ANESTHESIA LXII: THE EFFECT OF HEXAFLUORODIETHYL ETHER AND TRIFLUOROETHYL VINYL ETHER ON CEREBRAL METABOLISM

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HEXAFLUORODIETHYL ether (Indoklon) has been shown to evoke convulsive seizures in laboratory animals and man.[1, 2] Owing to the similarity of the seizures elicited by hexafluorodiethyl ether to those produced by electroshock, this agent has been used in the treatment of mentally ill patients.[3, 4] Trifluoroethyl vinyl ether (Fluoromar) is a volatile anesthetic which has had limited use in anesthesia.[5, 6] The two compounds bear a striking chemical similarity. In addition, neither compound is metabolized in the body. However, the response of the central nervous system to trifluoroethyl vinyl ether is characterized by cerebral depression and anesthesia, whereas hexafluorodiethyl ether induces intense stimulation and a *grand mal* convulsion.

Therefore, we considered it interesting to study these two similar compounds on brain oxidative metabolism at a cellular level, with the hope of contributing to the understanding of cerebral depression and stimulation.

METHODS

Tissue Respiration. Male albino guinea pigs (approximately 250 Gm.) were killed by decapitation. The brain *in toto* or each of four sections of the brain, namely, cerebrum, hypothalamus-thalamus, cerebellum, and medulla oblongata, was promptly placed in a cold medium to prepare 10 per cent homogenate suspensions using a Potter-Elvehjem[7] homogenizer. The medium was Krebs-Ringer phosphate, *p*H 7.4. The substrate, when present, was 0.2 per cent glucose (0.01*M*), 0.11 per cent sodium pyruvate (0.01*M*), 0.14 per cent sodium fumarate (0.01*M*), or 0.14 per cent sodium succinate (0.01*M*). The oxygen uptake of the brain tissues was measured by the direct Warburg technique.[8] The gas phase was air. After a 10-minute equilibration period readings were recorded every 10 minutes for one hour. Each experimental flask contained either 0.2 ml. of 0.1 per cent (v/v) of hexafluorodiethyl ether or 0.2 ml. of 0.35 per cent (v/v) of trifluoroethyl vinyl ether to yield final flask concentrations of 10.1 and 26.3 mg. per cent, respectively. The total volume per flask was 3.0 ml., including 0.2 ml. of 10 per cent potassium hydroxide in the center well for absorption of carbon dioxide. Four or more control flasks were run concurrently with the experimentally-treated flasks. The Q_{O_2} values represent μl. oxygen consumed per mg. final, dry weight per hour. Final, dry weights were calculated from 0.5-ml. aliquot samples of the 10 per cent homogenates dried overnight at 100 C.

Salamander Studies. ADMINISTRATION BY INHALATION: Adult (aquatic phase) salamanders, *Triturus viridescens,* each weighing approximately 2 Gm., were placed on 4-inch, 12-ply gauze pads in 3.4-liter exposure chambers. Hexafluorodiethyl ether (0.25–1.0 ml.) or trifluoroethyl vinyl ether (1.0 ml.) was dispersed on the pads, and the response of the animals was observed.

INTRA-ABDOMINAL ADMINISTRATION: Salamanders were also injected with hexafluorodiethyl ether (0.10–0.40 ml.) in a single or divided dose. The animal response was then noted. Pentylenetetrazol (Metrazol) (10 per cent) was given by the same route at a dose of 1.0 mg. per gram, and the effects were observed.

RESULTS

The results of the experiments on the whole brain homogenates of the guinea pig (table 1) indicated that hexafluorodiethyl ether, at the concentration studied, exerted no significant effect on tissue respiration. Trifluoroethyl vinyl ether, however, significantly depressed whole brain oxygen utilization in the absence of substrate (t value of 3.10) as well as in the presence of glucose, pyruvate and fumarate substrates (t values of 3.69, 4.50, and 5.06,

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TABLE 1

EFFECT OF HEXAFLUORODIETHYL ETHER AND TRIFLUOROETHYL VINYL ETHER ON GUINEA PIG WHOLE BRAIN OXYGEN UTILIZATION

Substrate (0.01 *M*)	Mean Q_{O_2} ± S.E.mean		
	Control	Hexafluorodiethyl Ether—Treated	Trifluoroethyl Vinyl Ether—Treated
None	2.44 ± 0.240 (16)	2.46 ± 0.279 (20)	2.16 ± 0.269 (20)*
Glucose	2.63 ± 0.361 (16)	2.75 ± 0.399 (20)	2.16 ± 0.380 (20)*
Pyruvate	2.72 ± 0.281 (18)	2.87 ± 0.324 (20)	2.28 ± 0.301 (20)*
Fumarate	2.80 ± 0.161 (20)	2.88 ± 0.185 (20)	2.43 ± 0.214 (15)*
Succinate	4.54 ± 0.636 (18)	4.21 ± 0.783 (18)	4.05 ± 0.799 (20)

Figures in parentheses refer to the number of determinations.
* Mean values differ significantly from those of the controls at a probability level of <0.05 based on the Student's *t* test.

respectively). There was no statistically significant change when succinate was the added substrate.

The possibility existed that hexafluorodiethyl ether or trifluoroethyl vinyl ether might exert a specific action on different areas of the brain, which effect could be masked by the use of whole brain preparations. Accordingly, a series of experiments was performed on four regions of the guinea pig brain, namely, cere-

TABLE 2

EFFECT OF HEXAFLUORODIETHYL ETHER AND TRIFLUOROETHYL VINYL ETHER ON OXYGEN UTILIZATION OF FOUR AREAS OF THE GUINEA PIG BRAIN

Area	Substrate (0.01 *M*)	Mean Q_{O_2} ± S.E.mean		
		Control	Hexafluorodiethyl Ether—Treated	Trifluoroethyl Vinyl Ether—Treated
Cerebrum	None	3.20 ± 0.451 (10)	2.82 ± 0.493 (10)	3.54 ± 0.598 (10)
	Glucose	3.59 ± 0.236 (12)	3.53 ± 0.305 (10)	3.45 ± 0.268 (10)
	Pyruvate	3.54 ± 0.247 (8)	3.64 ± 0.318 (10)	3.53 ± 0.287 (10)
	Fumarate	3.73 ± 0.842 (10)	2.77 ± 0.843 (10)*	4.24 ± 0.905 (10)
	Succinate	5.85 ± 0.529 (8)	5.74 ± 0.780 (10)	6.20 ± 0.840 (10)
Hypothalamus-thalamus	None	2.61 ± 0.266 (8)	2.43 ± 0.302 (10)	3.08 ± 0.310 (10)*
	Glucose	2.59 ± 0.192 (8)	2.62 ± 0.265 (10)	2.93 ± 0.244 (8)*
	Pyruvate	2.65 ± 0.113 (8)	2.71 ± 0.187 (10)	2.73 ± 0.143 (10)
	Fumarate	2.35 ± 0.230 (10)	2.14 ± 0.270 (10)	2.76 ± 0.253 (10)*
	Succinate	4.90 ± 0.916 (10)	4.30 ± 1.030 (8)	5.82 ± 1.178 (10)
Cerebellum	None	1.84 ± 0.309 (10)	1.80 ± 0.358 (10)	2.39 ± 0.429 (10)*
	Glucose	2.42 ± 0.377 (8)	2.87 ± 0.433 (10)	2.23 ± 0.400 (10)
	Pyruvate	2.39 ± 0.477 (12)	2.14 ± 0.472 (10)	2.86 ± 0.475 (10)*
	Fumarate	1.55 ± 0.236 (8)	1.51 ± 0.250 (8)	1.78 ± 0.285 (10)
	Succinate	4.21 ± 0.756 (10)	4.60 ± 1.028 (10)	3.46 ± 0.924 (10)
Medulla	None	1.33 ± 0.214 (12)	1.15 ± 0.267 (10)	1.26 ± 0.273 (10)
	Glucose	1.20 ± 0.502 (12)	0.90 ± 0.522 (10)	1.59 ± 0.568 (10)
	Pyruvate	1.33 ± 0.139 (10)	1.53 ± 0.184 (8)*	1.29 ± 0.179 (10)
	Fumarate	0.79 ± 0.146 (10)	0.89 ± 0.182 (10)	0.74 ± 0.178 (10)
	Succinate	4.43 ± 0.430 (12)	4.56 ± 0.202 (8)	4.29 ± 0.799 (8)

Figures in parentheses refer to the number of determinations.
* Mean values differ significantly from those of the control at a probability level of <0.05 based on the Student's *t* test.

brum, hypothalamus-thalamus, cerebellum and medulla oblongata. The convulsant induced a statistically significant increase in oxygen uptake by the medulla in the presence of pyruvate (t value of 2.29) but depressed the respiratory activity of the cerebrum when fumarate was added to the medium ($t = 2.55$). The anesthetic agent stimulated the hypothalamus-thalamus in the absence of substrate ($t = 3.20$) and in the presence of glucose ($t = 3.20$) or fumarate ($t = 3.62$). Similarly the oxidative metabolic activity of the cerebellum was significantly increased in the substrate-free medium ($t = 2.87$) or in the presence of pyruvate ($t = 2.10$). The data are shown in table 2.

In previous studies [2] concerned with the locus of action of Indoklon in the brain, the authors showed that this agent was capable of eliciting a convulsive response in the frog after the ablation of all areas of the brain excepting the medullary region. Pursuant to these studies, salamanders, *Triturus viridescens*, were subjected to the action of hexafluorodiethyl ether or trifluoroethyl vinyl ether. These animals lack a natural cerebral cortex, and have been used by Peters and co-workers [9, 10] to study the locus of the convulsive action of pentylenetetrazol and other analeptic agents. Exposure of 10 salamanders to hexafluorodiethyl ether vapor failed to produce convulsions. The response was principally that of depression. Six other salamanders were each injected intra-abdominally with this agent (0.10 to 0.40 ml.). One of the 6 animals given 0.30 ml. showed a transient tonic-clonic convulsion followed by depression while the remaining 5 were only depressed. Exposure of 5 salamanders to trifluoroethyl vinyl ether vapor induced hyperexcitability, including hyperreflexia, followed by marked depression. Five additional animals were each given pentylenetetrazol (1 mg. per gram body weight) intra-abdominally, and responded with tonic-clonic seizure.

Discussion

The experiments with Indoklon indicated that this convulsive agent does not markedly stimulate brain tissue respiratory activity. The only significant effects noted were a depressant action on cerebral oxygen uptake in the presence of sodium fumarate, and a stimulatory influence on the oxidative metabolism of the medulla oblongata in the presence of sodium pyruvate. In their study of convulsants (pentylenetetrazol, nikethamide and sodium fluoroacetate) on brain tissue glycolysis and respiration, Webb and Elliott [11] noted little effect except in high concentrations. Fluoroacetate appreciably inhibited the respiratory activity of guinea pig brain tissue. The final flask concentration of hexafluorodiethyl ether, namely, 10.1 mg. per cent was selected because it approximates the calculated blood level in a human subject exposed to a convulsive dose of this analeptic compound.

Trifluoroethyl vinyl ether significantly depressed the oxygen uptake by whole brain homogenates in the absence of substrate or in the presence of glucose, pyruvate or fumarate substrates, but showed no effect when succinate was added to the medium. This is in agreement with the early work by Quastel and Wheatley [12] and Creig.[13] Soskin and Taubenhaus [14] reported that sodium succinate counteracted barbiturate effect *in vivo* in rats. Their work has not been confirmed. The effect of trifluoroethyl vinyl ether on the different areas of the brain, however, was one of stimulation of the respiratory activity. The hypothalamus-thalamus, and cerebellum showed increased oxygen utilization. According to Webb and Elliott,[11] the pharmacologic effects of narcotic and convulsive agents may be more closely related to their effects on acetylcholine metabolism.

The results of these experiments bear out earlier investigation by Himwich and co-workers,[15] namely, that there is a gradient in respiratory activity as one ascends the neuraxis. The cerebrum had the highest oxygen utilization while the medulla the lowest. This relationship persisted with the various substrate conditions except with succinate where the medulla showed higher respiration than the cerebellum.

The results of this study suggest that the brain is capable of maintaining an essential homeostatic level of respiratory activity, necessary for neuronal function, in the presence of an anesthetic or convulsant refractory to catabolism. The pharmacologic response of animals to such agents, therefore, appears to depend more on some other mechanism of action, such

as an effect on the metabolism of the neurotransmitter substances, for example, acetylcholine and/or an effect on electrolyte exchange.

Summary

The effect of hexafluorodiethyl ether, a convulsant, and a chemically similar compound, trifluoroethyl vinyl ether, an anesthetic, have been studied with regard to their effect on the oxidative metabolism of the brain of the guinea pig. Hexafluorodiethyl ether exerted no significant effect on whole brain oxidative metabolism while trifluoroethyl vinyl ether depressed such activity in the absence of succinate substrate. Further the effect on isolated areas of the brain did not necessarily parallel that on the whole brain. Thus hexafluorodiethyl ether inhibited cerebral tissue respiration when fumarate was present, but stimulated medullary oxygen uptake in pyruvate-containing medium. Trifluoroethyl vinyl ether increased the respiration of hypothalamic-thalamic and cerebellar tissue.

With one exception, hexafluorodiethyl ether failed to convulse salamanders while trifluoroethyl vinyl ether markedly depressed these animals following an initial phase of hyperexcitability.

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