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Anticonvulsant Properties of Hydroxylamine and Elevation of Cerebral γ-Aminobutyric Acid in Cats.* (25106)

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In the preceding paper(1) it was shown that elevation of brain levels of γ-aminobutyric acid (γABA) could be achieved in the rat by injection of hydroxylamine (NH_2OH). Since a correlation exists between decreases in brain γABA and increased seizure susceptibility(2,3 also see 1 for other pertinent references), it was of interest to determine whether a decrease in induced seizure activity would be found when brain levels of this substance are elevated. The present report is concerned with the effect of NH_2OH on electrically-induced seizure afterdischarges in acute experiments in cats.

Methods. The cats used were immobilized with gallamine triethiodide [Flaxedil®] and anesthetized locally with procaine at painful points. Preliminary surgical procedures, including a wide bilateral craniectomy, were carried out under ether anesthesia. The ether was blown off during 1½ hours of artificial ventilation. Two samples of cortex, one on

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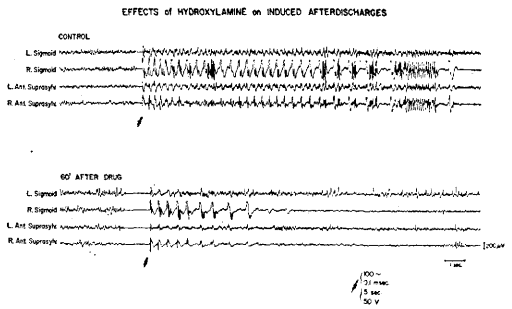

FIG. 1 (EEG records). At the S markings afterdischarges were induced by electrical stimulation near the R. sigmoid lead at parameters specified above. Recording was interrupted during stimulation. 10 mg/kg of NH_2OH were used.

each hemisphere but from nonhomologous loci, were then taken surgically as controls for γABA assay, hemostasis was completed, and multiple cortical electrodes were laid over exposed cortex for EEG recording. Two pairs of similar chlorided-silver ball electrodes (connected to Grass S4C stimulators and isolation units) were used for induction of afterdischarges. Fixed parameters for stimulation were: 100 c/s, 0.1 msec rectangular pulses, 5 sec. trains. Voltage was increased until consistent afterdischarge patterns were observed, with spread to at least one lead away from stimulated site. At least 3 control electrical seizures were obtained in each locus before administration of the test drug. Dilute neutralized solutions of NH_2OH or sodium nitrite in saline were given intravenously. Cortical EEG was monitored continuously thereafter. In each locus tested stimulations were repeated at 10 minute intervals at same fixed voltage as before drug administration for periods one to 4 hours. Two more cortical samples were taken for biochemical analysis at end of test period. Care was taken to obtain samples from cortical loci opposite to each control sample to minimize variations. All samples were weighed quickly and stored in 70% ethyl alcohol in the cold prior to assay for content of γABA.

Results. A brief (3-4 minutes) period of depression in electrocortical activity could be observed immediately after single doses of NH_2OH between 10-20 mg/kg were given. A gradual return to normal record took place in a few more minutes. This depression could be avoided by administering the same total dose in several injections spaced at 10-15 minute intervals. In some animals definite increases in seizure after discharges were observed within the initial 20 minute period after injection. After 30 minutes and up to 3 hours induced seizures were much shorter and spread to fewer leads than during control period and often were restricted to the lead next to the stimulating electrodes (Fig. 1 and Table I). In one cat it was necessary to raise the stimulating voltage considerably to elicit any seizures at all.

Levels of γABA in the brain samples are shown in Table II. Increases of 100% or more over control levels were obtained in 4 of the 5 NH_2OH-treated animals studied. The

TABLE I. Influence of Hydroxylamine and Sodium Nitrite on Electrically-Induced Seizure Afterdischarges in Cats.*

			Pre-drug		Post-drug (60 min.)†	
Drug	Dose, mg/kg	Locus of test	Mean seizure duration, sec.	Spread to channels, No.	Mean seizure duration, sec.	Spread to channels, No.
None		R. ant. suprasylv.	10	4	12	4
NH_2OH	20	R. sigmoid	18	5	4	2
		R. post. suprasylv.	12	2	5	1
"	10	L. sigmoid	10	2	5	1
		R. post. suprasylv.	8	3	3	1
"	20	L. middle suprasylv.	20	4	2	1
		R. ant. suprasylv.	7	4	0	0
$NaNO_2$	20	L. middle suprasylv.	12	4	28	4
		R. ant. suprasylv.	10	4	32	5

* Experimental parameters are described in text.

† Although results are shown for 60 min. period only, measurements were made in all instances for several hours at 10 min. intervals.

TABLE II. Influence of Hydroxylamine and Sodium Nitrite on Cerebral Levels of γ-Aminobutyric Acid in Cat.

Drug	Dose, mg/kg	Area	γ-Aminobutyric acid level: Pre-drug, mg %	γ-Aminobutyric acid level: Post-drug, mg %	Time, min.
NH_2OH	10	Post. ectosylv.	16.9	24.9	40
		Sigmoid	17.2	25.1	
"	"	Post. ectosylv.	22.4	41.7	60
		Sigmoid	18.3	46.0	
"	"	Post. ectosylv.	19.3	42.2	70
		Sigmoid	19.8	49.1	
"	20	Middle ectosylv.	19.6	44.9	90
		Ant. suprasylv.	21.0	41.0	
"	"	Post. ectosylv.	16.5	38.8	105
		Sigmoid	18.7	39.0	
$NaNO_2$	60	Post. ectosylv.	18.4	19.7	30
		Sigmoid	19.1	23.0	
"	20	Post. ectosylv.	22.6	33.0	75
		Ant. suprasylv.	21.4	24.0	

fifth animal was exposed to the drug for less than one hour and showed an increase of approximately 50%.

Reduction of seizure discharges and increases in cerebral γABA were not observed when solutions of sodium nitrite were injected into cats in amounts equivalent to NH_2OH. In 3 out of 4 samples there was no significant increase in cerebral γABA. Since sodium nitrite produced methemoglobinemia at least as extensive as that observed with NH_2OH, the results appear to rule out methemoglobinemia as a causative factor in electrographic changes. Actually, the methemoglobinemia appeared to be very mild and of short duration when doses of 10-20 mg/kg of NH_2OH were given. Use was not made of methylene blue to prevent methemoglobinemia because of the possibility of introducing complications in interpretation of EEG results. The preceding report(1) showed that methylene blue alone does not increase cerebral levels of γABA in rats.

From above data it is evident that NH_2OH can produce changes in neural excitability and content of γABA opposite to those induced by convulsant hydrazides(2,3). These findings are in agreement with a working hypothesis linking metabolism of γABA in a specific cerebral area to excitability of that area. Further work is in progress to extend the study of the anticonvulsant properties of NH_2OH, which may have important clinical value, and their mechanism of action.

Summary. Hydroxylamine when administered to immobilized cats in acute experiments in doses of 10-20 mg/kg markedly reduced duration and spread of electrically-induced afterdischarges while increasing cerebral levels of γ-aminobutyric acid. These findings increase the probability that a causal relationship may exist between metabolism of γ-aminobutyric acid in a particular cerebral area and its excitability.

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