work *in vivo* about the real GAD and ABAT activities and about the levels of several metabolites in certain brain regions and in some particular subcellular structures (like the nerve-ending particles) during convulsions. The possible influence of other factors on brain excitability, like the activity of other PALP-dependent enzymes, should also be considered.

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