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EFFECTS OF SEVERAL INHALATION ANAESTHETICS ON THE KINETICS OF POSTSYNAPTIC CONDUCTANCE CHANGES IN MOUSE DIAPHRAGM

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1 Miniature endplate currents were recorded with extracellular electrodes in mouse diaphragms in order to measure the kinetics of the conductance change produced by acetylcholine. Miniature endplate potentials (m.e.p.ps) were recorded intracellularly in the same fibres in which the currents were being recorded.

2 The general anaesthetics, ether, halothane, chloroform and enflurane at low (anaesthetic) concentrations increased the rate of decay of miniature endplate currents (m.e.p.cs) and reduced the amplitude of m.e.p.ps in this way.

3 At high concentrations the anaesthetics caused a reduction in the amplitude of both m.e.p.cs and m.e.p.ps, and a decrease in the rate of decay of the currents. With halothane and enflurane the decay of some currents became biphasic, with a prolonged tail.

4 It was proposed that the increased rate of decay of the conductance caused by the four agents at anaesthetic concentrations is due to an increase in the fluidity of the subsynaptic membrane. Prolongation of the currents at higher concentrations may be caused by an increase in membrane dielectric constant.

5 The effectiveness of the four anaesthetics in producing a 30% decrease in the time constant of decay of m.e.p.cs was shown to be related to their oil/water partition coefficients and followed closely the relationship between anaesthetic potency and oil/water partition coefficient. It is suggested therefore that the four anaesthetics may produce anaesthesia by changing the kinetics of postsynaptic conductance changes at synapses, perhaps by increasing membrane fluidity.

Introduction

It has been proposed that some pharmacological agents may depress synaptic transmission by increasing the fluidity of postsynaptic membranes (Gage, McBurney & Van Helden, 1974; Gage, 1976). Octanol, which has been shown to increase the fluidity of nerve and muscle membranes (Grisham & Barnett, 1973), increases the rate of closing of ionic channels activated by the interaction of acetylcholine with receptors so that the duration of endplate currents is decreased (McBurney & Gage, 1972; Gage *et al.*, 1974). It was suggested that the reduced duration of endplate currents was due to an increase in membrane fluidity and that any agents which increase membrane fluidity would have a similar effect.

The inhalation anaesthetics ether, chloroform, and halothane have been shown to increase the fluidity of phospholipid membranes (Johnson & Bangham, 1969; Trudell, Hubbell & Cohen, 1973). Ether and halothane also depress neuromuscular transmission (Karis, Gissen & Nastuk, 1966; Gissen, Karis & Nastuk, 1966; Galindo, 1971). The object of these experiments was to determine whether the inhalation anaesthetics depress neuromuscular transmission by reducing the duration of ionic current generated by the interaction of acetylcholine with receptors.

Brief descriptions of similar results in toad preparations have appeared elsewhere (Gage & Hamill, 1975; Hamill & Gage, 1975).

Methods

The preparation used for the experiments was the excised mouse diaphragm. Miniature endplate potentials (m.e.p.ps) were recorded with standard intracellular microelectrode techniques. Miniature endplate currents (m.e.p.cs) were recorded extracellularly using microelectrodes filled with 1 M NaCl in agar. These electrodes generally had a resistance of 0.2–1MΩ and a tip diameter of 10–100 μm. Care was taken when manipulating electrodes at junctional regions not to press too hard against the muscle fibre as pressure on nerve terminals may cause alterations

in the time course of m.e.p.cs (Katz & Miledi, 1973a). When m.e.p.ps and m.e.p.cs were recorded simultaneously, m.e.p.cs were first located by searching over endplate regions with an extracellular electrode and then an intracellular electrode was inserted into the muscle fibre where m.e.p.cs were being recorded. When this was done successfully, m.e.p.cs and m.e.p.ps could be recorded simultaneously. The intracellular electrode was positioned as close as possible to the extracellular electrode (within 100 μm).

Two methods were used to record spontaneous m.e.p.cs and m.e.p.ps which occur randomly at a low frequency. In some experiments m.e.p.cs were 'captured' by use of an on-line laboratory mini-computer (LAB 8/l, Digital Equipment) which was programmed to store and display the m.e.p.cs in a stationary position on the oscilloscope screen. These m.e.p.cs could be photographed for further analysis. In most experiments a hard-wired device which performed the same function (Neurograph N3, Transidyne General Corp) was used.

The time course of m.e.p.cs was measured from film of oscilloscope traces obtained during an experiment. The trace was magnified with a film projector and projected onto calibrated graph paper. A m.e.p.c. normally has two main phases consisting of a relatively rapid increase to a maximum amplitude (peak) followed by a slower decay. These two phases have been called (Gage & McBurney, 1975) the 'growth phase' and 'decay phase' respectively. Because the growth phase of the m.e.p.cs tends to be s-shaped, and the baseline noise obscures its early part, the 'growth time' was measured as the time for an m.e.p.c. to increase from 20 to 80% of its maximum amplitude. Using the Neurograph, it was possible to 'sweep out' individual m.e.p.cs after they had been stored and hence accurate measurements could be made of the growth time. M.e.p.cs were recorded as voltage deflections with peak amplitudes of 0.05 to 1 mV. The amplitude depends essentially on the resistance between the solution in the recording microelectrode and the grounded outside solution (Gage, 1976). Because the magnitude of this resistance is never known, currents are expressed in terms of voltage rather than amperage.

The time course of an m.e.p.c. was taken to reflect accurately the time course of the transient conductance increase produced by a quantum of acetylcholine (Gage & McBurney, 1975; Gage, 1976). Because of the variation in their time course, 20–30 m.e.p.cs were averaged for any one estimate of m.e.p.c. time course.

The anaesthetics, diethyl ether (ether); 2-chloro-1,1,2-trifluoroethyl-difluoromethyl ether (enflurane); 1,1,1-trifluoro-2 bromoethane (halothane) and chloroform were dissolved directly in the normal physiological mouse saline (Krebs solution) which was bubbled with a gas mixture of 5% CO_2 and 95% O_2, giving a pH of 7.2.

The bath temperature was controlled to within ±0.5°C. Experiments were usually done at temperatures below 30°C rather than at higher temperatures because this seemed to improve the durability of preparations. Later experiments have shown similar effects at 37°C (Hamill, Gage & Spence, 1976).

Results

As in amphibian muscle, m.e.p.cs in the mouse diaphragm had a rapid growth phase and slower decay phase (Gage & Armstrong, 1968; Gage & McBurney, 1972; 1975). The growth time (20–80%) ranged from 100 to 300 μs at 23°C. The decay was exponential as in amphibia (Takeuchi & Takeuchi, 1959; Magleby & Stevens, 1972a; Gage & McBurney, 1972). This is illustrated in Figure 1 in which the lower trace (points) show an m.e.p.c. recorded at 23°C. Inset is a graph of the logarithm of current amplitude against time from the peak and the decay is clearly exponential. The time constant of decay in this experiment was 1.4 milliseconds. It was found that m.e.p.cs in mouse diaphragm had a more rapid decay than m.e.p.cs in toad muscle (Gage & McBurney, 1972; 1975) at the same temperatures. In the upper trace is shown the m.e.p.p. generated by the current below. Its time course is slower than that of the current because of the capacity of the surface membrane of the muscle fibre.

Low concentrations of the anaesthetics

Low concentrations (see Table 1) of all four anaesthetics caused a more rapid decay of m.e.p.cs but no decrease in their amplitude. Because the integral of the current (charge displacement) was reduced, m.e.p.ps were reduced in amplitude (Gage & McBurney, 1973). These effects seen with 1 mM halothane are illustrated in Figure 2. The experiment was carried out at 20°C. A m.e.p.p. (above) and the m.e.p.c. (below) which generated it, were recorded in control solution (a). The time constant of decay of the m.e.p.c. was 1.9 milliseconds. After 15 min in 1 mM halothane (b) there was a noticeable increase in the rate at which m.e.p.cs decayed and the time course of their decay was still exponential. The time constant of decay of the m.e.p.c. in Figure 2b was reduced 37% to 1.2 ms by the halothane solution. The decreased decay time constant of the currents was associated with a reduction in m.e.p.p. amplitude (Figure 2b) as would be expected (Gage & McBurney, 1973). In this case (Figure 2) there was a 32% reduction in the amplitude of the m.e.p.p.

The decay of m.e.p.cs in the presence of low concentrations of all four anaesthetics remained exponential. This is illustrated in Figure 3 in which the logarithm of the amplitude of m.e.p.cs (recorded in

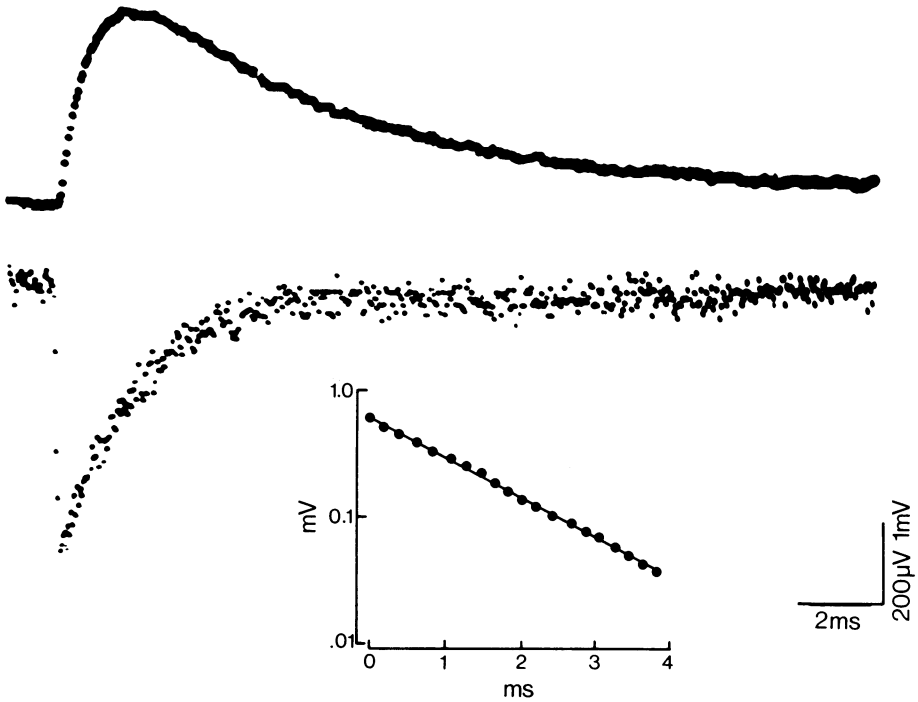

Figure 1 A miniature endplate potential (m.e.p.p.) (above) and miniature endplate current (m.e.p.c.) (below) recorded in control solution (23°C). Vertical calibration; 1 mV for the upper trace, 0.2 mV for the lower. Horizontal calibration; 2 ms. Inset is a semilogarithmic plot of current amplitude (mV) against time measured from the peak (ms) illustrating the exponential decay of the m.e.p.c.

four different experiments) has been plotted against time after the peak, in 10 mM ether, 1 mM chloroform, 1 mM enflurane and 1 mM halothane. There was no appreciable deviation from exponential decay and the time constant of decay was significantly reduced by each of the anaesthetics.

The pooled results from several experiments with low concentrations of each of the four anaesthetics are shown in Table 1. It can be seen that they all caused a significant decrease in the time constant of decay of m.e.p.cs. This effect was not accompanied by any change in the resting potential of muscle fibres and endplate potentials could still be elicited by stimulation of the phrenic nerve indicating that excitation-secretion coupling in the nerve terminals remained intact.

Higher concentrations of anaesthetics

The decrease in the time constant of decay of m.e.p.cs was found not to increase progressively with anaesthetic concentration. The changes in time course

Table 1 Reduction in the time constant of decay (τ) of miniature endplate currents caused by inhalation anaesthetics (23°C)

Anaesthetic	*Concentration (mM)*	*τ (ms) Control*	*Test*	*% Reduction in τ*
Ether	10	1.62 ± 0.02	1.065 ± 0.02	34%
Halothane	0.2	1.68 ± 0.02	1.18 ± 0.02	29%
Chloroform	0.5	1.51 ± 0.05	0.99 ± 0.01	34%
Enflurane	0.8	1.60 ± 0.02	1.14 ± 0.03	29%

Means are shown ± s.e. mean.

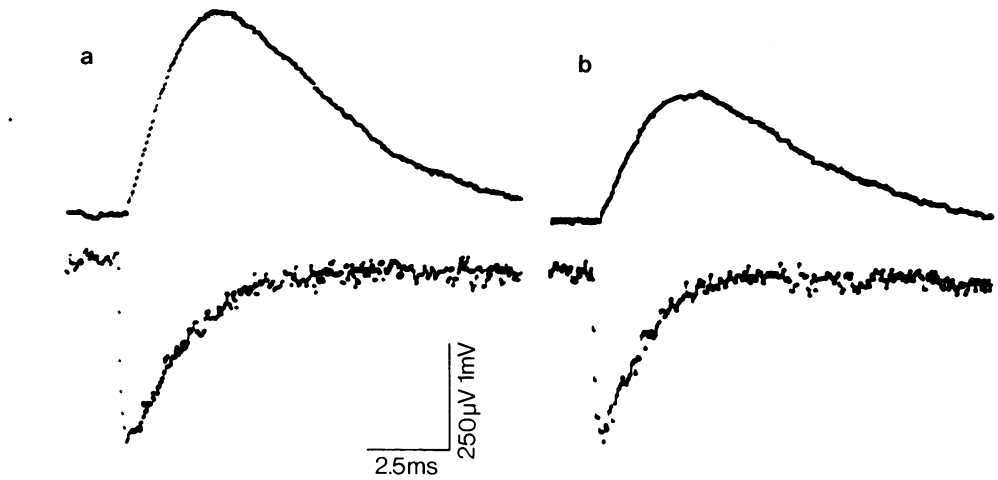

Figure 2 Halothane (1 mM) reduces the amplitude of m.e.p.ps (upper traces) by reducing the time constant of decay of m.e.p.cs (lower traces). Representative records are shown in control solution (a) and in 1 mM halothane (b). Vertical calibration; 1 mV for m.e.p.ps, 0.25 mV for m.e.p.cs. Horizontal calibration; 2.5 ms; experiments were done at 20°C.

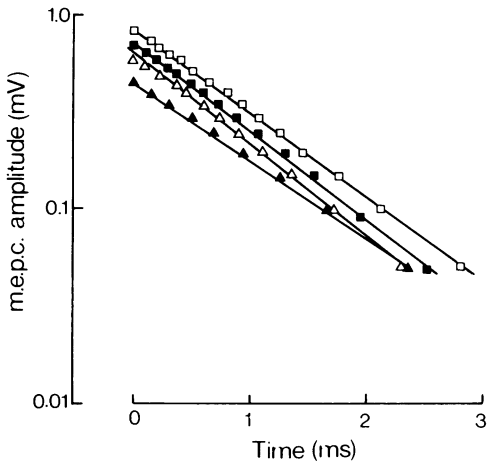

Figure 3 The amplitude of m.e.p.cs during the decay phase is plotted against time after the peak, for m.e.p.cs in 10 mM ether (□), 1 mM chloroform (■), 1 mM enflurane (△) and 1 mM halothane (▲), showing that the decay of m.e.p.cs remains exponential in the presence of the anaesthetics.

caused by higher concentrations were different for different anaesthetics and these will be described separately.

Ether. The effects of high concentrations of ether on m.e.p.cs are illustrated in Figure 4 with representative records obtained in one experiment. A m.e.p.c. in the control solution is shown in Figure 4a. In (b) (45 mM ether), there was a reduction in the amplitude of m.e.p.cs and an increase in the time constant of decay. This produced m.e.p.ps which were reduced in amplitude and had a slower time course than normal. These effects were even more pronounced in 55 mM ether (Figure 4c). Complete recovery (Figure 4d) was obtained by washing out the ether with control solution.

Averaged results obtained in four such experiments with a range of ether concentrations are shown in Figure 5 (23°C). The mean time constant of decay (τ) of m.e.p.cs in the control solution is shown as a horizontal line with ± 1 s.e. mean (broken lines). The points with vertical bars show the mean $\tau \pm 1$ s.e. mean measured from 20–30 m.e.p.cs in each of the four preparations. There is clearly a reduction in τ with ether concentrations from 2 to 25 mM. At concentrations above 40 mM, ether caused an increase in τ. However, the increases in τ were associated with a decrease in amplitude of m.e.p.cs so that no increase in amplitude of m.e.p.ps was produced by the lengthening of the current (Gage & McBurney, 1973). It was noticed that there was no significant change in the growth time of m.e.p.cs in spite of the large changes in τ.

Halothane differed from ether in two ways. Firstly, significant changes in the time constant of decay of m.e.p.cs occurred at much lower concentrations of halothane, 0.2 mM halothane producing much the same effect as 7.5 mM ether. Secondly, with higher

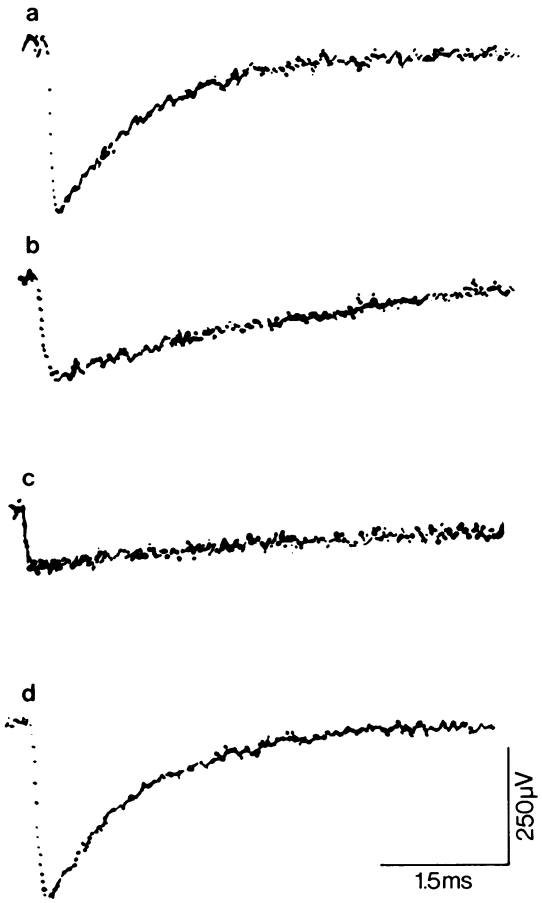

Figure 4 The effect of high concentrations of ether on m.e.p.cs. Currents are shown in control solution (a), in 45 mM ether (b), in 55 mM ether (c) and after washout of the ether with control solution (d). The figure illustrates the increase in decay time constant and reduction in amplitude of m.e.p.cs produced by high concentrations of ether. Vertical calibration; 0.25 mV. Horizontal calibration, 1.5 ms.

concentrations of halothane (more than 2 mM), at any one endplate the decay of some m.e.p.cs was no longer exponential but developed a slow component so that two rates of decay could be seen. Other m.e.p.cs had a prolonged exponential decay. Biphasic m.e.p.cs were

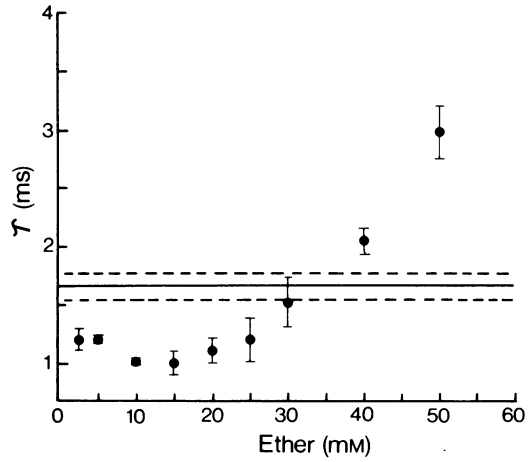

Figure 5 The effect of a wide range of ether concentrations (mM) on the time constant of decay (τ) of m.e.p.cs. The points and vertical bars show mean $\tau \pm 1$ s.e. mean in ether. The horizontal lines show the mean $\tau \pm 1$ s.e. mean in control solution. Experiments were done at 23°C.

never seen with ether concentrations up to 55 mM. An example of one of these 'biphasic' m.e.p.cs recorded in 20 mM halothane is shown in Figure 6b. An m.e.p.c recorded at the same site in control solution is shown for comparison in Figure 6a. Figure 6 also illustrates the reduction in amplitude of the m.e.p.p. (upper trace). The change in time course of the m.e.p.p. (the slowing of both rising and early decay phases) is as would be expected (Gage & McBurney, 1973) from the m.e.p.c. waveform.

The effect of a wide range of halothane concentrations on m.e.p.cs recorded in two experiments are shown in Figure 7. With concentrations above 5 mM, measurements were made only on m.e.p.cs having an exponential decay which could be described by a single time constant. Again it can be seen that there was a decrease in decay time constant at lower concentrations, but an increase at higher concentrations. The effects could always be rapidly and completely reversed by washing out the halothane with normal solution.

Chloroform produced a significant reduction in τ at a concentration of 0.5 mM (Table 1) and with

Table 2 Reduction in the time constant of decay (τ) of miniature endplate currents caused by a range of chloroform concentrations (25°C)

	Control		*Chloroform*		
Concentration (mM)	0	0.5	0.75	1	1.5
Mean (ms)	1.20	0.74	0.64	0.78	1.10
s.e. mean	0.04	0.03	0.04	0.05	0.07

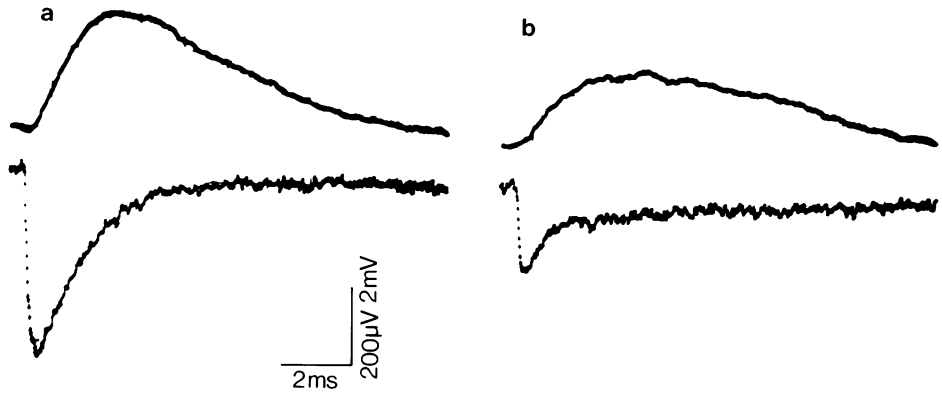

Figure 6 The effect of 20 mM halothane on m.e.p.ps (upper traces) and m.e.p.cs (lower traces). In 20 mM halothane (b), the m.e.p.c is biphasic and reduced in amplitude compared with the control (a), producing a prolonged m.e.p.p. also reduced in amplitude (b). Vertical calibration; 2 mV for upper traces, 0.2 mV for lower traces. Horizontal calibration; 2 ms.

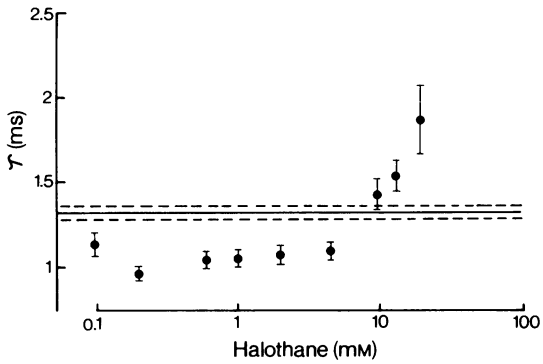

Figure 7 The effect of a range of halothane concentrations on the time constant of decay (τ) of m.e.p.cs. The points and vertical bars show mean $\tau \pm$ s.e. mean in halothane. The horizontal lines show the mean $\tau \pm$ s.e. mean in control solution. A logarithmic horizontal axis was used to cover the wide range of concentrations. Experiments were done at 25°C.

concentrations up to 0.75 mM there was an even greater reduction in τ (Table 2). Concentrations above 20 mM produced an increase in τ and in this respect chloroform was similar to ether, although chloroform did not produce as great an increase in τ as ether. The effects of a range of chloroform concentrations from one experiment are shown in Table 2.

Enflurane was similar to halothane in that shortening of m.e.p.cs was observed at low concentrations (0.75–2 mM). Biphasic and prolonged currents were observed at higher concentrations (10 mM).

Discussion

The inhalation anaesthetics, ether, halothane, chloroform and enflurane, reduced the amplitude of m.e.p.ps at all concentrations tested. At low concentrations (Table 1), they did this by reducing the time constant of decay of m.e.p.cs. Intravenous anaesthetics also increase the rate of decay of endplate currents (Seyama & Narahashi, 1975; Adams, 1975; Quastel & Linder, 1975; T. Torda & P.W. Gage, unpublished observations). At higher concentrations, the reduction in amplitude of m.e.p.ps produced by the inhalation anaesthetics was caused by a decrease in the amplitude of m.e.p.cs which also became prolonged, or biphasic with prolonged tails. Ether, and chloroform to a lesser extent, increased the time constant of decay of m.e.p.cs. Halothane and enflurane produced both biphasic and prolonged m.e.p.cs. Previously obtained results are not inconsistent with these observations if the possible influence of concentration is taken into account. Ether has been noted to reduce the average life-time of endplate channels (Katz & Miledi, 1973b) and ether and halothane have been reported to produce lengthened and biphasic currents in voltage-clamped fibres (Quastel & Linder, 1975).

The time course of an m.e.p.c. reflects the time course of the endplate conductance change generated by a quantum of acetylcholine (Gage & McBurney, 1975; Gage, 1976). Under normal conditions the rate of decay of this conductance is controlled by the rate

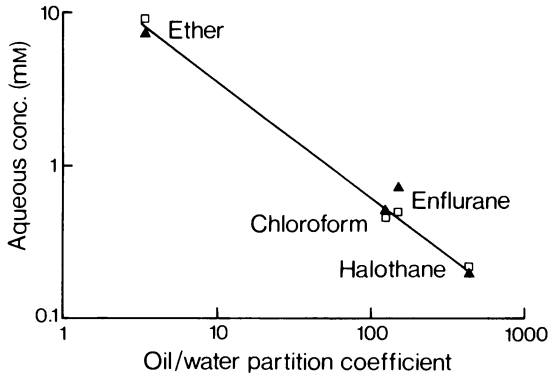

Figure 8 The aqueous concentrations (mM) of the four anaesthetics producing a 30% reduction in the time constant of decay of m.e.p.cs (triangles) are plotted against oil/water partition coefficients (taken from Secher, 1971; Dobkin *et al.*, 1971). Note the logarithmic ordinate and abscissa scales. The aqueous concentrations of the anaesthetics which produce anaesthesia (see Table 3) are plotted against partition coefficient on the same graph (squares) to show the close correlation between concentrations producing either effect.

of transition of ionic channels from open to closed conformation, as can be shown by comparing power spectra of m.e.p.cs and acetylcholine current noise (Katz & Miledi, 1972; Anderson & Stevens, 1973). Thus any increase in the rate of decay of m.e.p.cs is probably associated with an increase in the rate of this transition. (Another possibility is that an increase in the rate of decay of m.e.p.cs caused by pharmacological agents could be due to blocking of channels at a rate more rapid than the normal rate of closing). It has been proposed (Magleby & Stevens, 1972b) that the normal, rate-limiting step is a conformational change in a protein. If this is so, then low concentrations of the anaesthetics may increase the rate of this conformational change. Possible ways in which they might do this could be by interacting directly with the protein or with its environment. It is not possible at present to exclude either alternative but as ether, chloroform and halothane (Johnson & Bangham, 1969; Trudell *et al.*, 1973) have been shown to increase the fluidity of phospholipid membranes it seems not unlikely that they would also increase the fluidity of the lipid phase of postsynaptic membranes. If so, the change in time constant of decay of m.e.p.cs would be related to the concentration of the anaesthetics in membrane lipid. The good correlation which can be seen (Figure 8) between the aqueous concentrations which caused a 30% decrease in the time constant of decay of m.e.p.cs (triangles) and the oil/water partition coefficients of the anaesthetics is consistent with this hypothesis. It would appear that equal lipid concentrations of the anaesthetics have approximately the same effect on the rate of the conformational change which determines the life-time of ionic channels, as would be expected if the effect is due to an increase in membrane fluidity. However, the same correlation (Figure 8) might be obtained if the anaesthetics were acting at some hydrophobic protein site. A similar correlation between synaptic depression and hydrophobicity has been reported previously (Barker, 1974; 1975).

An increase in membrane fluidity does not explain the lengthening of currents or the biphasic m.e.p.cs caused by higher concentrations of these agents. It seems unlikely that these effects are due to inhibition of acetylcholinesterase. Anticholinesterases slow both the growth phase and decay phase of m.e.p.cs and the decay phase remains essentially exponential (Gage & McBurney, 1975) whereas the anaesthetics did not slow the growth phase of m.e.p.cs. Furthermore, halothane and enflurane gave some m.e.p.cs with a biphasic decay. Such an effect has not been reported for anticholinesterases.

Short-chain, aliphatic alcohols such as ethanol also produce an increase in the time constant of decay of m.e.p.cs (Gage, McBurney & Schneider, 1975). It was proposed (Gage *et al.*, 1975) that the effect was due to an increase in the dielectric constant of the membrane resulting from the entry of lipid-soluble substances with dielectric constants greater than 3 (the dielectric constant assumed for membrane lipid) into the postsynaptic membrane. If the rate-limiting reaction determining the decay of m.e.p.cs is accompanied by a decrease in the dipole moment of the reactant (Magleby & Stevens, 1972b; Gage & McBurney, 1975; Gage *et al.*, 1975) an increase in the dielectric constant of the environment of the reactant would slow the reaction rate (Glasstone, Laidler & Eyring, 1941). Ether and chloroform have dielectric constants greater than 3 (Weast, 1972) and may also prolong currents by increasing membrane dielectric constant. It may be that the biphasic m.e.p.cs are generated by two different populations of receptors, the prolonged phase being generated by those receptors which 'see' an increased dielectric constant in their environment and hence relax more slowly.

It is interesting that such different chemical agents as hexanol (Gage *et al.*, 1975), procaine (Maeno, 1966; Gage & Armstrong, 1968; Kordas, 1970; Maeno, Edwards & Hashimura, 1971; Deguchi & Narahashi, 1971; Adams, 1975), halothane and enflurane all produce biphasic m.e.p.cs but the origins of the effect may not be the same.

The question naturally arises as to whether the effects described here also occur at central synapses during anaesthesia. Certainly the low concentrations of anaesthetics are within the ranges of concentrations

which would be found in an aqueous phase equilibrated with the blood or alveolar gas of an animal anaesthetized to a level appropriate for surgical procedures. Concentrations in the aqueous phase given in Table 3 were calculated from blood concentrations and minimum alveolar concentrations (MAC; the concentration which abolishes motor responses to a painful stimulus in 50% of a test group of animals; Eger, Brandstater, Saidman, Regen, Severinghaus & Munson, 1965) associated with different levels of anaesthesia. Partition coefficients, MACs and blood concentrations are given by Secher (1971) for ether, halothane and chloroform, and by Dobkin, Byles, Ghanooni & Valbuena (1971) for enflurane. Where both blood concentrations and MACs were available, there was found to be little difference in aqueous concentrations calculated from either value. It can be seen that the anaesthetic concentrations in Table 1 are reasonably close to the calculated aqueous concentrations for anaesthesia in Table 3. This is illustrated also in Figure 8 in which aqueous concentrations, calculated from MACs and blood concentrations producing anaesthesia (squares), are plotted against oil/water partition coefficients for comparison with the concentrations producing a 30% decrease in the time constant of decay of m.e.p.cs (triangles). Considering the wide range of partition coefficients, the correlation between concentrations of the anaesthetics which produce a 30% reduction in the time constant of decay of m.e.p.cs and concentrations which produce anaesthesia is very good.

The good correlation does not prove, of course, that the anaesthetics produce the two effects in the same way. However, the general anaesthetics do increase membrane fluidity (Johnson & Bangham, 1969; Trudell *et al.*, 1973) and it seems reasonable to suppose that this is the cause of the increased rate of decay of the postsynaptic conductance change caused by the low concentrations of the anaesthetics. If the effects of the low concentrations described here are caused by an increase in membrane fluidity rather than by interaction with acetylcholine receptors, it would not be surprising if they had a similar effect on postsynaptic conductance changes in the central nervous system. As at the neuromuscular junction, this would cause a reduction in the amplitude of postsynaptic potentials and a decreased 'sensitivity' of the subsynaptic membrane to agonists. Although the reduction in amplitude of m.e.p.ps caused by the anaesthetics was less than 40%, and would have little effect on transmission at the neuromuscular junction where there is a high safety factor, such a reduction in the amplitude of postsynaptic potentials in the central nervous system would have a significant effect on synaptic transmission.

The different effects of the anaesthetics at different concentrations suggest that they had at least two different actions, one to shorten and the other to lengthen the duration of the conductance change. Although only shortening of currents was seen with low concentrations, both actions may normally be superimposed so that there is a net shortening of currents at low concentrations and a net lengthening at higher concentrations. It should be noted that the higher concentrations producing prolonged and biphasic currents are greater than those which would cause respiratory arrest in animals.

As suggested above, the anaesthetics at higher concentrations may increase the duration of the conductance change by increasing the membrane dielectric constant. This would prolong the conductance change at amphibian and mouse neuromuscular junctions if the decay of conductance is associated with a decrease in dipole moment of the receptor (Magleby & Stevens, 1972b; Gage & McBurney, 1975; Gage *et al.*, 1975). However, if at other synapses where the receptor protein is different, the decay of the conductance is associated with an

Table 3 Aqueous concentrations of the anaesthetics calculated from blood concentrations (37°C)

Anaesthetic		*Analgesia*	*Concentration for Anaesthesia*	*Resp. arrest*
Ether	Blood (mg%)	40–50	70–140	160–180
	Aqueous (mM)	6–7	10–21 (9)	23–26
Halothane	Blood (mg%)	5	14	22
	Aqueous (mM)	0.08	0.23 (0.2)	0.36
Chloroform	Blood (mg%)	5	15	30
	Aqueous (mM)	0.15	0.46	0.9
Enflurane	Blood (mg%)		15–25	
	Aqueous (mM)		0.3–0.6 (0.5)	

Aqueous concentrations calculated from minimum alveolar concentrations are shown in brackets. Data for ether, halothane and chloroform are from Secher (1971) and for enflurane, from Dobkin, Byles, Ghanooni & Valbuena (1971).

increase rather than a decrease in the dipole moment of the receptor, an increase in dielectric constant would increase the rate of decay of the conductance. At such a synapse, the two mechanisms seen at the toad (Gage & Hamill, 1975) and mouse neuromuscular junctions, instead of having the opposite effect, would be acting in the same direction and this would lead to an even greater decrease in the time constant of decay of currents and hence in the amplitude of postsynaptic potentials. Such a possibility does not seem implausible. For example, at crustacean neuromuscular junctions, the decay of synaptic current is shortened as the postsynaptic membrane is hyperpolarized (Dudel, 1974). By analogy with the explanation given for the voltage sensitivity at cholinergic neuromuscular junctions where hyperpolarization lengthens rather than shortens currents, this could be caused by an increase rather than a decrease in the dipole moment of relaxing receptors. In agreement with this idea, it has been found in preliminary experiments at crab neuromuscular junctions that ethanol shortens synaptic currents (Adams, Hamill & Gage, unpublished observations). Such an effect would be expected (Glasstone *et al.*, 1941) if ethanol increases membrane dielectric constant (Gage *et al.*, 1975) and the relaxation of the receptor is accompanied by an increase in the dipole moment of the reactant. At such synapses an increase in dielectric constant caused by general anaesthetics would similarly produce synaptic currents of briefer duration and hence synaptic depression.

In conclusion, it has been shown that low (anaesthetic) concentrations of ether, halothane, chloroform, and enflurane depress the amplitude of m.e.p.ps by increasing the rate of decay of the conductance change produced by a quantum of acetylcholine. If they do this by increasing membrane fluidity as proposed, they should have a similar effect at synapses in the central nervous system. At higher concentrations the general anaesthetics prolong the decay phase of m.e.p.cs. If this is caused by an increase in membrane dielectric constant (Gage *et al.*, 1975), the effect of these anaesthetics on synaptic currents at other synapses will depend on the sign and magnitude of any change in dipole moment of the receptor as it relaxes from open to closed conformation. Such properties of different receptors at different synapses, as exemplified already at peripheral neuromuscular junctions, may underlie some selective actions of different anaesthetic agents in the central nervous system.

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