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ANTICONVULSANT PROPERTIES OF DIACETYLMONOXIME (DAM)

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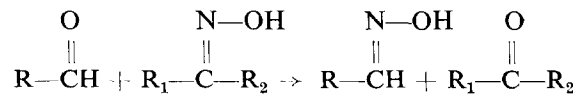
Abstract—Both diacetylmonoxime and hydroxylamine protect mice against the lethal effects of pentylenetetrazol (Metrazol); neither compound protects against the lethal effects of thiosemicarbazide. Hydroxylamine elevates the levels of γ-aminobutyric acid (GABA) in rats, while diacetylmonoxime does not. Neither compound elevates brain GABA levels in mice at anticonvulsant doses. The causal relationship between brain GABA levels and seizures is questioned.

RECENTLY two reports have appeared which demonstrate the ability of hydroxylamine (NH_2OH) to elevate the level of γ-aminobutyric acid (GABA) in rat brain[1] and the anticonvulsant properties of NH_2OH and elevation of cerebral GABA in cats.[2] The authors postulated that the anticonvulsant properties of NH_2OH are due to its ability to inhibit selectively vitamin B_6-dependent GABA-α-ketoglutaric acid transaminase (the enzyme presumed to be responsible for the metabolic utilization of GABA in brain), thereby increasing the level of brain GABA. The toxic properties of NH_2OH [1, 2] make it improbable that this compound could be used therapeutically for treatment of convulsant disorders. In view of these facts, attention was focused on several analogs of NH_2OH developed as cholinesterase reactivators. Diacetylmonoxime (DAM) was chosen from among these compounds because it is known to penetrate the blood brain barrier.[3] The following exchange reaction, well known to organic chemists,[4]



indicates that DAM might inactivate vitamin B_6 in a manner similar to NH_2OH. In addition, the toxicity of DAM in man has been studied by Jager and Stagg,[5] who reported that 30 mg of DAM per kg, given intravenously, produces transient coma. This report presents data obtained in preliminary experiments testing the effectiveness of DAM against convulsions in mice and rats caused by pentylenetetrazol (Metrazol) and thiosemicarbazide (TSC). Data on brain GABA levels, after treatment with DAM and NH_2OH, are also presented.

METHODS AND MATERIALS

White mice of the Swiss Webster strain weighing 23–28 g and Wistar rats weighing 150–280 g were used. DAM and NH_2OH hydrochlorides were used as obtained from Distillation Products, Inc. DAM was dissolved in distilled water and adjusted to pH 7;

NH_2OH was dissolved in distilled water and adjusted to pH 7·4. Metrazol solution (0·1 g/ml), as obtained from Knoll Laboratories, Orange, N.J., was diluted with saline. TSC, obtained from Aldrich Chemical Co., Milwaukee, Wis., was dissolved in saline.

Estimation of brain GABA was carried out by excising brains at various times after administration of DAM, NH_2OH or saline. The weighed brains were extracted with hot 95% ethanol and the extract was evaporated to dryness. The resulting material was taken up in a known volume of distilled water and an aliquot spotted for chromatography. One-dimensional chromatography was carried out in a phenol-water solvent.[6] A solution of 0·4% ninhydrin, 10% isopropanol and 5% collidine in water was used for color development.[7] Color was extracted from the paper with hot water and the optical density determined at 570 mμ. Recovery (94–108 per cent) of known amounts of GABA added to brain was achieved routinely.

RESULTS

Mice

DAM (400 mg/kg)* given intraperitoneally protected mice from the lethal effects of Metrazol (Table 1). This dose of DAM produced transient ataxia in most mice

TABLE 1. PROTECTION OF MICE AND RATS FROM CONVULSIVE AGENTS

Protective agent (mg/kg)	Species	Delay min	Convulsant (mg/kg)	Fraction surviving
Saline	Mouse	15	Metrazol (100)	2/31
DAM(400)			Metrazol (100)	18/19*
Saline			TSC(20)	0/10
DAM(400)			TSC(20)	0/10
Saline		60	Metrazol (100)	3/32
NH_2OH(32)			Metrazol(100)	16/32*
Saline			TSC(20)	0/10
NH_2OH(32)			TSC(20)	0/10
Saline	Rats	15	Metrazol(150)	0/10
DAM(300)			Metrazol(150)	8/10*
Saline		60	Metrazol(200)	0/10
DAM(300)			Metrazol(200)	6/10*
Saline			Metrazol(200)	0/8
NH_2OH(32)			Metrazol(200)	1/8
Saline		90	Metrazol(100)	3/10
NH_2OH(32)			Metrazol(100)	7/8*

Doses of DAM and NH_2OH were calculated as free base. DAM and NH_2OH were given intraperitoneally while Metrazol was given subcutaneously. "Delay" represents the time-interval between the doses of the protective agent and the convulsant. The fraction surviving was determined after 24 hr for mice and after one hour for rats. All saline-treated animals that died after injection with Metrazol did so within 30 min.

* Significantly different from control at $P = 0{\cdot}05$ level (Chi square).

immediately after injection; however, all mice appeared to recover and showed normal behavior by the time Metrazol was administered 15 min later. Metrazol produced only slight tremors and a few minor clonic convulsions in DAM-treated animals, whereas all mice given saline had repetitive clonic convulsions and usually

* Higher doses of DAM (800 mg/kg) produced a comatose state which lasted about one hour; all mice tested fully recovered from such treatment.

expired within 5–20 min after injection with Metrazol. NH_2OH (32 mg/kg), given intraperitoneally, also protected mice from the lethal effects of Metrazol. Neither DAM nor NH_2OH protected mice from the convulsions and lethal effects of TSC (Table 1).

No significant difference in brain GABA levels between saline-treated or DAM-treated mice was noted in animals sacrificed 30 min, 1 hr, 3 hr and 6 hr, respectively, after injection (Table 2). In doses which gave protection from Metrazol convulsions, NH_2OH also failed to raise brain GABA levels in mice (Tables 1 and 2).

Rats

Both DAM and NH_2OH protected rats from the convulsions and lethal effects of Metrazol (Table 1). All rats pretreated with saline convulsed with the doses of Metrazol used. Only 2 of the 10 rats pretreated with DAM and 1 of the 8 pretreated with NH_2OH convulsed with similar doses of Metrazol. Baxter and Roberts[8] have also reported protection in rats given NH_2OH.

In contrast to data obtained in mice, anticonvulsant doses of NH_2OH elevated brain GABA levels in rats; at doses which gave equal or better protection, DAM had no such effect (Table 2).

TABLE 2. BRAIN GABA LEVELS

Protector (mg/kg)	Time hr after injection	Species (number)	Brain GABA* μmoles/g
Saline	0·5	Mice (8)	2·3±0·055
DAM(400)	0·5	,,	2·2±0·066
Saline	1	,,	2·2±0·21
DAM(400)	1	,,	2·2±0·14
Saline	3	(4)	2·1±0·20
DAM(400)	3	,,	2·0±0·22
Saline	6	,,	2·0±0·26
DAM(400)	6	,,	2·2±0·24
Saline	1	(12)	2·2±0·16
NH_2OH(32)	1	,,	2·2±0·12
Saline	1	Rats (5)	2·3±0·14
DAM(300)	1	,,	2·2±0·08
Saline	1·5	(4)	1·8±0·03
NH_2OH(35)	1·5	,,	2·9±0·18†

Dosage and routes of administration were the same as those given in Table 1.

* Mean value ± s.e.

† Significantly different from control at $P = 0·05$ level (t-test).

DISCUSSION

DAM appears to have much in common with NH_2OH. Both compounds are carbonyl trapping agents; both protect mice against Metrazol convulsions without elevating brain GABA levels; and neither compound is capable of protecting mice from the lethal effects of TSC. These findings are consistent with the hypothesis that the two drugs produce their protective effects through similar mechanisms. Only NH_2OH is capable of elevating brain GABA levels in the rat, a fact which may have nothing to do with its protective effect.

The case for a causal relationship between brain GABA levels and seizures is currently being questioned. Results reported here, which show that NH_2OH and DAM do not elevate brain GABA levels in mice at doses which give good protection from lethal convulsions of Metrazol, indicate that GABA is not necessarily involved in the protective action of these drugs. In addition, recent results of Baxter and Roberts[9] show that thiosemicarbazide is capable of eliciting convulsions in rats at a time when brain GABA levels are still elevated from prior injection of NH_2OH. Further, Kessel[10] has reported that L-2,4-diaminobutyric acid elevates mouse brain GABA levels 2- to 3-fold, coincident with the occurrence of seizures. These findings have been confirmed by McKhann *et al.*[11]

DAM has been used in the treatment of anticholinesterase poisoning because of its ability to reactivate cholinesterase. It may also exert protective effects through an added anticonvulsant activity as well. The relationship between the anticonvulsant activity of DAM and its ability to reactivate cholinesterase is not clear.

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