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The Inhalation Convulsants: a Pharmacodynamic Approach

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SUMMARY

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Certain fluorinated ethers, e.g., $(CF_3CH_2)_2O$, are potent stimulants of the central nervous system. The presence of fluorine in the molecule is not necessarily indicative of this type of activity, since $(CF_3)_2CHOCH_3$, an isomer, is anesthetic. We have now found that the only reliable experimental parameter that could predict the type of activity in fluorinated ethers is the partial molal volume ($\bar{v}$) in a model solvent. Knowledge of $\bar{v}$ allows derivations of the solubility parameter (δ) and of the partition coefficient that are more dependable than had been hitherto possible. All the inhalation convulsants studied are characterized by low δ values (6.5-7.5) and incur large rates of expansion in molal volume (4-8%) in benzene solution (δ = 9.2). Their stimulatory activity could be ascribed to excess free energy ($\bar{F}^E$) in the biophase large enough to surmount the energetic barrier against the spontaneous influx of Na^+, to a preference for accumulating in that phase of a target organ that is compatible with their low δ, or to a combination of these effects.

INTRODUCTION

Hexafluorodiethyl ether, $CF_3CH_2OCH_2 \cdot CF_3$ (flurothyl, Indoklon), is a potent inhalation convulsant which is used in psychiatric shock therapy (1), its action being analogous to electroshock in effect, duration, and reversibility. Since its discovery by Krantz *et al.* (2), additional ethers and alkanes have been reported to display similar properties (3). All these embody fluorine in the molecule, occasionally with other halogens. In view of this, one is tempted to atrribute convulsant action to the presence of fluorine in these drugs. However, there are some notable exceptions, such as hexafluoroisopropyl methyl ether, $(CF_3)_2CHOCH_3$, an isomer of flurothyl, which is an anesthetic (4). Indeed, some of the best anesthetics in current use are fluorinated ethers or alkanes (5). There exists also a third class of compounds which appear to exert a dual convulsant-anesthetic effect in the same or different species (6-9), and perhaps a fourth, such as $(C_2F_5)_2O$, which is inert at acceptable concentrations (10).

As the two effects, depression of the nervous system on the one hand and its

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stimulation on the other, are properties of structually similar compounds, use of structure-activity relationships is not likely to afford a rationalization of convulsant property. In this respect the inhalation convulsants do not differ from their anesthetic counterparts, in which no definite pattern of chemical structure could be associated with activity or potency. The alternative approach is to seek some correlation between an intensive thermodynamic property of members of a series and their activity. This usually holds well when activity is pharmacologically of a uniform type. In any case, very few thermodynamic data have ever been published for the inhalation convulsants; and in one of the rate cases in which such data have been compared for a convulsant, $(CF_3CH_2)_2O$, and an anesthetic, $(CF_3)_2CHOCH_3$, one finds no clue to the opposite activity of the pair (4). Krantz attached some significance to the apparent difference in values of the oil/water partition coefficient of the pair. However, both these values are within the range of log P = 2, which characterizes most inhalation anesthetics (11).

The most striking property of fluorocarbons and related compounds is their unusual solubility. Most of these are poorly miscible with other organic solvents and are themselves poor solvents for most solids (12). Conceivably, then, a rational approach to the problem could dwell on the "regular solution" theory developed by Hildebrand, Prausnitz, and Scott (13), and advocated by various authors for a resolution of drug action. Examples are the proposal of Mullins (14) and Miller *et al.* (15) to link solvent property and biophase of action and the demonstration by Cammarata *et al.* (16) of a parabolic relationship between drug activity and solubility. We present evidence here to the effect that regular solution theory could provide a basis for understanding at the molecular level the attributes of the stimulatory activity of the inhalation convulsants.

MATERIALS AND METHODS

The ethers used in this study and their physical properties are given in Table 1. Most of these were prepared in this laboratory by standard procedures. Structure was verified by elemental and NMR analyses, and purity by gas-liquid chromatography.

Convulsant activity was determined on young chicks in preference to mice, since

TABLE 1

Physical constants of inhalation convulsants and anesthetics

Compound	Formula	B.p.	$n^{D}_{t°}$	$d_{25°}$	Concentration in air[a]
					μg/cc air
1	$C_2F_5CH_2OCF_2CHFCl$	93.5°	1.3015_{25}	1.5336	0.76
2	$C_2F_5CH_2OCF_2CH_2F$	76.5–77°	1.2807_{20}	1.4775	1.16
3	$CF_2ClCH_2OCF_2CH_2F$	88.5–89°	1.3235_{20}	1.4425	1.17
4	$CF_3CH_2OCH_2CF_3$	63.9°	1.2797_{20}	1.4151	2.47
5	$CF_3CH_2OCF_2CH_2F$	64.5°	1.2826_{25}	1.4073	2.67
6	$CF_3CH_2OCF_2CHFCl$	82°	1.3066_{25}	1.4899	1.10
7	$CF_3CH_2OCH_2OCH_2CF_3$	118°	1.4919_{20}	1.3785	0.48
8	$(CF_3)_2CHOCH_3$	50–50.5°	1.2750_{20}	1.3734	3.02
9	$CF_3CH_2OCH{=}CH_2$[b]	43.2°	1.3192_{20}	1.1291	2.94
10	$CF_3CH_2OC_2H_5$	50.0°	1.3007_{20}	1.0589	2.32
11	$CHFClCF_2OCHF_2$[c]	56.5°	1.3030_{20}	1.5242	2.65
12	$CHFClCF_2OC_2H_5$	88°	1.3425_{25}	1.2691	0.98
13	$CHCl_2CF_2OCH_3$[d]	104.7°	1.3839_{25}	1.4208	0.47

[a] At saturation at 37°.

[b] Fluroxene, Ohio Medical Products, Madison.

[c] Enflurane, Abbott Laboratories.

[d] Methoxyflurane, Abbott Laboratories.

the former species is more resistant to hypoxic death (17) and therefore the course of drug action could be more conveniently followed. In general, however, the convulsing concentrations were of the same magnitude for both species. One-day-old chicks weighing approximately 30 g were placed singly in plastic-covered 550-ml glass jars at an ambient temperature of 24–25°. The desired volume of agent was then introduced from a microliter syringe through the plastic cover, the liquid being ejected forcefully on the inside wall of the jar. This procedure ensured vaporization within seconds, which was proven by gas-liquid chromatographic analyses of the contents of such jars and others used in blank experiments with predetermined amounts of agent. Six chicks were used for each concentration of agent, and eight concentrations for each agent. A convulsing concentration was one that caused stretching of wings and episthotonus within 2 min from introduction of the agent. The same procedure was used for mice. These suffered violent seizures and died unless removed immediately to fresh air. Calibration curves for agent vapor in air were constructed by using a Packard model 417 Becker gas chromatograph with hydrogen flame detector and a copper column of 10% Carbowax 20M on Chromosorb 80/100.

The partial molal volume of a component is defined as the rate of increase in its content while being added at constant temperature, pressure, and mole numbers of all other components (13). The volume of a solution is not strictly equal to the sum of the volumes of its components, but is the fractional sum of its partial molal volumes. In practice, therefore, the partial molal volume is the *apparent* molal volume at low concentration. Shinoda and Hildebrand (18) described a dilatometric procedure which gave direct and accurate results. In the present study, use was made of 250-ml, benzene-filled dilatometers which could be injected directly with predetermined volumes of solute. These were constructed by sealing a 200-μl graduated capillary to the neck beyond the graduation mark. On the side of the flask at a point 2 cm distant from the neck was the injection port, which consisted of the upper part of a cone screw thread adapter (Quickfit ST 52/13) that had been sealed to the side. This was provided with a septum cut from a 3-mm-thick dense Neoprene sheet. This material was resistant to benzene at 25° for 24 hr and was not deformable, but sufficiently self-sealing to last four to six injections from a microsyringe. Each dilatometer contained a Teflon-coated magnetic stirring bar and could be filled with benzene, with the exclusion of air bubbles, after removal of the screw cap and septum. For each determination, two such dilatometers were equilibrated in a 250-liter thermostated water bath at 25° ± 0.1°. Then one of these was injected with a predetermined volume of agent, usually 100 μl, delivered from a constant-volume microsyringe, subjected to stirring for 1 min in an adjoining bath at 25° ± 0.1°, then replaced in the larger bath. Observation was then made of the rate of rise or fall of solvent level in the graduated capillary neck of both vessels. When the rates had become identical, the partial volume of solute was computed from the relation $v_{app} = (a_t - b_t) - (a_c - b_c)$, where a_t and a_c are readings in the test and control vessels at the time of injection, and b_t and b_c are the readings after the second equilibration. Readings were accurate to 0.2 μl. Reproducibility was routinely checked by injection of benzene as solute. Partial molal volumes were calculated by multiplying the molal volume of the agent at 25° ± 0.1° by the ratio $v_{app}/v_{injected}$. This procedure, although less accurate than the one described by Hildebrand, obviates the need for a temperature control to ±0.005° and ensuing problems. After a little practice, reproducibility was excellent.

RESULTS

In the series investigated, a graded response from convulsant to anesthetic activity was observed (Table 2). The most potent convulsants, compounds 1, 2, and 3, exhibited extremely steep log dose-response curves, with an induction time of 30 sec in chicks and 5–10 sec in mice. With

the less potent members, such as compounds 4 and 5, and at concentrations larger than EC_{50}, the animals quickly passed the stage of convulsions and assumed a condition of partial anesthesia characterized by loss of righting reflex, analgesia, hyporeflexia, and flaccidity. In the recovery process, the animals exhibited the same sequence of responses, but in reverse, with convulsions immediately preceding recovery. Compound 6 was convulsant in mice but anesthetic in chicks. The excitatory stage in the action of anesthetics 8–13 was more pronounced in mice than in chicks. Induction time for the anesthetics was 2–3 min. Thermodynamic potentials are expressed as the ratio of the EC_{50} to the concentration in air of the saturated vapor under identical conditions. Values of these are low for the more potent convulsants but are larger by about 1 log unit for the weaker convulsants and anesthetics (Table 2).

The partial molal volumes show a definite trend. The most potent convulsants show the greatest percentage expansion, while the most potent anesthetics show the least, or expansions of an order below the limits of sensitivity of the method used. Agents that are both excitatory and depressant display intermediate expansions (Table 3). These rates of expansion are best understood if interpreted in terms of δ, the solubility parameters of the agents concerned. The strict definition of δ is given by

$$\delta = \left(-\frac{E}{V}\right)^{1/2} = \left(\frac{\Delta H_v - RT}{V}\right)^{1/2} \quad (1)$$

where E is the internal energy of a pure compound, ΔH_v its heat of vaporization, and V its molal volume. Application of Eq. 1 in the present case requires prior knowledge of ΔH_v, which was lacking but which could be approximated by use of an equation advanced by Scott (20) for hydrocarbons, applicable also to fluorocarbons:

$$\Delta H_{v_{298^\circ}} = -2950 + 23.7T_b + 0.02T_b^2 \quad (2)$$

where T_b is the temperature of boiling; but values of δ thus obtained are certainly less dependable than experimentally derived ones. A more serious limitation to the derivation of δ from ΔH_v is its apparent failure to account for the low miscibility of fluorocarbons with hydrocarbons in what appeared as anomalous behavior (12). An alternative derivation, due to Hildebrand *et al.* (13), is based on the following relationship:

$$\frac{\bar{v}_2 - v_2^0}{v_2^0} = \frac{(\delta_1 - \delta_2)^2}{(\partial E_1/\partial V_1)_T} \quad (3)$$

where $\bar{v}_2$ is the partial molal volume of solute at infinite dilution, v_2^0 is its molal volume, δ_1 and δ_2 are the solubility parameters of solvent and solute, and $(\partial E_1/\partial V_1)_T$, in calories per milliliter is the internal pressure of solvent, which is 88.4 for benzene. Values of δ derived from $\bar{v}$ are given in Table 3, where they are also compared

TABLE 2

Potency and activity of selected inhalation convulsants and anesthetics in chicks or mice

Compound	Activity	Potency[a]	Thermodynamic potential[b]
		% vol in air	
1	Convulsant	0.05–0.08	0.005
2	Convulsant	0.15–0.30	0.007
3	Convulsant	0.16–0.32	0.008
4	Convulsant	0.40–0.50	0.009
5	Convulsant	1.0–1.7	0.02
6	Convulsant in mice	0.6–1.3	0.04
	Anesthetic in chicks	1.5–1.6	0.09
7	Convulsant	0.9–1.2	0.13
	Anesthetic	1.3–1.5	0.19
8	Anesthetic	2.0–3.4	0.04
9	Anesthetic[c]	3.4	0.06
10	Anesthetic	4.6–7.4	0.11
11	Anesthetic[d]	1.9	0.04
12	Anesthetic	1.6–2.0	0.08
13	Anesthetic	0.6–0.8	0.07

[a] EC_{50} is given as a range because of the steepness of log dose-response curves; the figures of convulsants are EC_{50} in chicks, unless otherwise stated.

[b] Calculated as the ratio of concentration at EC_{50} to that of the saturated vapor at 37° under identical conditions.

[c] See also Wallin *et al.* (19).

[d] See also Terrel *et al.* (6).

TABLE 3

Partial molal volume ($\bar{v}$), solubility parameter (δ), and excess free energy ($\bar{F}^E$) of inhalation convulsants and anesthetics in dilute benzene solution at 25°

Compound	v^0	$\bar{v}$	$(\bar{v} - v^0)/v^0$	δ calculated from		F^{Ea}
				v (Eq. 3)	H_v (Eqs. 1 and 2)	
	cc	*cc*			*(cal/cc)*$^{1/2}$	*cal mole*$^{-1}$
1	173.77	188.02	0.082	6.51	6.72	1361
2	157.02	170.68	0.087	6.43	6.77	1310
3	137.60	147.92	0.075	6.63	7.46	978
4	128.61	138.52	0.077	6.59	7.22 (6.92)[b]	944
5	129.33	136.57	0.056	6.98	7.22	673
6	145.31	153.16	0.054	7.02	7.13	728
7	153.79	158.56	0.031	7.55	7.56	432
8	132.52	137.69	0.031	7.55	6.85 (6.67)[b]	375
9	111.59	114.16	0.023	7.77	7.30	233
10	120.88	122.09	0.010	8.26	7.16	107
11	121.05	122.26	0.010	8.26	7.29	107
12	115.44	116.02	0.005	8.54	8.12	50
13	116.18	116.18	<0.005	>8.54	8.44	<50

[a] Calculated from δ derived from $\bar{v}$, Eq. 7.

[b] Calculated from H_v reported by Krantz *et al.* (4).

with values derived from ΔH_v, reported or approximated. It may be seen that convulsant property is definitely associated with low δ values while anesthetic property is associated with higher ones in a relatively narrow range extending from 6.5 to 8.5 (cal/cc)$^{1/2}$. Of particular interest is the pair of isomers, compound 4, a potent convulsant, and compound 8, an anesthetic. Until the present the opposite activity of these compounds could not be traced to any fundamental difference in the properties of the respective molecules. Also, reliance on ΔH_v reported (4) for the calculation of δ would lead to a situation in which the convulsant member would have the higher value of the two.

DISCUSSION

Two lines of thought, not necessarily mutually exclusive, emerge from the present results. One is concerned with bioavailability; thus the qualitative difference between convulsants and anesthetics could be ascribed to a quantitative difference in distribution in the biophase of relevance. That is, the low-δ convulsants tend to concentrate in one type of biophase while the high-δ anesthetics do so in another phase. There exists a sound theoretical basis in support of such an argument. A rigorous theoretical derivation of the partition coefficient, K, undertaken within the framework of this work and published elsewhere (21), shows that a compound i will distribute between two phases, 1 and 2 (at constant temperature and pressure), in accordance with the following relationship:

$$\ln K = \text{pn}\ \frac{C_{i_2}}{C_{i_1}} = \frac{2\delta_i v_i^0}{RT}(\delta_2 - \delta_1) + \bar{v}_{i_1}\left(\frac{\delta_1^2}{RT} - \frac{1}{v^0_1}\right) - \bar{v}_{i_2}\left(\frac{\delta_2^2}{RT} - \frac{1}{v_2^0}\right) \quad (4)$$

where δ_i, δ_1, and δ_2 are solubility parameters of solute i and phases 1 and 2; v_i^0, v_1^0, and v_2^0 are molal volumes of i and phases 1 and 2; and $\bar{v}_{i_1}$ and $\bar{v}_{i_2}$ are the *partial* molal volumes of i in phases 1 and 2. Thus K is a function of both δ and $\bar{v}$, and in this respect Eq. 4 has more sophistication than earlier work (22) on the relation between K and δ. For example, a simple exercise using Eqs. 3 and 4 could show that a potent convulsant such as $C_2F_5CH_2OCF_2CHFCl$ (compound 1) will partition between CCl_4 (δ = 8.6) and

CS_2(δ = 10.0) in a log ratio of 2.25, but that the anesthetic $CHCl_2CF_2OCH_3$ will do so in the same system in a log ratio of 0.26. Thus the target phase or organ could be different for convulsants and anesthetics, as argued by Schuck and Shulman (23) or by Seeman (24), who viewed specificity *in vivo* as a complex function of the precise time and space distribution of the drug in various regions of the brain. There is little evidence, however, to substantiate the requirement inherent in these views that the solubility parameter of nerve membrane originating in different organs could be of such extreme values as 9 in one case and 7 in the other. The solubility parameter of the relevant membrane phase has been assigned values close to 9 (14, 15), but one must remember that the partial constituents of that phase may differ among themselves in solubility parameter, since δ_{app} is the sum of the product of the volume fraction and δ (16):

$$\delta_{app} = \Sigma\Phi_i\delta_i \quad (5)$$

The other line of thought does not necessarily assume organ or phase specificity in the action of the inhalation convulsants and anesthetics. The two types of drugs are viewed as one homogeneous group as far as site of action is concerned, and stimulatory or convulsant activity is thought to be a function of the cohesive energy density of any particular member in the series. To illustrate this, we borrow some further concepts of regular solution theory (13). The process of mixing in such solutions is one that is accompanied by equivalent *expansion*, *absorption of heat*, and *increased fugacity*. The excess free energy in a two-component regular solution is given by the Hildebrand-Scatchard equation (13),

$$H^E = \bar{F}^E = (v_1x_1 + v_2x_2)\cdot\Phi_1\Phi_2\cdot(\delta_1 - \delta_2)^2 \quad (6)$$

which, at infinite dilution, would approximate to

$$\bar{F}^E = v_2(\delta_1 - \delta_2)^2 \quad (7)$$

Equation 7 has been applied to calculate $\bar{F}^E$ for the various drugs (Table 3) in benzene solution. If we make the reasonable assumption that the solubility parameter of the relevant membrane phase is close to that of benzene, then these values are an estimate of $\bar{F}^E$ at the biophase itself, following incorporation of these drugs. There is a *gradual* decrease in the value of $\bar{F}^E$ as one goes down from the most potent convulsant to the most potent anesthetic. Furthermore, values of $\bar{F}^E$ for all convulsants are in excess of 695 cal mole^{-1}, which is equivalent to 30 mV, the threshold potential for most excitable nerve membranes. Conceivably, then, convulsant activity will ensue when this excess free energy is of such magnitude as to surmount the barrier against the spontaneous influx of sodium ions. By the same token, all drugs, including the anesthetics, should be stimulatory to an extent commensurate with the value of $\bar{F}^E$. This is indeed the case, especially for compounds 6 and 7 which occupy a position intermediate between the anesthetics and convulsants and display both types of activity.

An important implication of Eq. 7 is that $\bar{F}^E$ is a function of both molal volume, v, and the solubility parameter of the agent. This would indicate that a decrease in v could be compensated by a decrease in δ, or vice versa. In fact, however, convulsant property seems to be associated with values of v in the range of 130–200 (3). Smaller molecules for which v is 100 or less, such as the mixed fluorochloromethanes, are either inert or slightly depressant irrespective of the value of δ. Cutoff also occurs as a result of a large increase in v or large differences between the cohesive energy densities of membrane and drug (14).

Thermodynamic data cannot be interpreted directly into mechanism of action. The most we can suggest at this stage is that convulsants provide enough free energy to open up the sodium ion channels by a lateral translation of molecules in the membrane. Anesthetics, which exert an opposite effect, stabilize membrane structure by a process akin to melting; i.e., they increase rotational energy. Whether the two opposite effects occur in the same organ remains an open question. We recall

the finding by Krantz *et al.* (4) that the action of flurothyl *in vivo* could be abolished by the administration of a structurally related anesthetic, and similar observations by Schuck and Shulman (23). In this context the reports by Rosner and Clark (25) and others (26) are of particular interest. These authors presented evidence that different anesthetics have different sequences of regional actions in the brain. These actions, excitatory or depressant, occur in the reticular formation, in nonspecific thalamic nuclei which project to wide sections of the cerebral cortex, and in the cerebral cortex itself. They postulated a dual depressant and excitatory effect, the former assuming predominance with time and increasing dosage. Remarkably, electroencephalographic evidence shows increasing signs of central nervous system irritability with the following anesthetics: diethyl ether, fluroxene, methoxyflurane, Forance, and Enflurane (27). Winters (28) contended that the anesthetic action of Enflurane, like that of nitrous oxide, is due to a cataleptoid central nervous system excitation. Several other findings show that even the pure anesthetics like halothane and pentobarbital show evidence of excitation when administered at very slow rates (29).

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