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Comparison of the Effects of General Anesthetics on the End-plate of Skeletal Muscle

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The ability of volatile anesthetics to depress carbachol-induced depolarization at the end-plate region of guinea pig lumbrical muscles was examined with the moving fluid electrode technique, and the concentration of each anesthetic agent required to depress depolarization by 50 per cent was determined. There was a close correlation between the values obtained and reported anesthetic potencies in man. Thus end-plate depolarization of guinea pig lumbrical muscle appears to provide an excellent *in-vitro* model for the study of the mechanism of anesthesia. (Key words: Neuromuscular junction; Potency, anesthetic; Anesthetics, volatile, diethyl ether; Anesthetics, volatile, enflurane; Anesthetics, volatile, fluroxene; Anesthetics, volatile, halothane; Anesthetics, volatile, isoflurane; Anesthetics, volatile, methoxyflurane.)

CONSIDERABLE INFORMATION is available on the relative potencies of a wide variety of anesthetics with regard to depression of the central nervous system. In particular, Eger and colleagues have provided careful measurements of the alveolar concentrations (MAC) of anesthetics associated with failure of 50 per cent of subjects to respond to a painful stimulus.[1] Such estimates of potency have been correlated with various physical characteristics such as solubility in olive oil. However, it is a long way between observation of a quantal response in an intact individual and the physicochemical event underlying the response at the cellular level. Thus, exploration of the mechanism of anesthetic action would be greatly facilitated if a simpler system than the intact central nervous system *in vivo* were available. Various simple systems have been tried, for example, the effects of anesthetics on bioluminescence of bacteria,[2] but their appropriateness as models for the central nervous system might be questioned. An *in-vitro* preparation of a few functioning cells from the central nervous system would be better, but does not, at present, seem practical. However, a considerable amount of our thinking about transmission of signals in the central nervous system is derived from studies of peripheral analogs, in particular the squid axon or the neuromuscular junction in the frog.[3,4] We have chosen to obtain quantitative estimates of potency in such a system, specifically an *in-vitro* preparation of a mammalian neuromuscular junction.

Methods

The experiments were carried out on isolated guinea pig lumbrical muscles incubated in Krebs' solution of the following composition (mM): Na^+, 138; K^+, 5.9; Ca^{++}, 2.5; Mg^{++}, 1.22; Cl^-, 123; $H_2PO_4^-$, 1.2; SO_4^-, 1.22; HCO_3^-, 25; plus glucose, 20.8 g/l, bubbled with 95 per cent oxygen and 5 per cent carbon dioxide and kept at 36–37 C.

Carbachol-induced depolarization was measured by Fatt's moving fluid electrode technique.[5] The muscle was mounted vertically in an organ bath that could be lowered or raised by a synchronous motor. One recording electrode was held in contact with the upper tendon while a second was kept in the bath fluid. The potential between these electrodes was recorded on the Y-axis of an XY recorder while the position of the fluid level along the length of the muscle was recorded on the X-axis. In the absence of carbachol, the muscle surface is equipotential, and the XY recorder yields a horizontal line as the bath level is lowered. However, if carbachol is present, it increases permeability of muscle end-plates in

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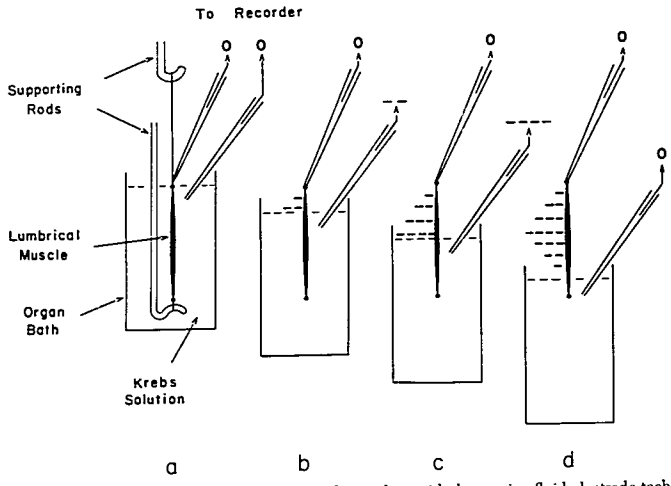


FIG. 1. Diagram to indicate mechanism of electrical recording with the moving fluid electrode technique. For further details, see text.

such a way that there is a net inward current.[4] Thus the extracellular fluid outside the end-plates becomes negative, and since the end-plates lie in a band across the middle of the lumbrical muscle, the contributions of the individual muscle fibers add to produce a band of negativity in the middle third of the muscle.

The diagram in figure 1 indicates how the moving fluid electrode scans the surface of the muscle to record such a potential. Suppose the muscle has been exposed for a minute or so in a solution of carbachol. The carbachol will have depolarized the end-plate region but, when the fluid is at the top of the muscle (fig. 1*a*), the saline solution shorts out all electrical signals and the electrodes are at the same potential. As the fluid surface is lowered (fig. 1*b*), the upper part of the muscle is now in air; thus there is less electrical shorting in this region, and the electrode in the bath fluid is now connected to a more negative (indicated by the negative signs in the figure) part of the muscle than the electrode on the upper tendon, so the recorder pen begins to leave the control baseline. When the fluid level is lowered to the point of maximal end-plate density (fig. 1*c*), the electrode in the fluid becomes several millivolts more negative than that on the tendon. As the fluid is lowered still further (fig. 1*d*), it is in contact with progressively less and less negative areas of muscle, and the recorder pen returns to baseline from the peak recorded in the middle of the muscle.

Negativity produced by carbachol at the end-plate region increases over a few minutes as the drug diffuses into the muscle. During the onset of depolarization, the muscle surface was scanned repeatedly until the electrical response stabilized. The drug was then washed out, and the muscle was allowed to recover for 20 minutes. The peak depolarizations obtained with a series of graded concentrations of carbachol were used to construct a control dose–response curve.

Next the preparation was equilibrated with a selected concentration of one of the volatile anesthetics to be studied. The magnitude of the depolarization produced by carbachol was quite sensitive to changes in anesthetic concentration, so a specially regulated vaporizing

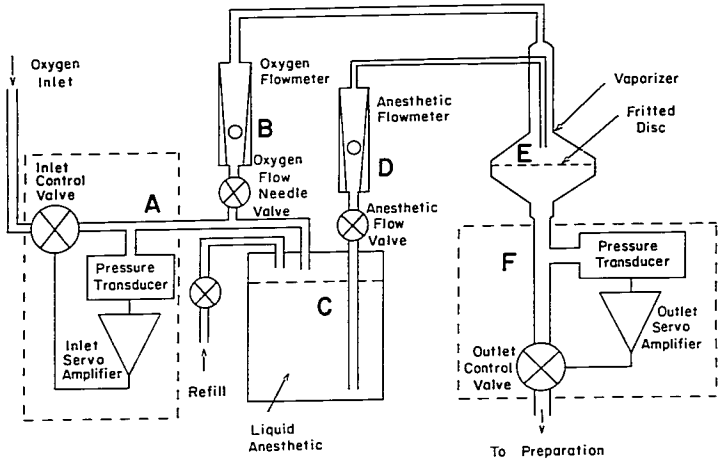


FIG. 2. Diagram of anesthetic vaporizer. For further explanation, see text.

apparatus was designed to provide reliably steady anesthetic concentrations (fig. 2) and to deliver the gas mixture at a pressure high enough to drive the gas through fritted glass gas-dispersion tubes in the organ bath. The principle of the apparatus was to use the same pressure difference to drive both oxygen and liquid anesthetic through needle valves into a vaporizing mixing chamber. Thus any slight change in driving pressure would affect the flows of both agents to essentially the same extent so their relative proportions in the final mixture would remain unchanged. The pressure regulators (*A* and *F*, fig. 2) consisted of a pressure transducer to sense the pressure to be controlled and an amplifier to control a valve accordingly. The amplifier and valve were made from a Grass Model III recorder amplifier and oscillograph unit. The pen on the latter was replaced by a small plastic finger that occluded a short length of flexible tubing as the oscillograph armature rotated. Thus circuit A would open the inlet control valve when pressure at the transducer fell below 800 torr, while that at F would open the outlet control valve when pressure at the transducer rose above 700 torr. Two mercury manometers (not shown in the diagram) were connected to provide a continuous indication of these two key pressures.

The higher pressure was then sent through a needle valve and flowmeter (*B*) into the vaporizing chamber (*E*), and this same pressure was also applied to the surface of the liquid anesthetic in a reservoir (*C*). The anesthetic was thus driven through a second needle valve and flowmeter (D) into the vaporizing chamber. The resulting gas mixture then flowed out through the outlet pressure regulator to bubble through the solution bathing the muscle. Changes in resistance of the fritted glass discs or screw clamps on tubing leading to them were automatically compensated by changes in the outlet control circuit *F* (*i.e.*, the control valve shifted so as to keep the total outflow resistance constant), so the pressure drop across the two key resistance valves *B* and *D* was shielded from variation outside the apparatus.

The two needle valves *B* and *D* were made by Nupro. The oxygen control valve *B* is not particularly critical, but the liquid flow through *D* can be quite small, so the Nupro "fine metering valve," essentially two

fine valves in series, was used at *D*. A filter was connected upstream from this fine valve to prevent resistance changes due to lodging of minute particles in the valve orifice. The two flowmeters were obtained from Gilmont and corrected for temperature, pressure, and fluid density and viscosity according to the manufacturer's formulas.

The concentrations of gas administered to the muscles were analyzed by gas chromatography and doubly checked by periodic extraction of samples of the bathing solution with heptane (hexane to separate methoxyflurane from the solvent peak) and assay by gas chromatography.[6]

The bathing solution was pre-equilibrated for at least 60 minutes in a separate reservoir with a given concentration of the anesthetic, and then the muscle was exposed to this solution (still bubbled with the appropriate gas mixture) for a further 45 minutes before the sensitivity to carbachol was determined. The particularly volatile agent diethyl ether seemed prone to excessive loss from the bath surface, so a Saran Wrap cover was used for all the diethyl ether experiments, and the anesthetic concentration in the bath solution was determined after each response to carbachol had been measured.

After a carbachol dose–response curve had been obtained in the presence of an anesthetic, the latter was washed out (by changing to fresh bathing solution and bubbling with anesthetic-free gas mixture), and a third carbachol dose–response curve was obtained to bracket the anesthetic values.

The dose–response curves were fitted by an iterative least-squares technique with the logistic function

$$E = M \frac{C^S}{C^S + K^S} (1 - \alpha) \qquad (1)$$

where E represents depolarization, M maximal depolarization in the absence of the anesthetic, C carbachol concentration, K the ED_{50} of the control carbachol dose–response curve, S a parameter that reflects the slope of the curve, and α an index of the effect of the anesthetic. This logistic function was chosen because it gives a good empirical fit to dose–response curves in general.

The $1 - \alpha$ term was added because the effect of the anesthetics appeared to be a general scaling-down of the carbachol dose–response curve, *i.e.*, low, medium, or high responses all seemed to be depressed to the same fraction of their control values. An α of zero then corresponds to no depression, while a value of one represents a maximal effect of the anesthetic—the carbachol response completely abolished.

Each anesthetic was examined at several concentrations and the values of α obtained were used to plot a dose–response curve for that anesthetic. In turn, these curves were fitted by the function

$$E = \frac{C^S}{C^S + K^S} \qquad (2)$$

where the effect E is now the observed α, C the concentration of the anesthetic, and K the ED_{50} of the anesthetic, *i.e.*, the concentration that halves the response to carbachol. S determines the steepness of the relation of α to anesthetic concentration while the maximum is now unity by the definition of α.

These anesthetic dose–response curves were estimated first with separate slopes S to test for parallelism (they were parallel) and then with a common slope to get final estimates of the anesthetic ED_{50}'s with standard errors.

While it is possible to suggest molecular models that could lead to the model of equation 1 (for example, an anesthetic effect that reduced the activity of the ionic channels at the end-plate will do it), note that equation 1 and particularly equation 2 are chosen empirically because they are the right general shape to fit the observations. Thus the reason for introducing these explicit expressions was to provide a basis for an *objective* statistical procedure for estimating the anesthetic ED_{50}'s and their standard errors, *i.e.*, to avoid fitting curves visually with all the associated pitfalls of such "eye-ball" estimates.

Results

All anesthetic agents shifted the carbachol dose–response curve downwards. Since nor-

mal responses could be obtained after washing out the anesthetics, the effect of these agents appears to be a reversible "scaling-down" of the depolarization caused by any given concentration of carbachol. Figure 3 shows an example for one concentration of each anesthetic studied.

The magnitude of depression of carbachol-induced depolarization increased with increasing concentrations of anesthetic. Such degrees of depression of the carbachol response measured over a range of concentrations for each anesthetic are plotted in figure 4. The dose–response curves obtained with the different anesthetics are parallel, *i.e.*, fitting the curves with a common slope changed the random variation about the curve insignificantly from that obtained when separate slopes were used (variance ratio = 0.76, 5 and 95 d.f.).

The concentrations of the anesthetics that depressed carbachol-induced depolarization by 50 per cent (ED_{50}) were obtained from the values in figure 4. Results are given in table 1, which also lists values of MAC for comparison.

Comparison of the relative abilities of these anesthetics to depress depolarization (ED_{50}) and their abilities to depress response to painful stimulation in man gives the regression in figure 5; the line, fitted by least squares, has a slope of 1.03 ± 0.15 SE.

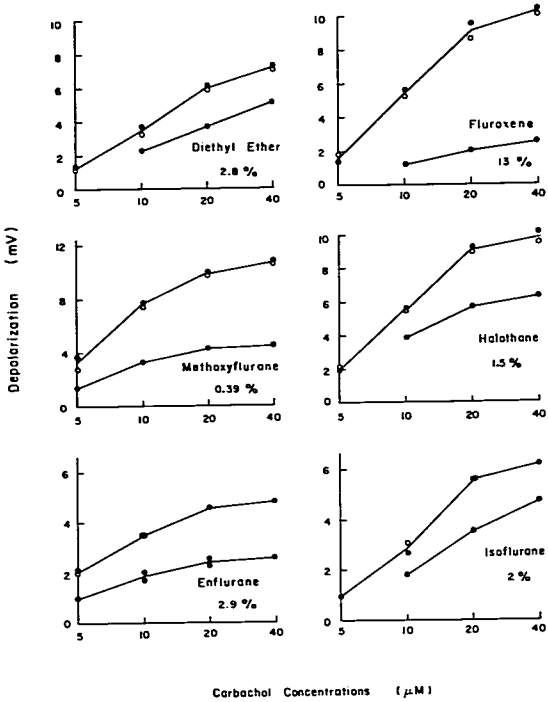


FIG. 3. Effects of anesthetics on carbachol-induced depolarization at the end-plate region of guinea pig lumbrical muscles. *Ordinates*: depolarization (mV). *Abscissae*: carbachol concentration (μM). Upper curve in each panel is the control dose–response curve (open circles are values obtained after the anesthetic had been washed out). Lower curve in each panel was obtained in the presence of the indicated concentration of the anesthetic. Each panel represents the results from one experiment.

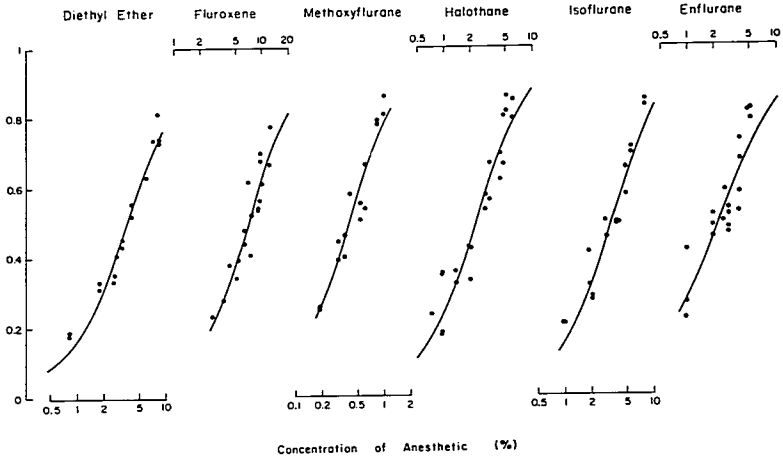


FIG. 4. Anesthetic dose–response curves. *Ordinates*: fractional depression of carbachol-induced depolarization. *Abscissae*: anesthetic concentration (vol per cent). Each point was obtained from statistical analysis of an experiment such as in figure 3.

Discussion

Although the action of volatile anesthetics on the central nervous system has been the object of much investigation, the mechanism is still not understood. Indeed, most of our knowledge of the functioning of the central nervous system has been derived from peripheral analogs, such as squid axon and frog neuromuscular junction.[3,4] In the present study, we have used another peripheral model —the neuromuscular junction of guinea pig lumbrical muscle. This preparation has the advantage of being a mammalian muscle and, as can been seen from the recovery responses of figure 3 (open circles), gives stable responses to carbachol during the 6–8 hours needed to perform an experiment.

The results of our study have both practical and theoretical significance.

Clinically, it is well known that less *d*-tubocurarine is needed to produce a neuromuscular block in the presence of a volatile

TABLE 1. ED_{50} and MAC Values

	ED_{50} ± SE (n) (vol per cent)	MAC ± SE* (n) (vol per cent)	ED_{50}/MAC† (95 per cent Confidence Limits)
Methoxyflurane	0.39 ± 0.02 (15)	0.16 ± 0.01 (17)	2.07–2.89
Enflurane	2.38 ± 0.12 (19)	1.69 ± 0.04 (19)	1.25–1.57
Halothane	2.39 ± 0.12 (22)	0.74 ± 0.03 (24)	2.83–3.67
Isoflurane	3.36 ± 0.18 (17)	1.16 ± 0.05 (32)	2.52–3.32
Diethyl ether	4.08 ± 0.23 (16)	1.92 ± 0.07 (17)	1.84–2.43
Fluroxene	7.24 ± 0.34 (18)	3.40 ± 0.05 (15)	1.92–2.36

* Values of de Jong and Eger.[7]

† Since ED_{50}/MAC is the ratio of two variables the distribution is skewed. Therefore, in place of a standard error, the 95 per cent confidence limits, calculated on the basis of Fieller's theorem,* are given.

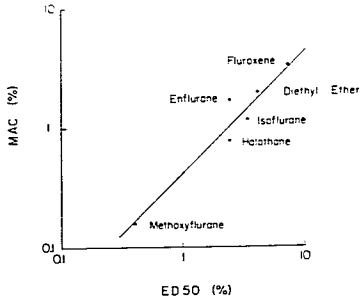
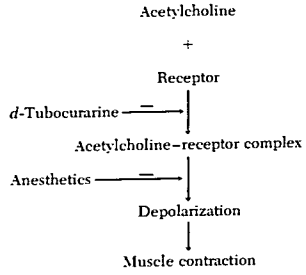


FIG. 5. Comparison of MAC and ED_{50}. *Ordinates*: MAC (vol per cent). *Abscissae*: ED_{50} (vol per cent). Number of observations per point given in table 1.

anesthetic. We can now view this more quantitatively in the following frame of reference:



Both *d*-tubocurarine and general anesthetics depress depolarization at the end-plate region, *d*-tubocurarine by combining with the receptor and preventing access of the acetylcholine molecules, and anesthetics by depressing depolarization at a step distal to the receptor.[6,9]

Now, an important feature of the synapse is its large margin of safety. This can be viewed several ways. One can say: 1) that much more transmitter is released than necessary barely to trigger the muscle fiber, or 2) that roughly four- to fivefold more receptors are available than would just allow the signal to activate the muscle fiber,[10] or 3) that the depolarization produced by the transmitter is considerably more than that required just to reach threshold. While all three views are equivalent, one or the other can be more appropriate in a given situation. In particular, if a drug acted presynaptically one would think in terms of amount of transmitter released. However, when curariform drugs are considered, thinking in terms of receptor occlusion is more convenient. When we come to the volatile anesthetics, the present results indicate that depolarization is the appropriate frame of reference.

Because of the large margin of safety, depolarization of the end-plate by the transmitter presumably will have to be depressed considerably before neuromuscular block would be produced by one of the volatile anesthetics. Since a concentration equal to MAC depresses depolarization by only about 25 per cent, one would not expect to see neuromuscular block resulting from the anesthetic alone at a concentration of 1 MAC. This expectation, of course, is in line with experience. However, depressing depolarization should act to lower the margin of safety. Our preliminary experiments with nerve–muscle preparations indicate that anesthetic concentrations that depress depolarization by about 50 per cent are needed to produce neuromuscular block. These observations imply that at 1-MAC levels, the volatile anesthetics studied will reduce the margin of safety roughly by half. Or, to put it another way, the required dose of a neuromuscular blocking agent will be roughly halved in the presence of the anesthetic. While these numbers can only be rough estimates until more quantitative information on the concentration–effect relationship for anesthetics on neuromuscular transmission is available, the order of magnitude of the potentiation of curariform agents to be expected from the anesthetic block of depolarization is still interesting. In particular, it appears unnecessary to look other than at the effect on depolarization to account for the extent of this drug interaction.

Note, in passing, that it is not very meaningful to compare the present ED_{50}'s with clinical impressions that potentiation of neuromuscular blocking agents is greater with some inhalation anesthetics than with others. In the clinical situation, the circulation, the nerve

terminal, and the postsynaptic muscle membrane are all involved, as well as the end-plate.

Comparison of MAC (a central nervous system effect) and ED_{50} of the anesthetic (a peripheral effect) shows an excellent fit (fig. 5). One particular feature deserves comment. Enflurane lies farthest above the regression line and, though the deviation is not statistically significant (the sample size is too small to permit a sensitive test of the deviation, particularly since a designed comparison was not involved), it is still interesting that the deviation is what one would expect if enflurane had either more effect on the muscle than expected or less effect in man. The known ability of enflurance to stimulate the central nervous system fits well with the second explanation.

The excellent correlation between MAC and ED_{50} indicates that the isolated guinea-pig lumbrical muscle may prove to be a very suitable model in which to study the mechanisms of action of general anesthetics at the cellular level.

Carbachol was purchased from K and K, diethyl ether from Squibb, methoxyflurane from Abbott, halothane from Ayerst, fluroxene and enflurane from Ohio Medical Products, and isoflurane was kindly donated by Ohio Medical Products.

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