

Amino Acid Levels in Hydrazine-Treated Rats

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Received December 4, 1967

Several studies have indicated that hydrazine produces an amino acid imbalance in experimental animals. The early work of Lewis and Izume (1926) suggested a hydrazine block of amino acid utilization since it impaired the ability of glycine to elevate the blood sugar of hypoglycemic rabbits. More recently, Amenta and Johnston (1963) have investigated the ability of liver slices from hydrazine-treated rats to metabolize ^{14}C -labeled amino acids. The ability of liver slices from treated rats to convert glycine or alanine to CO_2 was impaired by 60–70%. In contrast, the incorporation of labeled amino acids into cellular protein was increased. According to the authors these data suggest a block in amino acid metabolism, but no block in the ability to incorporate amino acid into protein. A number of previous studies with *in vitro* systems have shown that hydrazine and a number of its derivatives inhibit the conversion of amino acids to keto acids. This inhibition could be reversed by the addition of pyridoxal phosphate to the reaction mixture (Killam and Bain, 1957; McCormick and Snell, 1961). Banks and Stein (1965) have also demonstrated that liver RNA and liver protein are concomitantly elevated in hydrazine-treated rats, thus the authors suggest an *in vivo* stimulation of liver protein synthesis by hydrazine. Simonsen and Roberts (1967) have published data on the influence of hydrazine on the distribution of free amino acids in mouse liver. Increased levels of alanine, glutamic and aspartic acids, ornithine, glycine, and citrulline were noted in livers of hydrazine-treated rats. The relationship of hydrazine-induced amino acid imbalance to the acute convulsive response to hydrazine is not apparent although elevated levels of γ -aminobutyric acid in the brains of animals treated with hydrazine have been reported by Maynert and Kaji (1962).

In the present study, plasma and tissue amino acid levels were investigated in hydrazine-treated rats. The effects of hydrazine on the absorption and distribution of a tyrosine load was determined. In addition, the relationship of pyridoxine to the amino acid imbalance produced by hydrazine was investigated.

METHODS

Tyrosine loading (100 mg/rat) was essentially the method described by Udenfriend and Cooper (1952). Chemical estimation of tyrosine in tissues was carried out by the method of Chirigos *et al.* (1960). In order to avoid the possibility of direct chemical interactions, hydrazine was given subcutaneously (s.c.) and tyrosine intraperitoneally

(i.p.). Food was withheld for 16–18 hours prior to treatment to ensure relatively uniform tissue levels of amino acids and to obviate any effect of hydrazine on the rate of their gastrointestinal absorption. Relatively large doses of hydrazine in saline were injected in order to study amino acid imbalance at hydrazine levels somewhat comparable to those producing an acutely toxic response in treated animals. Injections of equal volumes of saline were given to control animals.

Tyrosine determination. At sacrifice, blood was collected in a heparinized syringe by cardiac puncture. To remove as much blood as possible from the brain, 15–20 ml of heparinized saline was slowly perfused by cardiac puncture after clamping off the hepatic vein and adjacent blood vessels just below the heart. The brain was immediately removed and homogenized in 2 volumes of 0.2 *N* HCl, after which 1 volume of 24% trichloroacetic acid was added. This is essentially the method described by Chirigos *et al.* (1960). The plasma was separated from the blood and proteins were precipitated by the addition of 3 volumes of 8% trichloroacetic acid. After addition of trichloroacetic acid, both homogenate and plasma samples were centrifuged at 2000 rpm for 10–15 minutes. Two milliliters of deproteinized solution was utilized for each determination of tyrosine. Added hydrazine did not interfere with this determination.

Free amino acids. Tissues were homogenized in 80% ethanol to extract free amino acids (Ruisseau *et al.*, 1957). Homogenate and plasma were deproteinized with tungstic acid and centrifuged; total α -amino nitrogen was determined in tissue and plasma extracts by the ninhydrin method described by Fisher *et al.* (1963). Hydrazine, at the concentrations present in animal tissues after treatment, did not interfere with this determination.

RESULTS

Figure 1 shows the amount of tyrosine in the plasma and brain of four groups of rats over the 3-hour period following the indicated treatment. The plasma concentration of tyrosine-loaded rats shows a marked rise to double or triple control levels at 1 and 2 hours after dosing. There is a trend toward normal concentrations at 3 hours. At the same time, brain concentrations show a continuous but slower rate of increase, being approximately four times control levels 3 hours after the tyrosine injection. In sharp contrast to this were the plasma and brain concentrations of tyrosine-loaded, hydrazine-treated rats. Plasma concentrations were almost 60 times control at 3 hours, and brain levels had increased 5- to 6-fold above controls and were double the brain levels of nonhydrazine-treated, tyrosine-loaded animals. The injection of 15 mg/kg of pyridoxine into tyrosine-loaded, hydrazine-treated rats did not markedly alter the tyrosine distribution. Unusually high values were obtained in plasma and brain of hydrazine-treated rats which were not tyrosine loaded, the brain levels being comparable at 1, 2, and 3 hours with those found in the tyrosine-loaded animals. In plasma, the tyrosine level at 3 hours in rats receiving only hydrazine was approximately four times greater than in animals given a tyrosine load.

Since the analytical method was not entirely specific for tyrosine but also measured tyramine and perhaps some other aromatic phenols and amines, it seemed essential to study the effects of hydrazine on total amino acid levels measured by an independent method.

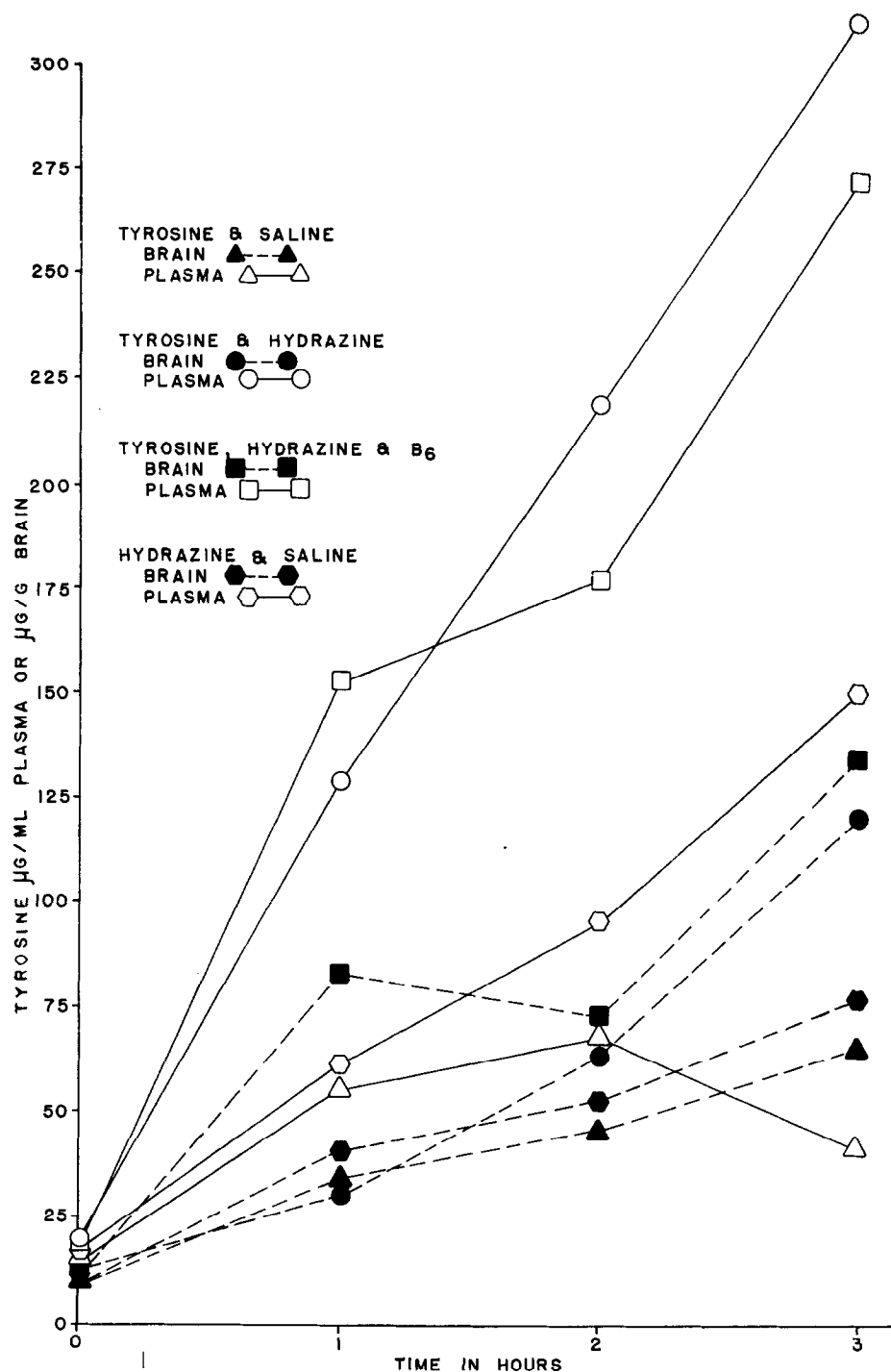


FIG. 1. Tyrosine levels in plasma and brain. Rats were dosed as follows: tyrosine, 100 mg/rat i.p.; hydrazine, 60 mg/kg s.c.; vitamin B₆ (pyridoxine HCl), 15 mg/kg i.p.

Total plasma α -amino acids as determined in normal, tyrosine-loaded and hydrazine-treated rats in several combinations are shown in Fig. 2. Tyrosine loading (i.p.) had no effect on total plasma amino acid levels. This is not totally unexpected since values for total free amino acids are in milligrams per 100 ml, whereas the tyrosine levels were previously measured in micrograms per milliliter. All rats receiving hydrazine had elevated serum α -amino acids when examined at 1, 2, or 3 hours after hydrazine injection. Pyridoxine (vitamin B₆) in the dose given had no effect on these abnormally high plasma amino acid levels in hydrazine-treated rats.

In an additional study, 8 hydrazine-treated animals received 500 mg of pyridoxine HCl(PY) per kilogram orally to see whether large doses of vitamin B₆ might reverse

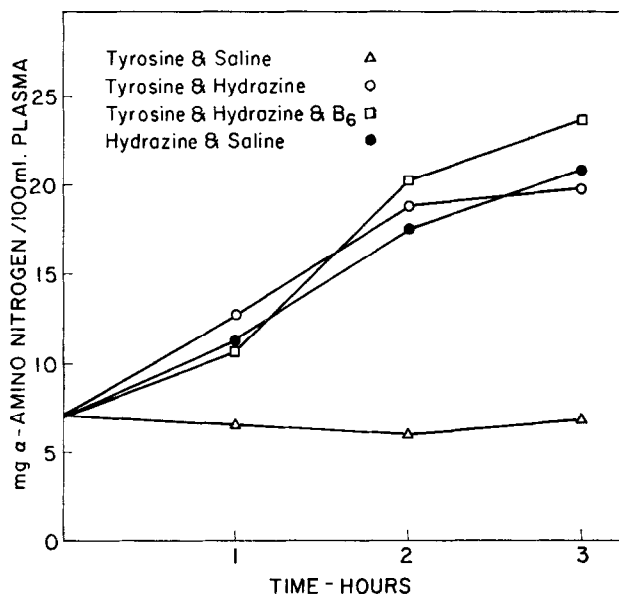


FIG. 2. α -Amino nitrogen levels in plasma. Rats were dosed as follows: tyrosine, 100 mg/rat i.p.; hydrazine, 60 mg/kg s.c.; vitamin B₆ (pyridoxine HCl), 15 mg/kg i.p.

this amino acid effect of hydrazine. No effect of vitamin B₆ was noted since plasma, liver, and brain amino acid levels were comparable to those in animals receiving hydrazine alone (Table 1).

In a further attempt to define a relationship between vitamin B₆ and amino acid levels in hydrazine-treated rats, animals were examined 18 hours after treatment. Plasma, liver, and brain amino acid levels at this time are also shown in Table 1. Hydrazine-treated (60 or 70 mg/kg) animals still had marked elevations of plasma amino acids 18 hours after treatment. The simultaneous injection of 15 mg of PY per kilogram had no effect on these elevated levels. Liver amino acid levels of hydrazine-treated rats were also elevated 18 hours after injection. Although brain levels of free amino acids in hydrazine-treated rats did not show the marked elevations present in serum and liver 3 hours and 18 hours after treatment, they were significantly higher than control values when the hydrazine dose was increased to 70 mg/kg.

TABLE 1
 α -AMINO NITROGEN CONCENTRATIONS AFTER HYDRAZINE INJECTION (MEAN \pm SE)

Treatment	Number of rats	Hours after injection	Plasma (mg/100 ml)	Liver (mg/gram)	Brain (mg/gram)
Saline	7	3	7.9 \pm 0.59	0.47 \pm 0.01	0.61 \pm 0.01
Hydrazine	7	3	23.8 \pm 1.7 ^a	0.96 \pm 0.13 ^a	0.61 \pm 0.03
Hydrazine + Pyridoxine HCl 500 mg/kg oral	8	3	21.4 \pm 1.7 ^a	0.85 \pm 0.06 ^a	0.66 \pm 0.06
Saline	6	18	6.6 \pm 0.43	0.45 \pm 0.02	0.57 \pm 0.01
Hydrazine	6	18	15.2 \pm 1.7 ^a	0.89 \pm 0.02 ^a	0.62 \pm 0.01
Hydrazine + Pyridoxine HCl 15 mg/kg s.c.	3	18	11.1 \pm 1.1 ^a	0.86 \pm 0.06 ^a	—
Hydrazine	8	18	13.2 \pm 1.2 ^a	0.78 \pm 0.02 ^a	0.67 \pm 0.01 ^a
Hydrazine + Pyridoxine HCl 15 mg/kg s.c.	9	18	12.2 \pm 0.7 ^a	0.82 \pm 0.01 ^a	0.68 \pm 0.03 ^a

^a Different from controls, $P < 0.01$.

DISCUSSION

Tyrosine loading of normal rats produced elevated plasma tyrosine levels which reached a peak at approximately 2 hours and then began to decline. Tyrosine levels in brain continued to increase over the subsequent 3-hour period. These findings are quite comparable to those reported by Chirigos *et al.* (1960) in tyrosine-loaded rats. In early studies tyrosine and hydrazine were both given intraperitoneally, and there was concern that perhaps the irritating effect of hydrazine had caused a more rapid uptake of tyrosine from the intraperitoneal cavity resulting in the marked increases in plasma and brain levels. For this reason, in the tyrosine-loading studies presented here, hydrazine was given subcutaneously and tyrosine intraperitoneally. Nevertheless, these marked plasma and brain elevations were found in all animals receiving hydrazine and tyrosine. The surprising finding was that hydrazine, without tyrosine loading, resulted in tyrosine plasma levels markedly higher than those found in the tyrosine-loaded rats. Brain levels of tyrosine in tyrosine-loaded and hydrazine-treated groups were quite comparable.

The rate of absorption of tyrosine is sufficiently slow that it had no effect on the level of total plasma amino acids in rats given an intraperitoneal dose of tyrosine. In all animals receiving hydrazine, however, plasma amino acid levels were markedly elevated within 1 hour after the injection of hydrazine. At 3 hours the plasma levels of treated rats were approximately three times control levels.

These findings may be related to the aminoaciduria previously reported in rats treated with *unsym*-dimethylhydrazine (Cornish and Barth, 1964). It is interesting that not only are plasma tyrosine and total amino acid levels elevated but tissue levels as well. Since these are 18-hour fasted rats, the free amino acids apparently arise from an endogenous source. A number of conditions that could account for these findings are an increased rate of protein degradation that cannot be compensated for by metabolism and excretion, or a continuing normal protein catabolism with a block in protein synthesis or a block in amino acid metabolism. The studies of Lewis and Izume (1926) and Amenta and Johnston (1963) suggest a block in amino acid metabolism. Likewise, the present findings of marked increases in plasma and liver amino acid levels in hydrazine-treated rats are consistent with a decreased ability of liver to metabolize amino acids by routes requiring pyridoxal phosphate as a coenzyme. If hydrazine reacts with pyridoxal or pyridoxal phosphate *in vivo* to produce an acute vitamin B₆ deficiency, one would anticipate a reversal of these effects in the presence of increased amounts of vitamin B₆. Although the ability of pyridoxal phosphate to reverse the *in vitro* inhibition of amino acid metabolism by hydrazine and its derivatives has been reported, the present studies utilizing a single dose of pyridoxine did not demonstrate such an *in vivo* effect. This may be partially due to problems of appropriate dosages of pyridoxine or to a continued hydrazone formation by hydrazine with newly formed pyridoxal phosphate, or it may be related to a more fundamental aspect of hydrazine toxicity that is not presently recognized. In the measurement of total free amino acids in brain, no marked elevations are noted in hydrazine-treated rats until the dosage was increased to 70 mg/kg. An increase was quite apparent, however, in the brain tyrosine levels of hydrazine-treated animals. In general, the elevated brain tyrosine values tend to parallel the plasma levels. It is interesting to note that

Simonsen and Roberts (1967) report that some, but not all, amino acids are elevated in the livers of hydrazine-treated rats. Tyrosine was found in the liver extracts of hydrazine-treated rats but was not seen in control samples, indicating that tyrosine was apparently elevated in these animals. This correlates with the present findings where tyrosine is markedly elevated in the plasma and the brain of hydrazine-treated animals. Although rather remarkable elevations of free amino acids exist, even 1 hour after hydrazine treatment, it is not yet possible to relate these findings to the early convulsive phase of hydrazine toxicity. However, such a pronounced effect on amino acid metabolism could conceivably play a role in liver degeneration which develops as a later phase of hydrazine toxicity.

SUMMARY

Tyrosine-loaded rats, injected with hydrazine, showed a marked elevation of plasma and brain tyrosine levels above that of a comparable tyrosine-loaded control group. An additional control group receiving only hydrazine had plasma and brain tyrosine levels greater than the tyrosine-loaded controls. The subsequent measurement of total α -amino nitrogen levels demonstrated that hydrazine injections, with or without tyrosine loading, resulted in approximately a 3-fold increase in plasma α -amino nitrogen levels when measured 3 hours after treatment. Eighteen hours after hydrazine injection plasma, brain, and liver α -amino acid levels were still above control values. Such findings are consistent with the known *in vitro* inhibition of amino acid metabolism by hydrazine, due to binding of the coenzyme pyridoxal phosphate.

ACKNOWLEDGMENTS

This work was sponsored by the Commission on Environmental Hygiene of the Armed Forces Epidemiological Board, and was supported in part by the Medical Research and Development Command, Office of the Surgeon General, Department of the Army of the United States.

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