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ENHANCEMENT BY L-GLUTAMATE AND L-ALANINE OF ARGININE PROTECTION AGAINST HYDRAZINE TOXICITY*

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Abstract—Pretreatment of mice with intraperitoneally administered solutions of L-arginine or L-glutamate decreased significantly the incidence of seizures and the lethality of single doses of hydrazine. Also there was some protection against lethality and attenuation of speed of toxic action of hydrazine after pretreatment with L-alanine. Protection given by mixtures of any two of the above amino acids appeared to be superior to that given by one alone. A remarkable protective effect was given by a mixture of L-arginine, L-glutamate, and L-alanine (4 mmoles/kg each). The above mixture gave significant protection when injected 3 min after a dose of hydrazine which killed 100% of the controls, but not when given at later times.

UNTIL recently no protective measures were available against the acute toxic effects of hydrazine. In a previous communication it was shown that pretreatment of mice with arginine can afford significant protection against lethality and seizure incidence resulting from injection of hydrazine. The effect appeared to be specific for L-arginine, since it was found that D-arginine, N-acetyl-L-arginine, or L-arginine ethyl ester did not give protection.[1] In a continuation of the latter work all of the naturally-occurring amino acids have been tested, as well as many related substances. A number of the experiments that may give some information relevant to the mechanism of action of hydrazine will be reported subsequently. In this present report only those experiments will be mentioned which have led to a greater degree of protection against hydrazine than that given by L-arginine alone.

METHODS

Swiss mice of both sexes of an inbred Swiss stock, weighing approximately 25 g, were used in all experiments. Every experiment to be reported was performed with at least 10 animals in each experimental group. In many instances larger numbers of mice, up to 20 per group, were employed. 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, in most instances, 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 experiment 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.

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L-Isomers of the amino acids of the highest degree of purity commercially available were employed.

A number of experiments in which amino acids other than L-arginine were given alone and in various combinations prior to the injection of hydrazine showed that L-glutamic acid and L-alanine also had some protective action. The results of three typical experiments are shown in Table 1. The mortality in the groups receiving

TABLE 1. PROTECTION AGAINST HYDRAZINE TOXICITY BY L-ARGININE, L-ALANINE, AND L-GLUTAMIC ACID

Expt. no.	No. of mice per group	Amino acids	Cumulative deaths (%)		
			30 min	60 min	24 hr
1*	15	None	66·7	73·5	93·5
		Arginine	13·3	26·6	26·6
		Glutamic acid	20·0	46·6	46·6
		Alanine	46·6	53·3	60·0
		Arginine + glutamic acid	6·7	6·7	20·0
		Arginine + alanine	20·0	20·0	20·0
		Alanine + glutamic acid	6·7	13·3	13·3
2*	10	None	20	80	90
		Arginine	0	20	40
		Glutamic acid	0	10	20
		Alanine	0	60	90
		Arginine + glutamic acid	0	0	20
		Arginine + alanine	0	0	20
		Alanine + glutamic acid	0	10	10
		Arginine + glutamic acid + alanine	0	0	0
3†	12	None	91·8	91·8	91·8
		Arginine	50·0	66·7	83·5
		Glutamic acid	75·0	91·8	91·8
		Alanine	83·3	91·8	91·8
		Arginine + glutamic acid	16·7	33·3	33·3
		Arginine + alanine	25·0	41·7	41·7
		Alanine + glutamic acid	33·3	50·0	50·0

* Hydrazine (3 mmoles/kg); 4 mmoles of each amino acid/kg.
† Hydrazine (3·5 mmoles/kg); 3 mmoles of each amino acid/kg.

the individual amino acids did not exceed that of the controls at any of the nine time-intervals recorded in these experiments. In both groups pretreated with arginine or glutamate (4 mmoles/kg) at the lower dose of hydrazine of 3·0 mmoles/kg (experiments 1 and 2) there was a marked decrease in the lethality at all of the times recorded. In the mice receiving alanine there also was a decrease from the controls, in five out of the six recorded times, in the proportion of animals dying. In experiment 2, 80% of the control animals succumbed within 50 min after the injection of hydrazine; in the mice pretreated with alanine, 40% died within this period, and 80% mortality was achieved only at 95 min. In experiment 3, in which a higher dose of hydrazine (3·5 mmoles/kg) and smaller amounts of amino acids (3 mmoles/kg) were employed, the protective effect of arginine was still evident, but the results obtained with glutamate and alanine were not significantly different from the controls.

In all three experiments in Table 1, with the exception of the 30-min period in experiment 2 in which no deaths occurred, the protection was greater when a mixture

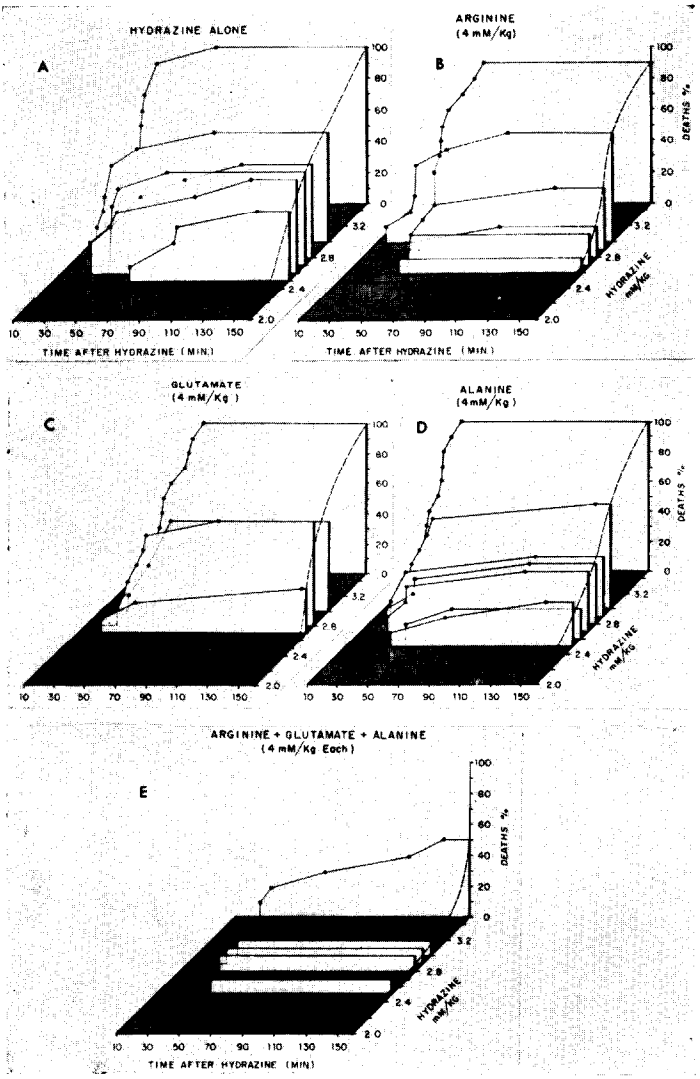

FIG. 1. A, Time course of deaths resulting from injection of various doses of hydrazine after pretreatment with saline; with L-arginine; B, with L-glutamate; C, with L-alanine; D, or a combination of the three amino acids, E.

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of glutamate and arginine was given than when either of these amino acids was given alone. Similarly, at seven of the nine times recorded, the mixtures containing alanine and glutamic acid or alanine and arginine, respectively, gave better protection than the individual amino acids. The most effective protection was obtained when all three amino acids (4 mmoles/kg) were given prior to the administration of hydrazine (3 mmoles/kg). None of the animals in the latter group died, and only one brief seizure was noted throughout the period of study.

The above observations were extended in an experiment performed at the same time with one large group of randomized mice (Fig. 1). Figure 1A shows a 3-dimensional plot of the cumulative mortality as a function of dose of hydrazine and time after intraperitoneal injection. The results in Fig. 1B show a similar experiment with the exception that the animals had received 4 mmoles of L-arginine i.p. per kg 30 min before the administration of hydrazine. The protective effect of arginine is particularly evident at the lower doses of hydrazine. Similar experiments with L-glutamate and L-alanine are shown in Figs. 1C and 1D respectively. The most potent protective effect was exerted by the mixture containing equimolar amounts of L-arginine, L-glutamic acid, and L-alanine (Fig. 1E). The results illustrate the remarkable protective effect when the above three substances are given together. Separate studies with the individual amino acids showed that increasing the amounts of any one of these amino acids beyond the 4 mmole/kg level did not enhance the protective action significantly.

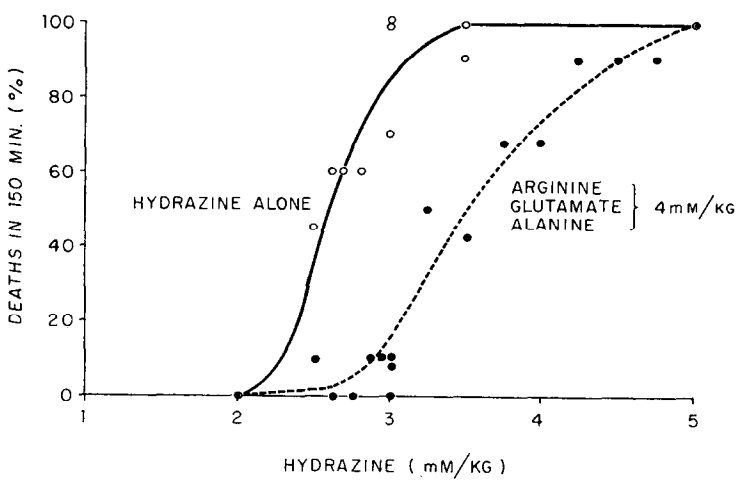

FIG. 2. Comparison of dose–mortality data for mice pretreated with saline or a mixture of L-arginine, L-glutamate, and L-alanine (4 mmoles/kg each).

Figure 2 shows the dose–mortality curves obtained from 11 groups of animals given hydrazine alone and from 17 groups pretreated with the mixture of amino acids prior to the injection of hydrazine. Each experimental point was obtained from an experiment utilizing 10–20 mice (average, 13). Of the many hundreds of experiments which we have performed with various substances, or combinations thereof, the mixture of L-arginine, L-glutamate, and L-alanine gave the greatest protective effect.

Table 2 shows the results obtained when different levels of the above mixture were employed. Essentially maximal protection was afforded when the mice were pretreated 30 min before injection of the hydrazine (3·5 mmoles/kg) with a mixture containing 3 mmoles of each of the three amino acids per kg. No deaths and no seizures were seen in the group receiving 5 mmoles. However, in general, there appeared to be little difference between the group receiving 3 mmoles/kg and those receiving higher levels of the mixture.

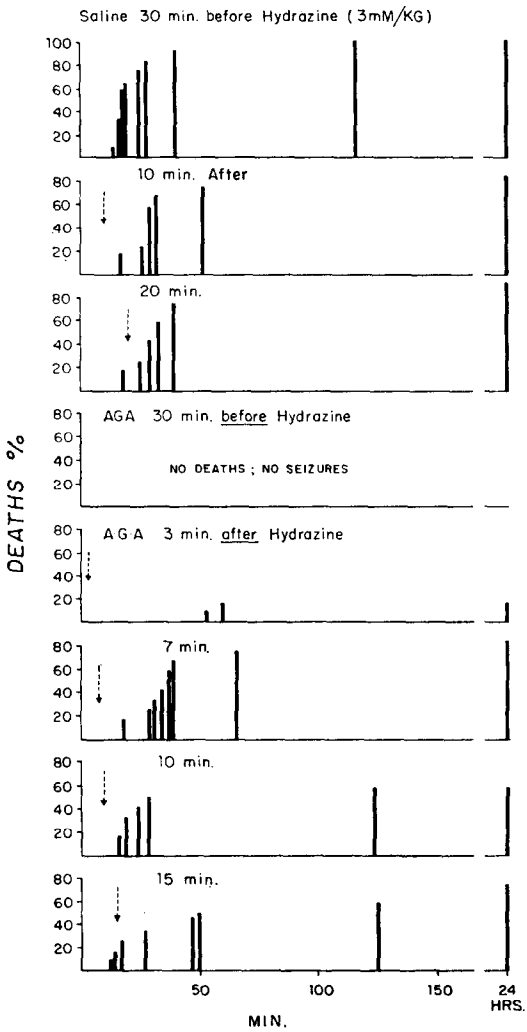

FIG. 3. Influence of time of administration of the amino acid mixture on the degree of protection.

It was desirable to determine whether post-treatment of the animals with the maximally protective mixture could afford protection against hydrazine. Figure 3 shows results of an experiment to test this possibility. The upper three charts in this figure demonstrate little difference in mortality when physiological saline (0·4 ml) was given

30 min before the test dose of hydrazine or 10 or 20 min after it. When the arginine–glutamate–alanine mixture (AGA, 4 mmoles/kg) was given 30 min before the hydrazine, no deaths and no seizures were observed. When this mixture was given 3 min after the hydrazine there was still protection against the lethal effects, and few seizures were observed. However, if the administration of the protective solution was delayed for as long as 7 min, the protection appeared to be greatly decreased.

TABLE 2. INFLUENCE OF EIGHT LEVELS OF PROTECTIVE AMINO MIXTURE ON EXTENT OF PROTECTION AGAINST HYDRAZINE*

Amino acid mixture (mmoles/kg)	Cumulative deaths (%)			
	30 min	60 min	90 min	24 hr
None	50	90	90	100
1†	40	70	70	90
2	0	50	60	70
3	10	10	10	20
4	0	10	10	10
5	0	0	0	0
6	10	10	10	20
7	0	10	10	30
8	10	10	10	20

* Hydrazine (3·5 mmoles/kg); 10 animals per group.
† Amount of each amino acid in a mixture containing L-arginine, L-alanine, and L-glutamic acid.

DISCUSSION

The experiments reported herein are merely typical studies of many more which have given essentially the same results. It is recommended that further pharmacological and toxicological studies be made of the maximally protective mixture of amino acids to ascertain whether this could lead to truly effective prophylactic or therapeutic measures in problems relating to hydrazine toxicity.

Subsequent reports will deal with studies of the mechanism of toxic action of hydrazine and of the protective action of L-arginine, L-glutamate, and L-alanine.

REFERENCE

1. E. ROBERTS, D. G. SIMONSEN and ETHEL ROBERTS, *Biochem. Pharmacol.* **12**, 1445 (1963).