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Biological action of 1,1-dimethylhydrazine*

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MANY papers have appeared in recent years concerning the toxicity and mechanism of action of hydrazine and 1,1-dimethylhydrazine (UDMH).[1-9] Such studies have been stimulated by the use of these compounds as rocket fuels. The acute effect of UDMH on experimental animals is primarily a central nervous system toxicity resulting in clonic–tonic convulsions and death.

A previous report[10] indicated that amino acid excretion in rats was markedly increased after the intraperitoneal injection of UDMH. The present study evaluates several other biochemical criteria which might serve as indexes of exposure if they correlate with UDMH toxicity. These include the measurement of plasma iron levels and serum, erythrocyte, and brain cholinesterase activities. The diuretic effect of UDMH and the effect of intraperitoneal injections of various glutamic acid metabolites as possible antagonists of UDMH toxicity were investigated. Since many of these metabolites are not able to penetrate the blood–brain barrier at appreciable rates, it seemed likely that the direct injection of these materials into the brain might provide further information on their relationship to UDMH toxicity.

MATERIALS AND METHODS

UDMH was dissolved in 27 mM sodium bicarbonate in 0·9% saline just prior to use.[9] Other injected materials were dissolved in 0·9% saline. Male albino rats of the Sprague-Dawley strain (Rawley Farms) weighing 225–325 *g* were used throughout the studies.

Effect of UDMH on serum iron levels. Rats were injected i.p. with 40 to 80 mg UDMH/kg and sacrificed 24 hr later. The unhemolyzed sera from these animals and from normal rats were analyzed for iron according to the method of Fischl.[11]

Effect of UDMH on tissue and blood cholinesterase. Rats were injected i.p. with 100 mg UDMH/kg, and 4 hr later, heparinized blood was collected from surviving animals by heart puncture. The brains were immediately removed, weighed, and frozen on dry ice. Each brain was homogenized in 24 volumes of cold distilled water and cholinesterase activity determined by a minor modification of the method of Frawley *et al.*[12]

Effect of glutamic acid and aminobutyric acid analogs on UDMH toxicity. Glutamic acid, α-aminobutyric acid (AABA), γ-aminobutyric acid (GABA), and γ-amino-β-hydroxybutyric acid (GABHBA) were studied for their effect on UDMH toxicity. Experimental groups consisted of six rats each, and all injections were intraperitoneal. Group I received 100 or 120 mg UDMH/kg. Group II received UDMH and the amino acid simultaneously. Group III received UDMH and the amino acid simultaneously plus a second dose of amino acid 1 hr later. Group IV received two intraperitoneal doses of amino acid 1 hr apart.

Intracerebral injection studies. Each rat was anesthetized with ether and an incision made along the sagittal line. The tissue was cleared from a point approximately 2 mm to the right of the sagittal suture and 2 mm posterior to the coronal suture. A small hole was drilled through the skull at that point and the intracerebral injection made with a stationary micrometer syringe and 26-gauge needle inserted to a depth of 3 mm from the skull surface. The test substances injected were UDMH, AABA, GABA, and GABHBA. Thirty μliters of sodium bicarbonate diluent and of 0·9% saline were injected as maximal volume controls. The test substances were given in minimal volumes depending upon solubility and dosage.

UDMH-induced diuresis. From 9 a.m. to 3 p.m. on two consecutive days, control urine samples were collected from each of six rats, the volumes recorded, and specific gravity determined gravimetrically. On the third day each rat was given 80 mg UDMH/kg i.p., and 6-hr urine samples were

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collected for two additional days. Animals were denied food and water during the 6-hr collection period. In a second study, 10 mg of UDMH was injected intracerebrally and urine samples collected as described above.

RESULTS

Effect of UDMH on serum iron levels. Analysis of samples from 15 normal rats gave an average concentration of 433 ± 26 μg iron/100 ml serum, compared to 455 ± 16 for UDMH-treated rats. (All numerical results are given as the mean ± standard error.) There is no significant difference between treated and control groups.

Effect of UDMH on tissue and blood cholinesterase. Plasma cholinesterase levels of UDMH-treated rats (0·85 ± 0·08) were slightly above those of control animals (0·63 ± 0·06). Brain and erythrocyte cholinesterase levels of UDMH-treated rats were 0·94 ± 0·02 and 0·18 ± 0·01 respectively, not significantly different from those of control animals (brain: 0·91 ± 0·01, erythrocytes: 0·16 ± 0·01).

Effect of glutamic acid and aminobutyric acid analogs on UDMH toxicity. Control rats given 500 or 1,000 mg glutamic acid, AABA, GABA, or GABHBA i.p. per kg showed no abnormal behavior. Rats given both glutamic acid and UDMH convulsed and died at the same rate as rats given only UDMH. Animals receiving both AABA and UDMH exhibited an increased number of convulsions and a greater mortality rate (5/6) than rats receiving only 100 mg of UDMH per kg (2/6). GABA or GABHBA (1,000 mg/kg) provided no significant protection against an LD_{100} dose of UDMH (120 mg/kg).

TABLE 1. INTRACEREBRAL INJECTION OF UDMH AND GLUTAMIC ACID METABOLITES INTO NORMAL RATS

Substance injected	Amount (mg)	No. convulsing	No. dead
0·9% saline (30 μliters)		0/2	0/2
UDMH diluent (30 μliters)		0/6	0/6
UDMH	0·2–4·0	0/9	0/9
UDMH	6·0–8·0	12/19	5/19
UDMH	10·0	7/7	2/7
AABA	0·2–6·0	0/6	0/6
GABA	1·0	0/3	0/3
GABA	2·0	0/3	0/3
GABA	4·0–9·0	0/6	4/6
GABHBA	1·0	0/3	0/3
GABHBA	2·0–6·0	1/7	0/7

Intracerebral injections. Effects of the intracerebral injection of a number of compounds are shown in Table 1. Thirty μliters of 0·9% saline or of UDMH diluent produced no noticeable response in control rats. Four milligrams of UDMH elicited no response, but 6 to 8 mg caused convulsions (12/19) approximately 0·5 hr after injection. These convulsions were more severe as the dosage was increased. The minimal quantity of UDMH (6 mg) that produced convulsions when given intracerebrally had no effect when given intraperitoneally.

Two tenths to 6 mg of AABA had no effect when injected intracerebrally into normal rats. Respiratory arrest, convulsions, and death occurred frequently when rats received from 4 to 9 mg of GABA intracerebrally. Doses of 2 to 6 mg of GABHBA generally produced respiratory arrest, which was followed by hyperactivity in resuscitated animals. When these compounds were injected intracerebrally into rats treated with 120 mg UDMH/kg, no protective effects were noted.

Diuresis studies. The average 6-hr urine volume of animals receiving 80 mg UDMH/kg was 6·8 ml, compared to 1·8 ml for control rats (Table 2). In general, urine samples showed a considerable drop in specific gravity with the increase in urine output.

TABLE 2. EFFECT OF THE INTRAPERITONEAL INJECTION OF UDMH (80 mg/kg) ON RAT URINE VOLUME

Control day 1		Control day 2		Day of injection		Day after injection	
Vol. (ml)	Sp. Gr.	Vol. (ml)	Sp. Gr.	Vol. (ml)	Sp. Gr.	Vol. (ml)	Sp. Gr.
0·3		1·2	1·044	6·5	1·024	1·4	1·038
3·4	1·021	1·6	1·041	6·8	1·014	1·2	1·039
0·8	1·040	2·4	1·040	9·4	1·013	0	
2·7	1·039	1·2	1·016	4·6	1·017	2·2	1·036
1·5	1·043	2·6	1·043	7·5	1·016	0·6	
2·7	1·028	1·2	1·049	6·0	1·017	2·2	1·039
1·90 ± 0·49*	1·034 ± 0·004	1·70 ± 0·26	1·039 ± 0·005	6·80 ± 0·65†	1·017 ± 0·002†	1·27 ± 0·36	1·038 ± 0·001
Combined controls		1·80 ± 0·26	1·037 ± 0·003				

* Average ± one standard error of the mean.
† Significantly different from combined control, with $P < 0·005$.

Five rats injected intracerebrally with UDMH diluent excreted normal amounts of urine (1·2 ± 0·5 ml) after the injection. Four of six rats surviving a 10-mg intracerebral dose of UDMH excreted 3, 7, 20, and 26 ml of urine respectively during the subsequent 6-hr period. A similar dose of 10 mg of UDMH given i.p. did not alter the urine volume.

DISCUSSION

A study of brain, plasma, and erythrocyte cholinesterase levels after UDMH injection indicates that cholinesterase inhibition does not play a role in UDMH convulsions nor can such measurements be used to assess the degree of acute exposure. The slight rise noted in plasma cholinesterase levels of UDMH-treated rats may be related to convulsive seizures or to alterations of fluid balance brought on by diuresis. Similarly, serum iron levels in UDMH-treated rats were not different from controls and are not of value in assessing acute UDMH toxicity.

Six or more milligrams of UDMH injected intracerebrally brings on convulsive seizures within half an hour. The usual time to onset of convulsions after the intraperitoneal injection of UDMH is 1·5 to 2 hr.[10] Thus, the direct injection of UDMH into brain markedly reduces the latent time before convulsions occur and will induce convulsions at dosages well below those required by other routes of injection. This finding suggests that the convulsive effect of UDMH is mediated directly in the brain and is not the result of UDMH metabolites formed in the liver or elsewhere in the body. On the basis of the metabolic blocks in glutamic acid metabolism produced by UDMH[7, 8] it appeared possible that the intracerebral injection of one or more of these metabolites might alleviate the symptoms of UDMH toxicity. However, neither the intraperitoneal nor the intracerebral injection of varying amounts of AABA, GABA, or GABHBA protected rats from the toxic effects of UDMH. In normal rats these compounds are biologically active, producing convulsions and respiratory arrest in control animals when injected intracerebrally at relatively low dosages.

Although urine output is markedly increased in UDMH-treated rats, the mechanisms involved and any relationship to the convulsive state remain to be clarified. An intracerebral injection of 10 mg of UDMH produced a marked diuresis in rats. This amount given intraperitoneally has no diuretic effect, thus suggesting that the diuresis occurring in UDMH-treated rats may be initiated in the brain or in the pituitary.

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