Convulsant Effect of Hydroxylamine in Epileptic Monkeys and Rats.* (28024)

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Interest in the possible role of γ-aminobutyric acid (GABA) in cerebral physiology has led to a variety of studies and findings. Since GABA of exogenous origin passes the blood-brain barrier with difficulty(1), the administration of hydroxylamine (NH_2OH) has been suggested as a means of raising brain cortical levels of GABA in rats, cats and monkeys within 1-1½ hours after injection (2,3,4), and has also been shown to exert an anticonvulsant effect in cats as evidenced by a reduction in electrically induced afterdischarges at a time when cortical levels of GABA were increased(3). Large doses of hydroxylamine caused occasional convulsions in rats within 5 minutes when there was no increase in cerebral cortical GABA(2,4). The present study was undertaken to study the clinical responses of epileptic and non-epileptic monkeys and rats to injection of hydroxylamine.

Method. Hydroxylamine hydrochloride, freshly prepared in 2% concentration in physiological saline and neutralized to pH 7.0 with NaOH, was injected at a rate of 1 ml/min intravenously into 12 monkeys. Dosage used was 7.5 mg/kg calculated as hydroxylamine.

Four monkeys had been made chronically epileptic by cerebral application of alumina cream(5) by multiple injections or disc (Fig. 1). Four monkeys had been prepared similarly with multiple injections or discs of asbestos powder or talc, and had never shown evidence of clinical epilepsy either spontaneously or after standard prodding or intramuscular Metrazol(6). The remaining 4 monkeys were unoperated normals. Routine serial electroencephalograms (EEG's) were made, without anesthesia with animal on restraining board, before and after parenteral drug administration and animals were observed for at least 90 minutes following injection. The clinical and EEG responses of all monkeys were evaluated following standard intramuscular Metrazol doses of 24 or 40 mg/kg(6).

Nine male Sherman rats made chronically epileptic by local cerebral cobalt application (7) and 6 unoperated normal rats received intravenous injections of 5 mg/kg hydroxylamine (1% hydroxylamine hydrochloride, neutralized, at rate of 0.5 ml/min). Intramuscular Metrazol (2% solution) in dose of 20 mg/kg was injected and rats observed for at least 1 hour thereafter.

Results. Monkeys. Breathing became rapid about 20 seconds after start of hydroxylamine injection, and within 60 seconds the monkeys appeared cyanotic and dyspneic with occasional generalized jerks and movements. Movements continued for the next minute or two, after which the animals became quiet but remained cyanotic or pale for the ensuing 60 to 90 minutes.

All 4 epileptic monkeys had severe generalized tonic-clonic seizures soon after the intravenous injection of hydroxylamine was completed(¾, ¾, 1½ and 15 minutes respectively). None of the remaining 8 monkeys (4 operated non-epileptic and 4 normal) had similar seizures (Table I).

In the epileptic monkeys no changes in EEG could be detected until the sudden acute onset of the generalized tonic-clonic seizure, when diffuse convulsive high amplitude spike seizure patterns occurred. In 2 of the 4 nor-

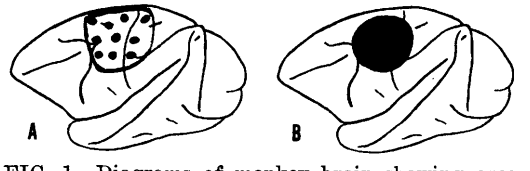

FIG. 1. Diagrams of monkey brain showing area of application to left cerebral hemisphere of alumina cream, asbestos powder or talc powder by multiple injection (A) or disc (B).

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TABLE I. Effects of Injection of Hydroxylamine (7.5 mg/kg i.v.) into Chronic Epileptic, Non-Epileptic Brain-Operated and Normal Monkeys.

	Sex	Wt, kg	Operative treatment right motor	Metrazol i.m., mg/kg		Hydroxylamine i.v., 7.5 mg/kg
Epileptic						
1	♂	4.0	Alumina multiple	24	+	+
2	♀	3.0	" "		+	+
3	♂	3.2	Alumina disc		+	+
4	♀	3.5	" "		+	+
Brain-operated non-epileptic						
5	♀	4.3	Asbestos multiple	40	0	0
6	♀	5.0	" disc		0	0
7	♂	6.6	Talc multiple		0	0
8	♀	4.5	" disc		0	0
Unoperated						
9	♂	4.0		40	0	0
10	♂	2.4			0	0
11	♀	3.4			0	0
12	♂	2.4			0	0

+ indicates clinical seizure; 0 indicates no clinical seizure,
Metrazol challenge performed at least 6 wk prior to hydroxylamine test.

mal monkeys moderately increased diffuse theta rhythms were noted for 1-9 minutes following injection, after which beta rhythms prevailed; in the other 2 normals no significant change in EEG pattern could be detected.

Rats. Five of 9 epileptic rats developed clinical seizures (minor clonic or generalized convulsions) within 1-5 minutes (average 3 minutes) following intravenous injection of hydroxylamine, whereas none of 6 normal unoperated rats developed seizures. Metrazol (20 mg/kg i.m.) produced seizures in all 9 epileptic rats in at least 1 of 2 trials in 3-13 minutes (average 8 min), while none of the 6 normal rats similarly tested showed clinical seizures.

Discussion. Our findings indicate that hydroxylamine has an early convulsant action in primates which is similar to that which occurs in rats. Epileptic monkeys proved to be more susceptible than normal or non-epileptic brain operated monkeys to the early convulsant effects of intravenous hydroxylamine. This increased susceptibility of epileptic monkeys has been reported previously for other analeptic agents such as intramuscular Metrazol, intravenous or intramuscular picrotoxin, intravenous semicarbazide and intravenous Mikedimide(8).

Hydroxylamine may be considered to have a dual action since it has been noted to have both convulsant and anticonvulsant actions. The convulsant action usually occurs early (5-10 min) after parenteral administration in animals, while the anticonvulsant action is delayed(3). Since our experiments were concerned only with the immediate convulsant action of hydroxylamine, inferences regarding its presumed anticonvulsant effects cannot be made on the basis of our findings. In rats, the convulsant effects occur at a time when the brain cortical levels of GABA are normal(2,4), usually within 5 minutes after intraperitoneal injection when there is evidence of pronounced cyanosis and/or dyspnea.

The anticonvulsant effects of hydroxylamine have been reported in cats 1 to 2 hours after injection(3). Gross reduction in susceptibility to electrically induced seizures occurred ½ to 3 hours after hydroxylamine injection, at a time when significant increases in brain levels of GABA could be demonstrated. Marked reduction in clinical seizures occurred at about the same time in awake restrained macaque monkeys stimulated through chronically implanted electrodes.

It is difficult to invoke a common pathophysiologic mechanism for the early convulsant and the relatively late anticonvulsant action of hydroxylamine. The early convul-

sant action is presumably unrelated to GABA levels of brain cortex, whereas the later anticonvulsant action may be associated with increased cortical GABA. In our own experiments the same dosage of drug reported to have a late anticonvulsant effect for monkeys proved to have an early convulsant action in epileptic monkeys, so that the method of administration and dosage of drug were probably not significant factors in this regard. The early convulsant effects, moreover, are usually associated with obvious physiological changes such as hyperpnea and methemoglobinemia which were shown in rats to be unrelated to brain GABA levels(4).

Summary. 1) Chronic epileptic monkeys (alumina cream) showed greater susceptibility than normal or non-epileptic brain-operated monkeys to the early convulsant effects of hydroxylamine. 2) Rats made epileptic (intracerebral cobalt) showed a similar increased susceptibility to hydroxylamine compared to unoperated controls.

1. Tower, D. B., *Neurochemistry of Epilepsy,* C. C Thomas, Springfield, Ill., 1960, 249-250.
2. Baxter, C. F., Roberts, E., *Inhibition in the Nervous System and γ-Aminobutyric Acid,* Pergamon Press, 1960, 358-364.
3. Eidelberg, E., Baxter, C. F., Roberts, E., Saldias, C. A., *ibid.,* 365-370.
4. Baxter, C. F., Roberts, E., *J. Biol. Chem.,* 1961, v236, 3287.
5. Kopeloff, L. M., Chusid, J. G., Kopeloff, N., *Neurology,* 1954, v4, 218.
6. Chusid, J. G., Kopeloff, L. M., Kopeloff, N., *J. Appl. Physiol.,* 1953, v6, 139.
7. Kopeloff, L. M., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 500.
8. Kopeloff, L. M., Chusid, J. G., *ibid.,* 1959, v101, 468.

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Effect of Oxidative Phosphorylation Inhibitors on Synthesis of Liver Mitochondria Phospholipids.* (28025)

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Liver mitochondria are composed of 21% phospholipids on a dry weight(1,2). Purified cytochrome oxidase contains phospholipids (3) and is reactivated by purified phospholipids(4). Recently a number of other enzymes, DPNH-cytochrome c reductase, succinic cytochrome c reductase, succinic coenzyme Q reductase and DPNH diaphorase have been shown to contain 26-83% lipid and the lipids are predominantly phospholipids (5). All of these enzymes are involved in the electron transfer system. Mitochondria(6) and the electron transport particles(7) are very active in phosphorylation. Kennedy has demonstrated that synthesis of phospholipids takes place in isolated mitochondria and requires the maintenance of oxidative phosphorylation(8). Bilirubin(9), dinitrophenol(10), arsenate(11,12) and oligomycin (13,14) have been shown to inhibit oxidative phosphorylation. In view of these observations, experiments were undertaken to determine the effect of inhibition of oxidative phosphorylation on synthesis of liver mitochondria phospholipids.

Methods. Male albino rats of Sprague-Dawley strain, weighing 100-140 g, were divided into 4 groups and received a single daily injection of the following compounds. The animals in Series I were injected intraperitoneally with arsenate (14 mg/kg), bilirubin (3.3 mg/kg), in Series II rats were injected with oligomycin (0.83 mg/kg), in Series III, dinitrophenol (30 mg/kg) and in Series IV, dinitrophenol (30 mg/kg for 3 days). Control rats for each group received intraperitoneal injections of saline except the control animals for Series II which received

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