ANESTHESIA LXII: THE EFFECT OF HEXAFLUORODIETHYL ETHER ON BRAIN CHOLINESTERASE ACTIVITY

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THAT the living cell can serve as the site of consciousness is the most intriguing and baffling phenomenon in biology. Equally puzzling is the fact that a hiatus in consciousness can be achieved by general anesthetic agents. It appears that the elucidation of the second phenomenon, and possibly the first, will be achieved by the study of the action of anesthetics at a cellular level. Acetylcholine has been shown to play a role in neuronal transmission in the synapses in the brain. We were, therefore, prompted to study the action of the unusual convulsive agent, hexafluorodiethyl ether (Indoklon), similar in chemical constitution to many anesthetic agents, on brain cholinesterase.

Hexafluorodiethyl ether evokes convulsive seizures in animals and man.[1, 2] Hexafluorodiethyl ether was shown to elicit no significant effect on the oxidative metabolism of the brain of the guinea pig.[3] In an endeavor to elucidate the mechanism of the convulsive action of this agent, we have studied the cholinesterase activity of rat's brain. Tower [4] in his studies of slices of human epileptogenic and normal cerebral cortex found significantly higher levels of cholinesterase activity in the epileptogenic foci than in the normal cortex. It occurred to us that changes in the level of this enzymatic activity in the brain might be evoked by the hexafluorodiethyl ether seizure.

METHODS AND MATERIALS

Determination of Cholinesterase Activity. The cholinesterase activity of whole brain homogenate preparations was determined according to a manometric technique modified after the methods of Nachmansohn and Rothenberg [5] and Tower and Elliott.[6] Male albino rats (approximately 200 Gm. each) were decapitated and the entire brain removed. Following removal of the pituitary gland the brain was placed in ice-cold medium previously gassed with 95 per cent N_2 and 5 per cent CO_2. The brain was homogenized according to the Potter-Elvehjem technique [7] for approximately two minutes to give a 10 per cent suspension that was subsequently diluted to 0.25 per cent. Aliquot samples of 2.6 ml. were transferred to Warburg flasks.

The medium consisted of 150 m*M* NaCl, 25 m*M* $NaHCO_3$ and 40 m*M* $MgCl_2 \cdot 6H_2O$. The *pH* was 8.0 The substrate was 27.5 m*M* acetylcholine chloride (Merck), and was freshly prepared on the day of each determination. Four-tenths milliliter (0.4 ml.) of the substrate was pipetted into the sidearm of each Warburg flask. The final volume of each flask was 3.0 ml. The anaerobic gas phase was established by gassing all flasks for 10 minutes with 95 per cent N_2 and 5 per cent CO_2.

For each determination 16 flasks were analyzed concurrently, namely, 1 medium-substrate thermobarometer, 3 autohydrolysis controls (medium, tissue and no substrate),[4] untreated control flasks containing medium, tissue and substrate, and 8 experimental flasks with medium, tissue after convulsion and substrate. After an equilibration period of 10 minutes the substrate was tipped in from each sidearm. Preliminary studies indicated that the first 10-minute interval represented anaerobic glycolytic activity, after which the brain tissue maintained a uniform rate of CO_2 evolution dependent upon hydrolysis of the acetylcholine. The bath temperature was maintained at 37.5 C.

The final dry weight (mg.) of brain tissue was determined by taking aliquot samples of the 10 per cent homogenate as well as equal aliquots of the medium and drying overnight at 100 C. The amount of tissue per flask was then determined by reducing the values of the 10 per cent homogenates to 0.25 per cent. The authors considered this more re-

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TABLE 1

THE EFFECT OF CONVULSIVE TREATMENT ON RAT BRAIN CHOLINESTERASE (ACH-ASE) ACTIVITY

Treatment	Det'n (n)	Mean ACh-ase Activity $\mu l.CO_2$ mg. hour	Percentage Change	t*
A. *In vitro studies*				
Control	17	74.45±5.46	+10.68	3.11
Hexafluorodiethyl ether	25	82.40±6.03		
B. *In vivo studies*				
Control	8	81.52±3.16	—	—
10-sec. hexafluorodiethyl ether	14	94.61±4.06	+16.06	5.74
30-sec. hexafluorodiethyl ether	10	94.53±5.06	+15.96	4.60
Control	8	77.06±5.22	+16.21	3.99
Electroshock	12	89.55±4.45		
Control	7	79.68±4.25	+7.73	2.03
Pentylenetetrazol	10	85.84±4.45		

* Significance is based on 't' test for paired means with the differences from control being at least at the 1 per cent level of probability.

liable than the weighing of small aliquots of a 0.25 per cent homogenate.

The "cholinesterase activity" of brain tissue is reflected by the "Q_{CO_2} activity" which is the microliters of CO_2 evolved per milligram of final dry weight of brain tissue per hour. This activity is dependent upon the hydrolysis of free available acteylcholine in the brain tissue under the influence of acetylcholinesterase as well as other esterases capable of splitting acetylcholine (Tower and Elliott [6]).

CONVULSIVE TECHNIQUES

A. *Hexafluorodiethyl Ether in Vitro.* An initial concentration of 130 mg. per cent of hexafluorodiethyl ether in substrate-medium was prepared. Three-tenths milliliter (0.3 ml.) of this solution was placed in the side-arm of each flask, which, when tipped in, yielded a final flask concentration of 13 mg. per cent.

B. *Hexafluorodiethyl Ether in Vivo.* Each rat was exposed to 0.5 ml. hexafluorodiethyl ether (100 per cent) dispersed on a 4-inch gauze pad (12-ply) in a 3.4 liter chamber (Krantz, *et al.*[2]). One group of rats was exposed for 10 seconds, and a second group, 30 seconds.

C. *Electroshock.* Each rat received 120 mAmp. for a 0.5-second interval administered by corneal electrodes. The seizures were induced with an Electroshock Seizure Apparatus, Model 2C (Hans Tech. Associates, Palo Alto, Calif.).

D. *Pentylenetetrazol (Metrazol).* In this series, each rat received an intraperitoneal dose of 50 mg./kg. of a 10 per cent solution of pentylenetetrazol. In all 3 *in vivo* experiments, each rat was guillotined during the tonic phase of the convulsive response.

RESULTS

From table 1 hexafluorodiethyl ether *in vitro* or *in vivo* significantly increased the cholinesterase activity of rat brain tissue, as did electroconvulsive treatment. Pentylenetetrazol, however, did not significantly increase this activity although the rats did exhibit clonic-tonic convulsions. Furthermore, there was no appreciable difference in cholinesterase activity of brain tissue following either a 10-second or a 30-second exposure to the vapors of hexafluorodiethyl ether.

DISCUSSION

The early work of Quastel *et al.*[8, 9] revealed that brain tissue can synthesize acetylcholine, and that this humoral factor exists in two forms. The first is a free, active, soluble

form; the second is a pharmacologically inactive form, attached to tissue solids, which can be converted into the free, active form. McLennan and Elliott [10] more recently reviewed the role of acetylcholine synthesis by brain tissue and observed the action of convulsant, narcotic and anticonvulsant agents of acetylcholine synthesis. They noted that a low concentration of picrotoxin or pentylenetetrazol increased the synthesis while higher concentrations inhibited the elaboration of free acetylcholine. Richter and Crossland [11] reported that pentobarbital anesthesia elevated and electroshock convulsion diminished the total acetylcholine content in rat brain. These observers believe that this supports the view that activity in the central nervous system involves the liberation and breakdown of acetylcholine. Elliott *et al.*[12] confirmed this view by observing that pentylenetetrazol or picrotoxin decreased total brain acteylcholine in pentobarbitalized cats but not in unmarcotized cats. More recently Tower [4] reported increased cholinesterase activity and decreased bound, inactive acetylcholine content in human cerebral cortical tissue removed from epileptogenic foci.

Summary

Hexafluorodiethyl ether, administered *in vitro* or *in vivo*, and electroshock convulsions evoke a significant increase in rat brain cholinesterase activity. This did not prevail with pentylenetetrazol. The effect of prolonged exposure of rats to hexafluorodiethyl ether did not differ significantly from a shorter interval of exposure with regard to cholinesterase activity. It would appear that hexafluorodiethyl ether and electroconvulsion induce a mobilization of free, active acetylcholine from the bound, inactive state in brain tissue.

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