Acknowledgements—This work was supported in part by grants from the National Institute of Neurological Diseasesand Blindness, B-1575-(C3), and the National Science Foundation, G-9867.

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Free amino acids in brain of mice treated with L-glutamic acid-γ-hydrazide

(*Received* 10 *July* 1962; *accepted* 20 *July* 1962)

DIFFERENT carbonyl trapping agents can elevate or depress the levels of γ-aminobutyric acid (GABA)* in the brain of adult rodents. Hydroxylamine and aminooxyacetic acid injected into rats increase GABA in the brain,[1, 2] whereas thiosemicarbazide lowers the levels of this amino acid and decreases GAD activity.[3] To the authors' knowledge, the effects of GAH on the free amino acid pattern of the brain have not been investigated, and it was considered of interest to carry out experiments in this direction. Theoretically, GAH might block PyP-dependent enzymes such as GABA-T and GAD it could be an antimetabolite of glutamic acid and glutamine, and it could induce changes in their cerebral concentrations or in those of their metabolically related amino acids.

Adult, unfasted mice (local strain), previously fed with a commercial diet (Purina Laboratory Chow), were used. Different groups of animals were injected intraperitoneally with GAH (hemihydrate, A grade; California Corp. for Biochemical Research; 160 mg/kg body weight). The animals were killed by decapitation after 3·5, 6·5 and 24 hr, respectively. The controls in each case were injected with saline solution. In the first experiments the maximum changes in the GABA and alanine levels were obtained more than 6·5 hr after the injection of the drug. Other groups of mice were injected with different doses of GAH (40, 80, 360, 720 and 1440 mg/kg, respectively) and sacrificed after 6·5 hr. After decapitation the brains 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 resulting suspensions, after treatment by the method of Awapara,[4] furnished extracts free of lipids and protein. These extracts were dried with the aid of an infrared lamp and the dried residues were dissolved in enough distilled water to give a 10- to 20-fold concentration The paper chromatographic methods used for the free amino acid analysis of the extracts have been described elsewhere.[5] The bidimensional descending technique was used (80% phenol and acetic acid : butanol : water, 1 : 4 : 1, by vol) for running duplicate series of chromatograms (Whatman no. 1 paper). The concentration of each amino acid in the chromatograms (glutamine included) was

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

measured by the colorimetric method of Naftalin[6] in a Beckman B spectrophotometer, at 570 mμ. All the steps were carried out simultaneously for the brains from GAH-treated and control mice. GAH appears in the chromatograms at a definite location distant from that of the analyzed amino acids.

Several experiments on the effects of GAH *in vitro* on the GABA-T and GAD activities as well as on the glutamic-oxalacetic and glutamic-pyruvic transaminase activities were carried out on brain homogenates of mice. GABA-T activity was estimated by an adaptation of the procedures of Bessman *et al.*[7] and Baxter and Roberts[8] to brain homogenates. GAD activity was evaluated by measuring the increase in GABA according to the conditions established by Roberts and Frankel[9] and Rindi *et al.*[10] with slight modifications.[5] Glutamic-oxalacetic and glutamic-pyruvic transaminase activities were evaluated by the procedure of Awapara and Seale.[11]

Mice injected with doses of GAH ranging between 40 and 360 mg/kg showed symptoms of depression. Higher doses, moreover (720 and 1440 mg/kg), induced convulsions and hesitant walking. Some of the animals remained in this state for several hours and eventually died.

The results of the amino acid analysis are summarized in Tables 1 and 2, which show a striking elevation of the concentration of cerebral GABA and alanine, as compared with that found in the

TABLE 1. FREE AMINO ACID CONTENT OF BRAINS FROM GAH-TREATED (160 MG/KG) AND CONTROL MICE*

Group	After HGT injection (hr)	Aspartic acid	Glutamic acid	GABA	Glutamine	Alanine
		(mg the amino acid/100 g wet tissue)				
(1) Control (4)	3·5	52·8±4·51	189·7± 3·98	25·7±0·73	52·8±2·61	1·0±0·08
Treated (4)		53·8±3·17	188·1±11·57	81·2±7·11	33·6±5·15	9·6±0·95
(2) Control (27)	6·5	50·4±1·43	186·5± 2·41	25·1±0·74	54·4±1·64	1·4±0·11
Treated (8)		51·3±1·60	179·5± 3·41	107·9±4·81	33·6±0·93	12·1±1·05
(3) Control (7)	24	49·4±3·51	194·4±11·10	20·0±1·43	56·3±2·20	1·1±0·19
Treated (7)		57·2±3·27	193·3± 7·84	79·3±5·85	71·1±2·97	6·8±0·98

* Values are means ±s.e.m. Number of animals is shown in parentheses.

Statistical significance of results according to *t*-test.

GABA	*P*	Glutamine	*P*	Alanine	*P*
25·7– 81·2	<0·001	52·8–33·6	<0·02	1·0– 9·6	<0·001
25·1–107·9	<0·001	54·4–33·6	<0·001	1·4–12·1	<0·001
20·0– 79·3	<0·001	56·3–71·1	<0·01	1·1– 6·8	<0·001

brains of control animals. The maximum increase was observed in the brains of mice injected with 360 mg/kg in which the levels of GABA and alanine were 6 and 10 times higher than those found in controls, respectively. The level of aspartic acid was practically not altered by GAH-treatment; in the case of glutamic acid, doses higher than 360 mg/kg induced a significant decrease of its cerebral concentration. Increased GABA and alanine levels persisted after 24 hr (Table 1). Brain glutamine concentration after GAH-treatment diminished significantly within a period of 6·5 hr.

TABLE 2. LEVELS OF SOME AMINO ACIDS OF BRAINS FROM CONTROL MICE AND MICE TREATED WITH DIFFERENT DOSES OF GAH (6·5 HR AFTER INJECTION)*

Group	Number of animals	Aspartic acid	Glutamic acid	GABA	Glutamine	Alanine
		(mg amino acid/100 g wet tissue)				
Control	27	50·4±1·43	186·5± 2·41	25·1±0·74	54·4±1·64	1·4±0·11
Treated with GAH:						
Subgroup dose (mg/kg)						
A 40	8	50·9±3·23	179·8± 7·80	43·2±4·15	40·4±2·38	3·1±0·29
B 80	8	51·4±3·68	196·5± 6·18	64·1±5·60	49·0±4·70	4·2±0·61
C 160	8	51·3±1·60	179·5± 3·41	107·9±4·81	33·6±0·93	12·1±1·05
D 360	4	51·6±1·71	191·0±11·05	154·2±6·42	32·1±3·27	15·2±0·67
E 720	4	53·0±3·11	148·0± 5·80	126·9±5·29	35·7±5·08	12·9±0·62
F 1440	4	51·6±5·57	150·8± 1·74	107·6±3·36	38·1±2·17	11·3±0·68

* Values are means ±s.e.m.

Statistical significance of results according to *t*-test.

GABA	*P*	Glutamine	*P*	Alanine	*P*	Glutamic acid	*P*
50·4 – 43·2, 64·1, 107·9, 154·2, 126·8, 107·6	<0·001	54·4 – 40·4	<0·001	1·4 – 3·1	<0·001	186·5 – 148·1, 150·8	<0·001
		49·0	<0·5	4·2			
		33·6	<0·001	12·1			
		32·1	<0·001	15·2			
		35·7	<0·001	12·9			
		38·1	<0·01	11·3			

Preliminary experiments *in vitro* with brain homogenates of mice showed that GAH ($3·2 \times 10^{-3}$ M) inhibits the activity of GABA-T (40 per cent) and GAD (94 per cent); such inhibitions were partially reversed by addition of PyP to the homogenates. Glutamic-oxalacetic and glutamic-pyruvic transaminase activities were not altered by the same concentration of GAH.

The changes of the free amino acid pattern of the brain after GAH administration could be attributed chiefly to its inhibitory action *in vivo* on the GABA-T activity. It should be considered that the effects of some carbonyl trapping agents *in vivo* can be different from their effects *in vitro*. Baxter and Roberts[12] have found that hydroxylamine and aminooxyacetic acid inhibit the activity of GABA-T and GAD *in vitro* whereas *in vivo* these compounds inhibit only the former activity. The effects of GAH on the enzymatic activities *in vivo* mentioned above are currently being studied.

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Acknowledgements—The authors wish to thank Dr. José Laguna and Dr. Barbarin Arreguín for their cooperation and assistance in the preparation of this paper.

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Effect of catecholamines and their chloro-analogs on the *in vitro* release of histamine from cells of rat peritoneal fluid

(*Received* 30 *July* 1962; *accepted* 1 *August* 1962)

THE HISTAMINE-RELEASING activity of dichloroisoproterenol (DCI), a powerful inhibitor of the effects of epinephrine on β-receptors,[1, 2] as well as that of dichloroepinephrine and dichloroarterenol, has not been previously reported. Epinephrine has been studied in this regard, but conflicting results about its activity have been presented by Koch and Szerb[3] who found it active in the perfused rat lung whereas Mongar and Whelan,[4] could not demonstrate its activity on a variety of rat tissues *in vitro*. The results presented below indicate that dichloroepinephrine, dichloroarterenol and DCI are comparatively potent releasers of histamine from cells of rat peritoneal fluid. In contrast, the corresponding catecholamines were found to be devoid of this activity.

METHODS

Adult Wistar rats were exsanguinated and their peritoneal fluid cells collected by washing the peritoneal cavity with Krebs–Ringer phosphate buffer, pH 7·3, containing 0·1% glucose. These cells contain histamine which is predominantly bound to the mast cell fraction of the cellular population. After centrifugation the cells were resuspended in buffer and representative samples placed in the incubation flasks. After the addition of the amines, the flasks were kept at 37 °C for 15 min with continuous, gentle agitation. Blanks contained cells in buffer only. After incubation the cell suspensions were centrifuged and washed twice with fresh buffer. Histamine was estimated by bioassay on the guinea-pig ileum. Since all the amines tested inhibited the response of this preparation to histamine, residual rather than liberated histamine was measured. For this the washed cells were heated to boiling with 0·1 N HCl for 5 min to liberate residual bound histamine. Blanks were similarly extracted, and their residual histamine content, to which a value of 100 per cent was assigned, furnished the basis of reference for the estimation of the release occurring in the treated samples. The spontaneous release occurring during incubation of the blanks was always less than 10 per cent.

Drugs. L-Adrenaline, British Drug Houses; dichloroepinephrine (DL-β-hydroxy-N-methyl-3,4-dichlorophenylethylamine), dichloroarterenol (DL-β-hydroxy-3,4-dichlorophenylethylamine), and dichloroisoproterenol (DL-β-hydroxy-N-isopropyl-3,4-dichlorophenylethylamine), Lilly Research Laboratories; DL-arterenol and DL-isoproterenol, Sterling-Winthrop Laboratories. With the exception of adrenaline all drugs were in the form of the hydrochlorides.

The results shown in Table 1 indicate that epinephrine, arterenol ,and isoproterenol were unable to release significant amounts of histamine when tested at 3- or 10-mM levels; in contrast, their chloro-analogues were highly effective at 3 Mm, and practically ineffective at 1-mM concentrations. Pretreatment with iproniazid *in vivo* (50 mg/kg i.v. 2 hr before removal of the peritoneal cells), or *in vitro* (preincubation with 3 mM iproniazid), did not alter these results, indicating that intracellular destruction of the catecholamines by the amine oxidase, said to exist in mast cells,[5] could not be the reason for their ineffectiveness. Results similar to those shown in Table 1 were obtained when the *in vitro* effects of DCI and isoproterenol on the morphology of mast cells of rat mesentery were