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SHORT COMMUNICATIONS

Protection against hydrazine toxicity by α-ketoglutarate and oxalacetate: Enhancement of arginine protection*

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IN PREVIOUS communications we have reported that significant protection against hydrazine toxicity in mice was achieved by pretreatment of mice with arginine[1] and that the degree of protection was enhanced if the mixture also contained glutamic acid and alanine.[2] It was thought possible that the hydrazine might exert its toxic action, at least in part, by interference with the function of the tricarboxylic acid cycle in liver and/or brain and that the glutamic acid and alanine might have a protective action because of conversion to α-ketoglutarate and pyruvate. The present experiments were performed in order to determine whether the possible increase in tricarboxylic acid cycle intermediates produced by administration of α-ketoglutarate, pyruvate, and oxalacetate alone or in combination with each other would protect mice against acute hydrazine toxicity.

METHODS

Swiss mice of both sexes of an inbred Swiss stock, weighing approximately 25 g, were used in all experiments. The animals were starved for approximately 18 hr prior to a single i.p. injection of about 0·1 ml of a freshly prepared hydrazine solution (pH 7·0). The injection of hydrazine was preceded by 30 min by the injection of physiological saline (0·1–0·4 ml) or by a similar volume of neutral test solution. After the initiation of the experiments the animals were observed continuously for a 3-hr period and at 0·5-hr intervals for an additional 3 to 4 hr. The final observations were made 24 hr after the injection of hydrazine. The keto acids and the the L-isomers of arginine and glutamic acid employed in this study were of the highest degree of purity commercially available.

RESULTS AND DISCUSSION

Several experiments showed that when mice were pretreated with 4 mmoles oxalacetate or α-ketoglutarate/kg and then given 3 mmoles or less of hydrazine/kg significant protection was given by both compounds. Under the same conditions pyruvate gave no protection whatsoever. Typical results are shown in experiment 1, Table 1. When larger doses of hydrazine were employed, the above amounts of oxalacetate no longer were effective, but α-ketoglutarate still showed a protective action (Expt. 2, see groups (gr.) 5–7; Expt. 3, see gr. 14–17). No protection was given in similar experiments by succinate, fumarate, or malate; citrate was toxic at this level.

In experiment 2 isomolar amounts of α-ketoglutarate (gr.7) and L-arginine (gr. 8) gave the same degree of protection. However, mixtures of oxalacetate and arginine (gr. 9), α-ketoglutarate and arginine (gr. 12), and oxalacetate and α-ketoglutarate (gr. 10) gave better protection than any of the substances given alone. The degree of protection given by the latter two mixtures or by a combination of arginine, α-ketoglutarate, and oxalacetate (gr. 13), was at least as great as that afforded by the best protective mixture previously obtained (arginine, glutamate, and alanine; gr. 11).[2] Pyruvate gave no protection by itself (expt. 1, gr. 4; expt. 3, gr. 16) nor did it enhance the protective action of α-ketoglutarate (expt. 3, gr. 17 and 18). However, there was decreased lethality in the animals (gr. 19) receiving both oxalacetate and pyruvate, although neither substance alone was effective. No deaths or seizures were observed in the animals which received a mixture of arginine, α-ketoglutarate, and oxalacetate (expt. 2, gr. 13) and the animals in this group appeared to be in the best condition of any of the experimental groups at the 24-hr period.

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TABLE 1. PROTECTION OF MICE AGAINST HYDRAZINE TOXICITY

Expt. no.	No. of mice per group	Group	Compounds	Cumulative deaths (%)		
				30 min	60 min	24 hr
1*	10	1	Saline	20	50	60
		2	Oxalacetate	0	0	10
		3	α-Ketoglutarate	10	10	20
		4	Pyruvate	20	60	60
2†	12	5	Saline	41·7	66·7	75
		6	Oxalacetate	50	75	75
		7	α-Ketoglutarate	8·3	33·3	50
		8	Arginine	8·3	50	50
		9	Oxalacetate + arginine	8·3	8·3	33·3
		10	α-Ketoglutarate + oxalacetate	0	8·3	8·3
		11	Arginine + glutamate + alanine	8·3	8·3	8·3
		12	Arginine + α-ketoglutarate	0	0	0
		13‡	Arginine + α-ketoglutarate + oxalacetate	0	0	0
3§	11	14	Saline	36·3	72·7	100
		15	Oxalacetate	81·8	100	100
		16	Pyruvate	72·7	90·9	90·9
		17	α-Ketoglutarate	36·3	45·4	45·4
		18	α-Ketoglutarate + pyruvate	18·2	45·4	54·5
		19	Oxalacetate + pyruvate	27·2	36·3	36·3
		20	Oxalacetate + pyruvate + α-ketoglutarate	18·2	18·2	18·2
		21	Oxalacetate + α-ketoglutarate	0	0	0

* Hydrazine (3 mmoles/kg); 4 mmoles of each test substance/kg.
† Hydrazine (3·2 mmoles/kg); 4 mmoles of each test substance/kg.
‡ All the animals in this group appeared to be in excellent condition at the 24-hr period.
§ Hydrazine (3·2 mmoles/kg); 4 mmoles of each test substance/kg.

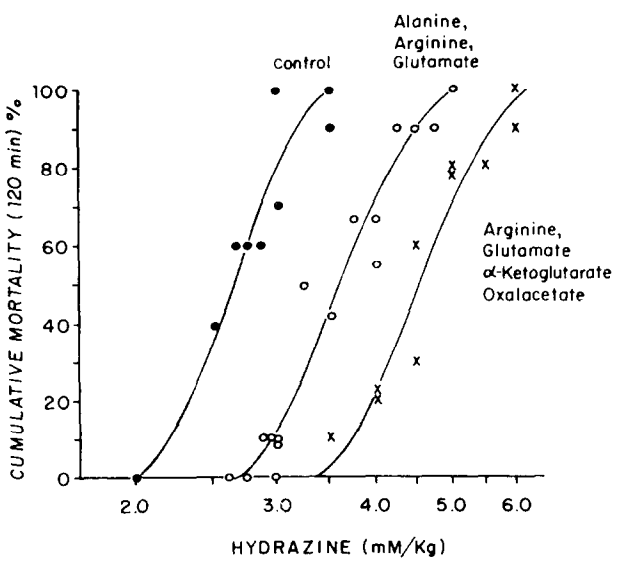

FIG. 1. Comparison of dose-mortality data in 120 min for mice pretreated with saline; a mixture of L-arginine, L-glutamate, and L-alanine; or a mixture of L-arginine, L-glutamate, α-ketoglutarate, and oxalacetate. The dose of hydrazine is plotted on a logarithmic scale. Each test substance was given at 4 mmoles/kg.

Subsequent experiments were performed with various mixtures of the above keto acids with the amino acids found previously to be protective. It was established that an isomolar combination of arginine, glutamate, α-ketoglutarate, and oxalacetate gave considerably better protection against hydrazine toxicity than did the previously best combination, which contained arginine, glutamate, and alanine. The results of experiments performed at various levels of hydrazine are shown in Fig. 1. The LD_{50} values in mmoles hydrazine/kg estimated from the curves are as follows: saline controls, 2·65; arginine–glutamate–alanine, 3·58; arginine–glutamate–oxalacetate–α-ketoglutarate, 4·54.

These experiments suggest that two intimately related phases of metabolism may be directly or indirectly effected by hydrazine. The protective effects of arginine suggest that nitrogen metabolism may be disturbed in the intoxicated mice and that arginine may act by facilitating the conversion of ammonia to urea. The results with the keto acids indicate that the operation of the tricarboxylic acid cycle may be partially inhibited and that exogeneously administered oxalacetate and α-ketoglutarate may enhance the function of the cycle by increasing availability of limiting substrates. Since it is not possible to elucidate biochemical mechanisms at the cellular level only from toxicity studies in intact animals, biochemical investigations of the tissues of hydrazine-poisoned animals have been begun. Preliminary results show that the activity of the condensing enzyme (citrate synthesis) is unaffected in the livers of hydrazine-poisoned mice.

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Changes in pentobarbital distribution with time in neural and non-neural tissue of the cat brain*

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SEVERAL studies [1–5] have dealt with the penetration of barbiturates into the central nervous system and their regional distribution in the brain, but none has considered what types of cells the drugs go to. This paper will describe the concentration of phenobarbital in the medial geniculate bodies of cats after cortical lesions have caused neuron loss.[6,7]

METHODS

All cortical areas known as temporal, insular, AI, AII, and SII were removed unilaterally in cats,[6] and six weeks later they were given 39 mg pentobarbital/kg i.v. in 1–2 min. Their brains were exposed and then removed at various intervals after injection. Four cats were killed at 5 min, one at 10, four at 15, two at 20, and three at 30. The brains were chilled in ice-cold water, and the medial geniculates were stripped of their pia, removed, and weighed. Pentobarbital was measured by its u.v. extinction.[8] The tissue was homogenized with 15μg pentobarbital in 1 ml distilled H_2O as internal standard, the homogenizer was rinsed with 1 ml 1M NaH_2PO_4 and then 5 ml ethylene dichloride and the mixture put into a 25-ml separatory funnel. The phenobarbital was extracted into the ethylene dichloride and then into 1·5 ml 0·5 N NaOH. The funnels were shaken 10 min each time. The NaOH extract was

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