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The Action of Volatile Anesthetics and Convulsants on Synaptic Transmission: a Unified Concept

JACOB RICHTER, EMMANUEL M. LANDAU, AND SASSON COHEN

Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

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SUMMARY

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The effects of convulsant and anesthetic fluorinated ethers on synaptic transmission were studied at the frog neuromuscular junction by intracellular recording. The resting potential, input resistance, and spike threshold of the muscle fibers were unaffected by either methoxyflurane (MF, anesthetic) or hexafluorodiethyl ether (HFE, convulsant) in the concentration range from 1 nM to 1 mM. The effects of both drugs on synaptic transmission were biphasic, the end plate potential (EPP) amplitude being enhanced by low concentrations and reduced by high concentrations of the drugs. The increase in EPP amplitude was related to an increased quantal content (m), whereas reduction of the EPP was related to a decrease in the miniature EPP amplitude, which presumably was due to reduced postsynaptic sensitivity to acetylcholine. The difference in the actions of the two drugs was quantitative rather than qualitative, the effective concentrations of HFE being 10-10,000 times higher than those of MF for a corresponding effect. A model is proposed whereby these agents partition between the bulk lipids of the membrane and specific membrane subregions, in accordance with δ, the solubility parameter of the drug, and those of each of the holding phases. It is concluded that the fluorinated ethers act on specific sites in the membrane, probably belonging to the membrane proteins relevant to synaptic transmission.

INTRODUCTION

General anesthetics are believed to exert their effect on the nervous system primarily by affecting synaptic transmission (1-4). This effect is "nonspecific," as it is produced by a large variety of drugs which bear no structural similarity to the natural neurotransmitters. Two major mechanisms have been implicated. In one, the anesthetic affects synaptic transmission by increasing the fluidity, and hence disorder, of the membrane lipids (5, 6). This increased fluidity is believed to shorten the duration of the synaptic conductance event caused by a quantum of acetylcholine (7-9). In the other, the anesthetic binds to definite sites in the membrane ultrastructure that are relevant to synaptic transmission (10); such binding is believed to modulate the function of the whole structure. A good example of this is the effect of some general anesthetics on

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TABLE 1

Physical and chemical constants of drugs used

Density (d), solubility parameter (δ), molal volume (V_s), and octanol/water partition coefficient (K).

Compound	Formula	Name	d (25°)	δ[a]	V_s[a]	K[b]
				(cal/ml)$^{1/2}$	ml	
1	$CF_3—CH_2—O—CH_2—CF_3$	Hexafluorodiethyl ether[c]	1.415	6.59	128.6	135
2	$CF_3—CH_2—O—CH_2—O—CH_2—CF_3$	Hexafluoroethylal[c]	1.379	7.55	153.8	
3	$CF_3—CH_2—OCH=CH_2$	Fluroxene[d]	1.129	7.77	114.2	90
4	$CHFCl—CF_2—O—CHF_2$	Enflurane[e]	1.524	8.26	122.3	94
5	$CF_3—CH_2O—CH_2—CH_3$	Trifluorodiethyl ether[c]	1.059	8.26	122	100
6	$CHCl_2—CF_2—O—CH_3$	Methoxyflurane[e]	1.421	>8.54	116.2	400

[a] From ref. 13.

[b] From ref. 14.

[c] Synthesized by Dr. A. Goldschmid (see ref. 14).

[d] Fluoromar; Ohio Medical Products, Madison.

[e] Abbott Laboratories.

the enzymatic activity of the luciferin-luciferase system (11).

We have now approached the problem by studying the effects of such "nonspecific" agents on a wide range of membrane properties: the resting potential, input impedance, and spike threshold of the muscle fibers, the rate of transmitter release, and the size of spontaneously occurring postsynaptic potentials. We have found that methoxyflurane, a powerful anesthetic, selectively affects certain of these parameters. However, another, equally "nonspecific" ether but also a powerful convulsant, hexafluorodiethyl ether,[1] elicits effects similar to those of MF[2] but at concentrations 10–10,000 times higher. Both drugs have close octanol/water partition coefficients (see Table 1) and hence would be expected to cause, at equal concentrations, a comparable degree of membrane disorder. However, large differences in their potency could arise if they had different affinities for discrete membrane sites. We found the potency of these and additional drugs to correlate with their solubility parameter, δ. In this respect the solubility parameter appears to be a more precise guide than other physicochemical correlates for predicting the nature and intensity of drug action.

[1] Known in clinical use as Indoklon.

[2] The abbreviations used are: MF, methoxyflurane; HFE, hexafluorodiethyl ether; EPP, end plate potential; MEPP, miniature end plate potential.

MATERIALS AND METHODS

Preparations and solutions. Experiments were performed at room temperature (20–24°) on the sartorius nerve muscle preparation of the frog (*Rana ridibunda*), set in a 13-ml Perspex tissue bath. Usually the bathing solution was frog Ringer's solution of the following composition: NaCl, 116 mM; KCl, 2.5 mM; $CaCl_2$, 1.8 mM; and Tris-maleate, 4 mM (pH 7.4). For measurement of end plate potentials the $CaCl_2$ concentration was reduced to 1 mM, and 2 mM $MgCl_2$ was added. For measurement of spike threshold the muscle fibers were detubulated by treatment with 1 M ethylene glycol according to Howell and Jenden (12).

Drugs. Six fluorinated ethers were studied (Table 1). The drugs were dissolved in the bathing solution within 1 hr before the experiment. The preparation was then perfused with 100–150 ml of the test solution from a closed bottle for 5–10 min. The effect of the drugs on EPPs and MEPPs always developed during the washing-in period and remained stable for as long as 25 min, indicating that no significant evaporation could have taken place during the 5-min period needed for recording. For washout, 250–300 ml of the control solution were employed. The washout was stopped when the EPP returned to within 10% of the control value and remained stable. Experiments in which this recovery was not achieved were discarded.

Intracellular recording. Microelectrodes filled with 2.5 M potassium citrate were used. Signals were amplified via an NF-1 Biolectric electrometer and displayed on a 5031N Tektronix storage oscilloscope. Signals were photographed from the oscilloscope screen with a Polaroid camera (MEPPs) or transmitted to a computer for averaging transients (CAT-400C-TMC). Usually 50–100 signals were automatically averaged and then plotted with an *x-y* recorder (Hewlett Packard 7035B) (EPPs, spikes, and passive properties).

Measurement of passive properties. In this type of experiment two microelectrodes were inserted into the same fiber. The distance between the electrodes was less than half the diameter of the fiber. Hyperpolarizing current pulses of 10-msec duration and 100-namp amplitude were passed by a Wavetek stimulator (model 112). The injected current (I) was measured across a 1-Mohm resistor. The resulting hyperpolarization (V) was recorded, and the effective input impedance was calculated as V/I. In each experiment 100 voltage traces were averaged.

Determination of spike threshold in muscle fibers. In this type of experiment, two microelectrodes were inserted in the same fiber, as in the previous experiment. The muscles were detubulated (15). A conditioning hyperpolarizing current kept the membrane potential, prior to the depolarizing step, at −110 mV. Depolarizing current pulses of 100-msec duration were applied, and their amplitude was adjusted to depolarize the fiber to the treshold level; 50–100 spike responses were averaged in each cell, and 8–10 cells were recorded at each concentration. The same distance between the two microelectrodes was maintained in each experimental series.

Recording of EPPs and MEPPs. EPPs and MEPPs were recorded intracellularly in synaptic areas. EPPs were elicited by stimulating the sciatic nerve with supramaximal pulses from a Grass SD-9 or S-4 stimulator, at a rate of 0.5 Hz or less. Pulse width was 0.05 msec, and the stimulation electrodes were silver wires.

Determination of EPP quantal content and MEPP frequency. The quantal content (m) was determined as the ratio of the

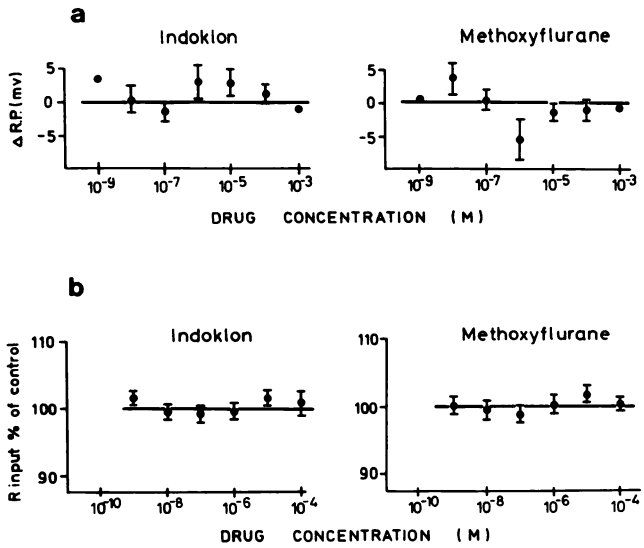


FIG. 1. *Effect of MF and HFE on passive properties of membrane*

a. Changes in the resting potential (R.P., ordinate) are shown as a function of the concentrations of either HFE (left) or MF (right). Concentrations (abscissa) are on a logarithmic scale. Vertical bars indicate ±1 SD (three to six experiments per point). Points where no bars are shown are from single experiments at a given concentration.

b. Effective input resistance (R) of the muscle fibers (as percentage of the control value, ordinate) plotted against concentration of either HFE (left) or MF (right). Concentrations (abscissa) are on a logarithmic scale. Vertical bars indicate ±1 SD (three or four experiments per point).

average EPP ($\bar{V}$) and the average MEPP amplitude ($\bar{a}$). To determine $\bar{V}$, 100 EPPs were averaged and the average amplitude was corrected for nonlinear summation (16). When the cell resting potential decreased during the experiment, the EPPs were normalized with respect to the initial driving force (resting potential, −15 mV). To determine $\bar{a}$, 85–100 MEPP amplitudes were measured from Polaroid photographs and averaged. MEPP frequency was determined by counting at least 85 MEPPs.

RESULTS

Fluorinated ethers do not affect passive membrane properties and spike threshold. In the concentration range of 1 nM–1 mM, neither (MF nor HFE had a consistent effect on the resting potential, the observed changes being smaller than ±5 mv (Fig. 1a). The input impedance and the spike threshold were equally unaffected (Figs. 1b and 2a and b). In this respect our results are at variance with those of others (17, 18), who used a different preparation or a higher agent concentration.

Fluorinated ethers exert a dual effect on the EPP amplitude. In contrast to the above findings, the EPP amplitude was clearly affected by both MF and HFE. This effect was biphasic, at low concentrations (10 nM) MF increased the EPP amplitude

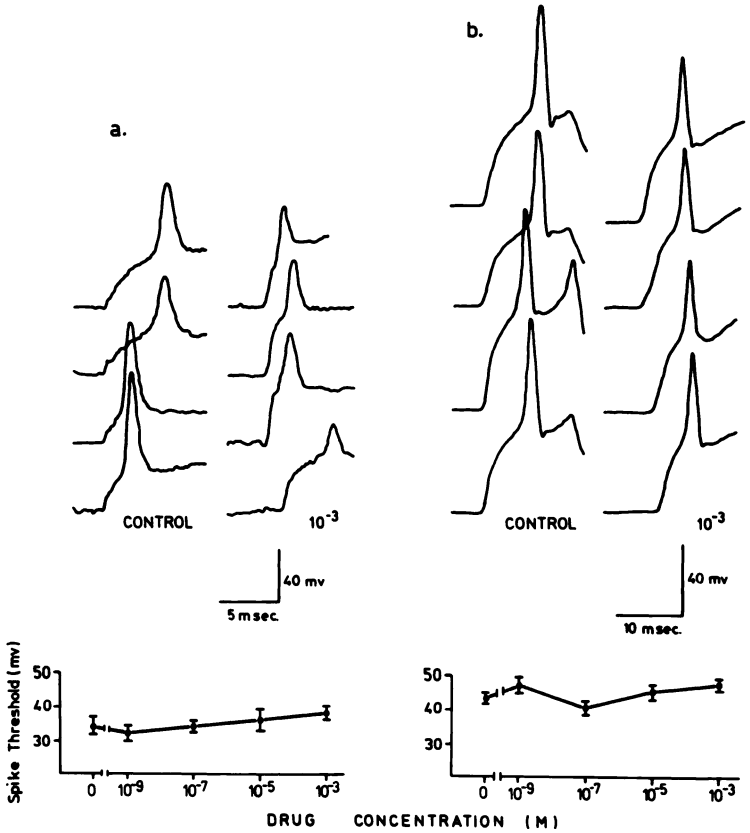


FIG. 2. *Spike thresholds in muscle fibers*

a. Effect of MF. The spike threshold is expressed as the depolarization which when added to a holding potential of −110 mV is required to produce a spike (ordinate). The concentrations of MF are on a logarithmic scale (abscissa). Each point is the average of thresholds found in 8–10 different muscle fibers. Vertical bars indicate ±1 SEM. The inset shows sample records from a number of control cells (left) and cells at 1 mM MF (right). The records were obtained by averaging 50 spikes with the CAT computer. Calibration: vertical, 40 mV; horizontal, 5 msec.

b. Effect of HFE. The axes of the graph and the insets have the same meaning as in Fig. 2a. Sample records shown in the inset were obtained by averaging 100 spikes. Calibration: vertical, 40 mV; horizontal, 10 msec.

by 25% (Fig. 3a) but at higher concentrations (0.1 mM) the same drug decreased the EPP amplitude by 60% (Fig. 3b). The increase was attributed to enhanced transmitter release (increased m), and the decrease was attributed to a parallel decrease in MEPP, which is usually taken to indicate a reduced postsynaptic effect of acetylcholine (19). Both effects were entirely reversible.

The same phenomenon was observed with HFE, but the increase in EPP amplitude occurred at a higher concentration (0.1 mM) than with MF, as did the decrease (1 mM) (Fig. 4a and b).

A complete dose-response curve was determined for each of the two drugs (Fig. 5a). In each case the drug effect was at least biphasic and followed essentially the same course, except that the dose-response curve for HFE was shifted toward higher concentrations with respect to that of MF. Thus the maximum increase in EPP amplitude occurred at approximately 10 nM

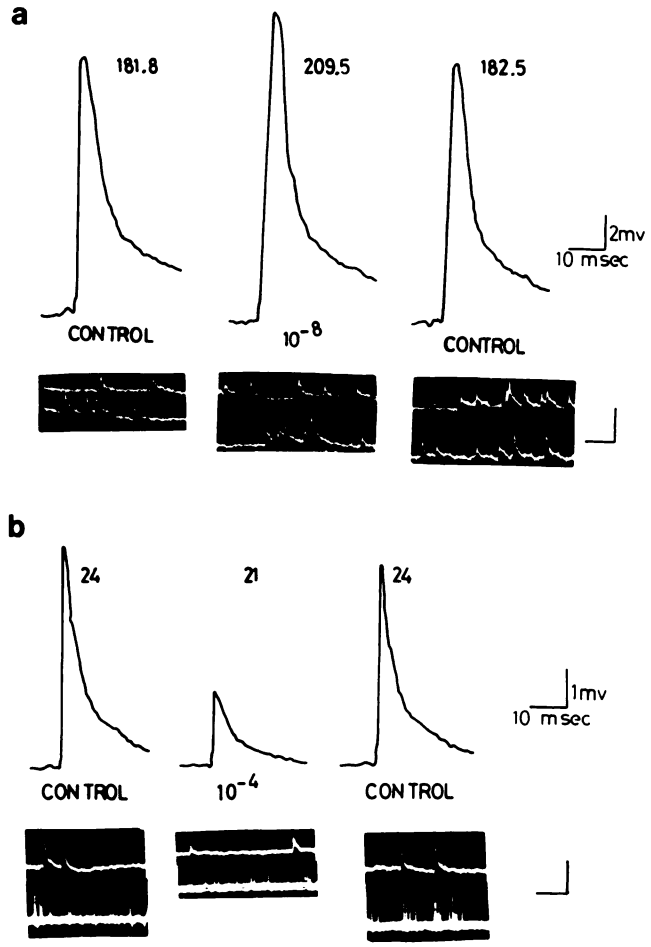


FIG. 3. *Effect of MF on synaptic transmission*

a. Upper row: each value is the average of 100 EPPs. From left to right: control; 10 nM; after washout. The number above each EPP trace is the mean quantal content. Calibration: vertical, 2 mV; horizontal, 10 msec. Lower traces: sample MEPPs recorded immediately after the EPPs. Calibration: vertical, 0.1 mV; horizontal, 20 msec.

b. Upper row: average EPPs as in Fig. 3a. From left to right: control; 0.1 mM; after washout. EPP quantal content is marked above each trace. Calibration: vertical, 2 mV; horizontal, 10 msec. Lower traces: sample MEPPs as in Fig. 3a. Calibration: vertical, 0.25 mV; horizontal, 20 msec and 1 sec. An amplitude histogram of the MEPPs at 0.1 mM showed a normal distribution.

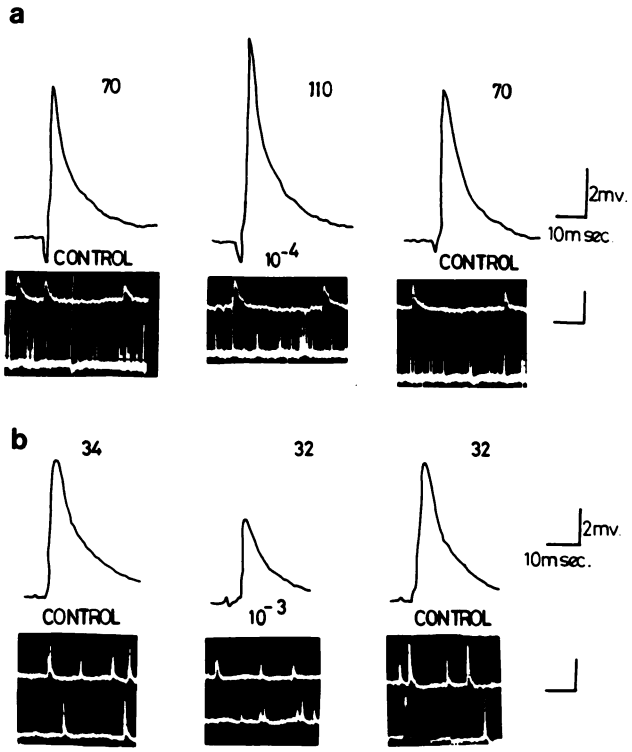


FIG. 4. *Effect of HFE on synaptic transmission*

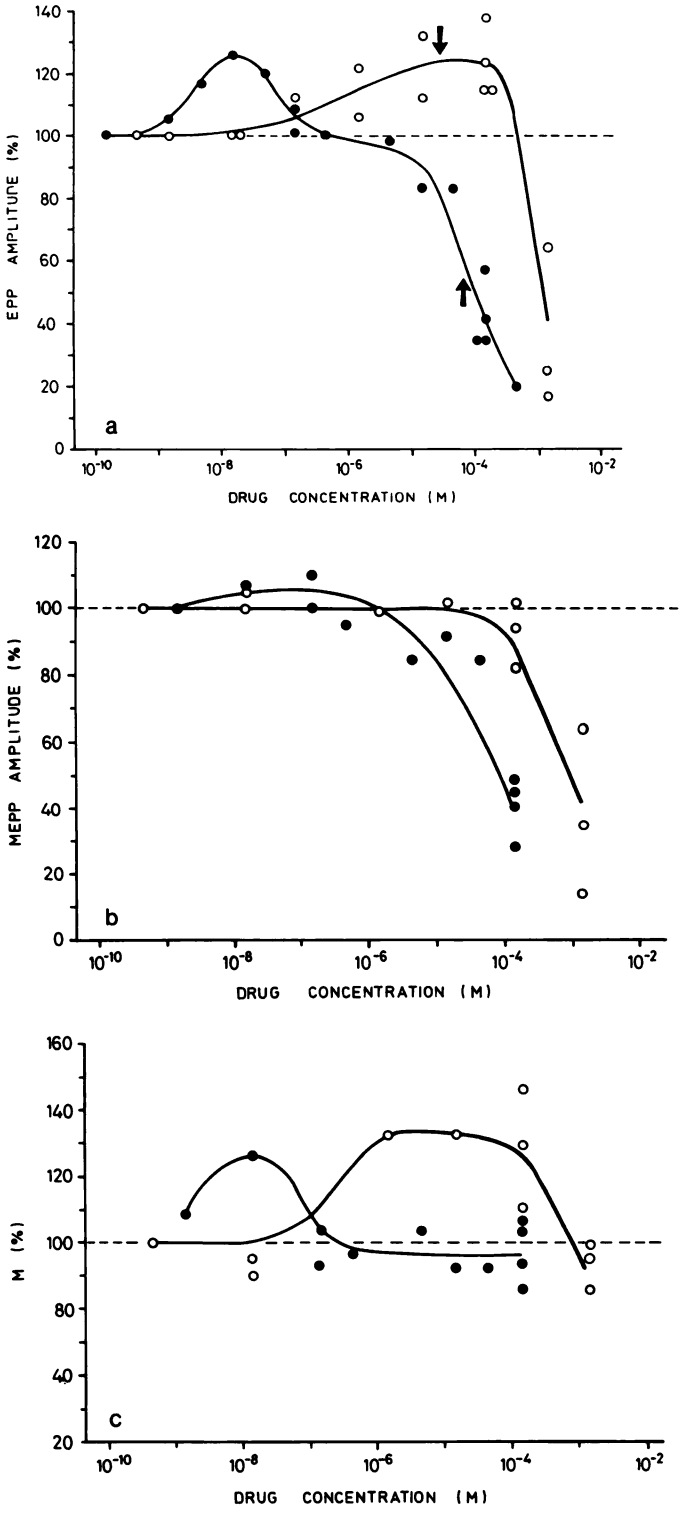
a. Upper row: average EPPs as in Fig. 3. Quantal content is marked above each trace. From left to right: control; 0.1 mM; after washout. Calibration: vertical, 2 mV; horizontal, 10 msec. Lower traces: sample MEPPs as in Fig. 3. Calibration: vertical, 0.25 mV; horizontal, 20 msec for the upper trace and 1 sec for the lower.

b. Upper row: average EPPs as in Fig. 4a. Quantal content is marked above each trace. From left to right: control; 1 mM; after washout. Calibration: vertical, 2 mV; horizontal, 10 msec. Lower traces: sample MEPPs. Calibration: vertical, 0.25 mV; horizontal, 50 msec. An amplitude histogram constructed for the MEPPs at 1 mM exhibited a normal distribution.

MF but at 0.1 mM HFE, followed by strong depression at 0.1 mM and 1 mM, respectively. At these high concentrations the MEPP amplitudes were correspondingly depressed by both drugs (Fig. 5b). The quantal content (m) exhibited a biphasic course (Fig. 5c), rising to a maximum and then declining to about the control level. The peak increase in m for both drugs coincided with the peak increase in the MEPP amplitude. MEPP frequency was measured in all the experiments of Fig. 5 and was found to be unaffected by either MF or HFE (±3% of control). This finding indicates that the increase in transmitter release is probably not associated with poisoning of presynaptic mitochondria (22). In view of the enhancement in post-tetanic potentiation found after treatment with MF (3), the increased m may be due to augmented calcium influx into the nerve terminals during stimulation.

The ratios between equipotent concentrations of the two drugs were different for each of the three synaptic phenomena described; thus the HFE:MF ratio for causing a 50% increase in m was about 100, that for a 50% decline of m back to control was about 10,000, and that for 50% of the MEPPs was approximately 10.

MF occludes effect of HFE. The structural similarity of the two drugs and their comparable dose-response curves (Fig. 5) suggested that they both act at common membrane sites, where they produce a comparable effect when occupying an


EPP AMPLITUDE (%)
140
120
100
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a
10^{-10}
10^{-8}
10^{-6}
10^{-4}
10^{-2}
DRUG CONCENTRATION (M)
MEPP AMPLITUDE (%)
120
100
80
60
40
20
0
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DRUG CONCENTRATION (M)

equal fraction of the sites. The difference between the effective bath concentrations of the drugs would then reflect different affinities for these particular sites. If so, it could be predicted that the combined and equal bath concentrations of both drugs will be as effective as MF alone plus an increment of 0.01–10% (see Fig. 5). In other words, at those concentrations where the effects of the two drugs differ in quality of action (0.1 mM), the effect of HFE will be occluded. This prediction was borne out by experiments. The combined effect of MF and HFE, both at 0.14 mM, and the predicted values are shown in Table 2. The calculated values agree with the observed ones; noteworthy is the reduced m (85%) as opposed to m with HFE alone (135%).

Correlation between synaptic effects and solubility parameter of fluorinated ethers. The difference of 1, 2, and 4 logarithmic units between the equipotent bath concentrations of MF and HFE (Fig. 5) could not be attributed to the difference between their oil/water partition coefficients, since that of MF is only 3 times larger (Table 1). Instead we considered their solubility (δ), which was found recently to correlate well with the pharmacological effect of fluorinated ethers *in vivo* (13). Knowing δ, which is a measure of the van der Waals interactions between molecules (23), one can derive the distribution of a nonpolar solute between two nonpolar or slightly polar solvents (24). It is generally believed that the volatile anesthetics act in the hydrophobic region of the excitable membrane (25).

If in the present case the specific sites of action of these drugs are simulated by a phase having a higher δ value than the bulk lipid of the membrane, then the higher will be the δ value of the drug, the more it will tend to associate with these sites, and the lower will be its effective bath concentration (see DISCUSSION). Cohen *et al.* (13) found that MF has a higher δ value than HFE (Table 1). This explains the results shown in Fig. 5, where the dose-response curve for HFE (low δ) is shifted to the right with respect to that for MF (high δ).

To test further the possibility of a correlation between δ and the synaptic events, we studied the effect of three additional fluorinated ethers of intermediate δ values (drugs 2, 3, and 4 in Table 1). The experiments were conducted at a single concentration (0.14 mM) where the difference between the effects of MF and HFE on the EPP and MEPP amplitude was greatest. If we assume that the dose-response curves of the fluorinated ethers are similar in shape but shift to the right with decreasing δ, we would expect their synaptic effect at the given concentration to vary systematically with δ. EPPs and MEPPs were

TABLE 2

Observed and predicted values for combined treatment with MF and HFE, both at 0.14 mM

All values are expressed as a percentage of the control value before treatment with the drug, and results are means ± standard errors of three experiments. The predictions are based on data from Fig. 5.

	Expected	Observed
EPP amplitude	46	47 ± 7
MEPP amplitude	44	52 ± 9
m	98	85 ± 10

FIG. 5. *Dose-response curves of MF (●) and HFE (○)*

a. EPP amplitudes as percentage of the control value (ordinate) are plotted against drug concentration (molar, abscissa, logarithmic scale). Each point is from a different experiment like those shown in Figs. 3 and 4. The upper arrow indicates the probable concentration of HFE *in vivo* for clinical purposes (see ref. 20). The lower arrow indicates the same for MF (21). The lines were fitted by eye.

b. MEPP amplitudes as percentage of control (ordinate) are plotted against drug concentration (molar, abscissa, logarithmic scale). Each point corresponds to a given point in Fig. 5a. However, not each point in Fig. 5a has a corresponding point in Fig. 5b. MEPP amplitude histograms were constructed for those points where the MEPPs were reduced to 40% or less. In each case the distribution was normal. The lines were fitted by eye.

c. EPP quantal contents as percentage of control (ordinate) plotted against drug concentration (molar, abscissa, logarithmic scale). Each point corresponds to a given point in both Fig. 5a and b. The lines were fitted by eye.

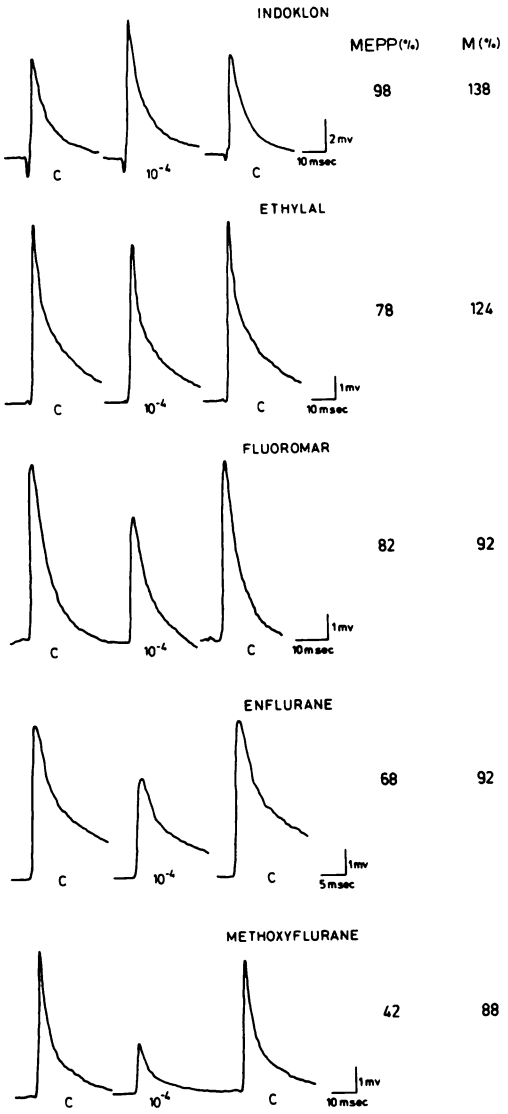


FIG. 6. *Effect of five fluorinated ethers on synaptic transmission*

Each row represents an experiment with a different ether. The name of the ether is shown above each row. Each series contains an average EPP for the control, after exposure to 0.14 mM drug, and after washout. The numbers at the end of each row describe the MEPP amplitude and the quantal content (m) in the presence of the drug as percentage of control. Calibrations of time and voltage are shown near each row.

recorded after treatment with each of these drugs (Fig. 6), and this expectation was confirmed. Thus Fig. 7 shows a good correlation between δ and (a) the EPP amplitude ($r = 0.96, p < 0.001$), (b) the MEPP amplitude ($r = 0.87, p < 0.001$), and (c) the quantal content m ($r = 0.83$, $p < 0.001$). Another ether (drug 5) was not considered because its effect was not reversible.

DISCUSSION

An interesting finding in this study is the high degree of selectivity exhibited by the "nonspecific" drugs. For example, MF considerably enhances transmitter release at 3 nM, but brings it halfway back to the control level at 60 nM, then decreases postsynaptic sensitivity by 50% at 80 μM. On the other hand, the membrane impedance was not affected by concentrations up to 0.1 mM, and the resting potential and spike threshold remained unchanged even at 1 mM. The results of Karis, Gissen, and Nastuk (26) for the effects of ether on neuromuscular transmission in the frog and those of Richards *et al.* (3) for the effect of MF on synaptic transmission in mammalian brain are in general agreement with our results. It is difficult to envisage how nonspecific disordering of the membrane lipids (25) can lead to such a high degree of selectivity. Also it is difficult to understand how drug concentrations as low as 1 nM or even 0.1 mM can cause appreciable disordering of the membrane lipids, if one recalls that the concentration required to produce nerve blockade or 50% antihemolytic effect on red blood cells approaches 10 mM (27). A more likely possibility is that the fluorinated ethers act on definite membrane sites, as suggested earlier by Mullins (10) (cf. refs. 11 and 28). Such sites are probably located on the relevant gating molecules such as the presynaptic calcium channel and the postsynaptic acetylcholine receptor, and serve to modulate their action. A similar mechanism has been recently proposed by Boggs and her co-workers, based on spin label studies of lipid vesicles and nerve membranes (29, 30). It will be interesting to see whether this concept of modulatory sites can be applied to other phenomena in membrane physiology.

Our findings indicate that the activity of fluorinated ethers cannot be predicted

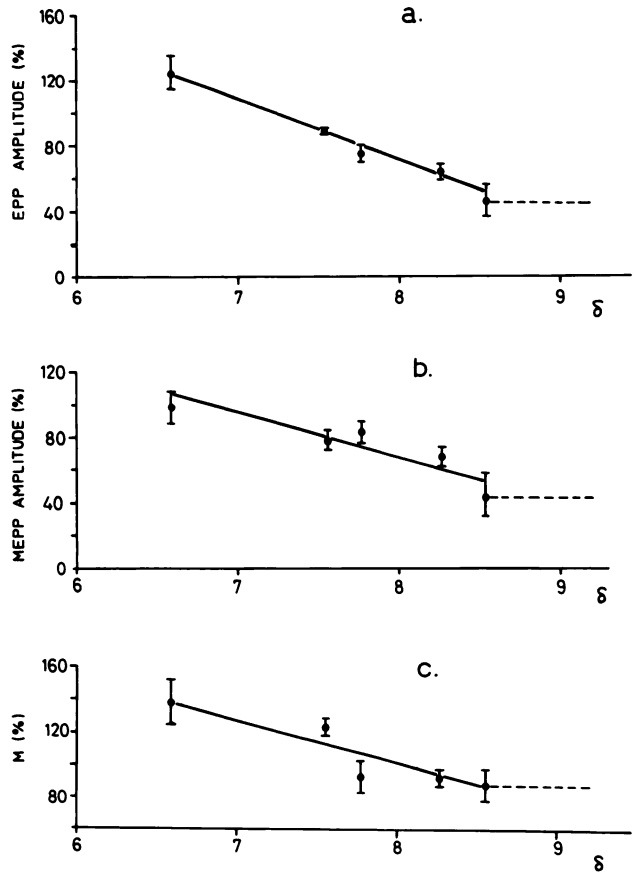


FIG. 7. *Correlation between characteristics of synaptic transmission and solubility parameter, δ*

EPPs and MEPPs were measured at a 0.14 mM concentration of drugs 1, 2, 3, 4, and 6 (Table 1). Each point is the average of three or four experiments like those shown in Figs. 3 and 4. Vertical bars denote ±1 SD. There is some uncertainty concerning the δ value of MF, which lies between 8.54 and 9.2, as indicated by the broken horizontal line. The straight lines connecting the points were fitted by eye. Each point in Fig. 7a corresponds to the given points in Fig. 7b and c. The first point on the left in all the figures belongs to HFE (drug 1), and the other points, from left to right, belong to drugs 2, 3, 4 (enflurane), and 6 (MF).

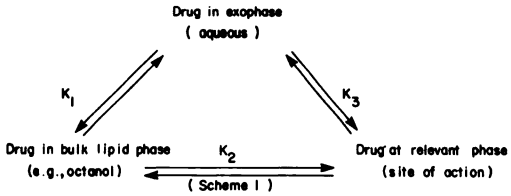
a. Correlation between EPP amplitudes (as percentage of control; ordinate) and δ values (abscissa). The correlation coefficient (r) was 0.96, which is significant at the 0.001 level (t-test). The correlation was calculated by taking the results from the experiments individually (n = 16).

b. Correlation between MEPP amplitude (as percent of control; ordinate) and δ values (abscissa). r was 0.87, which is significant at the 0.001 level (t-test; n = 15).

c. Correlation between the quantal content (as percentage of control; ordinate) and δ values (abscissa). r was 0.82, which is significant at the 0.001 level (t-test; n = 15).

from their octanol/water partition coefficient (K). Thus HFE is less effective than enflurane in reducing the MEPP amplitude (Fig. 6) even though its K is higher (Table 1). Also the presynaptic potency of MF is 2–4 log units greater than that of HFE (Fig. 5c), while their K values are both within $\log K = 2$ (Table 1). Therefore one is led to the conclusion that any partition of relevance to the action of these drugs is between the exophase and a specific membrane subregion, or between the bulk lipid and a specific site, the mere presence of the agent in the bulk lipid phase being rather circumstantial. This situation at equilibrium can perhaps be

illustrated as in Scheme 1, where K_1 is the



octanol/water partition coefficient (the macroscopic partition coefficient), K_2 is the microscopic partition coefficient, which may be derived from δ values of the corresponding phases and that of the agent (24), and K_3 is the partition coefficient between the exophase and the relevant phase and is equal to $K_1 \times K_2$; hence K_3 is sensitive to both K_1 and δ.

This difference in potency between MF and HFE may contribute to the resolution of a long-standing paradox in the theory of anesthesia, namely, that closely related fluorinated ethers produce either anesthesia or convulsions, or both (31). This discrepancy has been attributed to differences in the time and space distribution of these drugs (25). On the other hand, our results indicate that anesthetics and convulsants affect synaptic transmission in a similar manner; the different nature of the effects elicited *in vivo* can be related to different effective concentrations in cholinergic and other synapses in the central nervous system. Note that the presumed concentrations of HFE and MF *in vivo* fall in a range where the first depresses and the second enhances synaptic transmission (see Fig. 5). Thus an inversion of the biological effect within a series of drugs can be achieved by a shift in effective concentrations, provided that their dose-response curves are biphasic.

This approach also allows for an inversion of the order of potency with a change in the constitution of the site of action. Thus MF is more effective than HFE in suppressing excitatory synapses, but the reverse could be true for inhibitory synapses, in which HFE could be the more effective of the two.

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REFERENCES

1. Larrabee, M. G. & Holaday, D. A. (1952) *J. Pharmacol. Exp. Ther.*, 105, 400–408.
2. Larrabee, M. G. & Posternak, J. M. (1952) *J. Neurophysiol.*, 15, 91–114.
3. Richards, C. D., Russell, W. J. & Smaje, J. C. (1975) *J. Physiol. (Lond.)*, 248, 121–142.
4. Richards, C. D. & White, A. E. (1975) *J. Physiol. (Lond.)*, 252, 241–257.
5. Trudell, J. R., Hubbell, W. L. & Cohen, E. N. (1973) *Biochim. Biophys. Acta*, 291, 321–327.
6. Ueda, I., Shieh, D. D. & Eyring, H. (1974) *Anesthesiology*, 41, 217–225.
7. Gage, P. W., McBurney, R. N. & Van Helden, D. (1974) *Life Sci.*, 14, 2277–2283.
8. Gage, P. W., McBurney, R. N. & Schneider, G. T. (1975) *J. Physiol. (Lond.)*, 244, 409–429.
9. Gage, P. W. and Hamill, O. (1975) *Neurosci. Lett.*, 1, 61–65.
10. Mullins, L. J. (1971) in *Handbook of Neurochemistry*, Vol. VI, *Alterations of Chemical Equilibrium in the Nervous System* (Lajtha, A., ed.), pp. 395–421, Plenum Press, New York.
11. Ueda, I. & Kamaya, H. (1973) *Anesthesiology*, 38, 425–435.
12. Howell & Jenden (1965) *Fed. Proc.*, 26, 553.
13. Cohen, S., Goldschmid, A., Shtacher, G., Srebrenik, S. & Gitter, S. (1975) *Mol. Pharmacol.*, 11, 379–385.
14. Goldschmid, A. (1975) "Structure Activity Relationship in Halogenated Anesthetics," Ph.D. thesis, Tel Aviv University.
15. Anderson, C. R. & Steven, C. F. (1973) *J. Physiol. (Lond.)*, 235, 655–691.
16. Martin, A. R. (1955) *J. Physiol. (Lond.)*, 130, 114–122.
17. Chalazonitis, N. (1967) *Anesthesiology*, 28, 111–123.
18. Inoue, F., Pinsky, C. & Sanders, H. D. (1967) *Can. J. Physiol. Pharmacol.*, 45, 758–766.
19. Waud, B. E. & Waud, D. R. (1975) *Anesthesiology*, 42, 275–280.
20. Karliner, W. & Padula, L. J. (1962) *J. Neuropsychiatr.*, 3, 159–162.
21. Secher, O. (1971) *Acta Anaesthesiol. Scand. Suppl.*, 42, 1–95.
22. Alnaes, E. & Rahamimoff, R. (1975) *J. Physiol. (Lond.)*, 248, 285–306.
23. Hildebrand, J. H., Prausnitz, J. M. & Scott, R. L. (1970) *Regular and Related Solutions*, Van Nostrand Reinhold, New York.
24. Srebrenik, S. & Cohen, S. (1976) *J. Phys. Chem.*, 80, 996–999.
25. Seeman, P. (1972) *Pharmacol. Rev.*, 24, 583–655.

26. Karis, J. H., Gissen, A. J. & Nastuk, W. L. (1966) *Anesthesiology*, 27, 42–51.
27. Roth, S. & Seeman, P. (1971) *Nature*, 23, 284–288.
28. Balasubramanian, D. & Wetlaufer, D. B. (1966) *Proc. Natl. Acad. Sci. U. S. A.*, 55, 762–765.
29. Boggs, J. M., Yoong, T. & Hsia, J. C. (1976) *Mol. Pharmacol.*, 12, 127–135.
30. Boggs, J. M., Roth, S. H., Yoong, T., Woong, E. & Hsia, J. C. (1976) *Mol. Pharmacol.*, 12, 136–143.
31. Krantz, J. C., Rudo, F. G. & Loecher, C. K. (1967) *Proc. Soc. Exp. Biol. Med.*, 124, 820–822.