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The Effects of Diethyl Ether, Enflurane, and Isoflurane at the Neuromuscular Junction

B. E. Waud, M.D., and D. R. Waud, M.D., D. Phil.†*

The actions of diethyl ether, enflurane, and isoflurane at the neuromuscular junction were examined in isolated guinea pig lumbrical muscles. These anesthetics depressed the ability of carbachol to depolarize the endplate region; this depression of depolarization did not show competitive kinetics. None of the anesthetics altered the affinity of the acetylcholine receptor for *d*-tubocurarine, *i.e.*, the dissociation constant of *d*-tubocurarine was unchanged. Since diethyl ether, enflurane, and isoflurane produced no observable alteration of the receptor, the antagonism of the drug-induced depolarization of the neuromuscular junction appears to be exerted at a stage subsequent to reaction with the receptor. (Key words: Anesthetics, volatile, diethyl ether; Anesthetics, volatile, euflurane; Anesthetics, volatile, isoflurane; Neuromuscular relaxants, *d*-tubocurarine; Neuromuscular junction.)

DIETHYL ETHER, enflurane, and isoflurane have all been found to block neuromuscular transmission to some extent.[1-3] However, because the effect examined has been the response of muscle to single or tetanic nerve stimulation, it is not possible to tell whether the action of the anesthetic is a) presynaptic, b) on the acetylcholine receptor itself, or c) on muscle membrane distal to the receptor. Karis *et al.*[4] demonstrated that diethyl ether interfered with the depolarizing action of acetylcholine or carbachol at the frog endplate, *i.e.*, there was a postsynaptic effect, but one cannot determine from their experiments whether this interference was at the receptor itself or on some structure associated with the subsequent change in membrane permeability. Waud *et al.*[5] have demonstrated that, although halothane depressed markedly the ability of carbachol to depolarize the endplate region, there was no effect on the acetylcholine receptor.

The purpose of the present experiments was to determine whether diethyl ether, enflurane, and isoflurane behave like halothane, or whether these agents, which are well known for their ability to produce muscle relaxation, react with the acetylcholine receptor itself. Specifically, the effect of diethyl ether, enflurane, or isoflurane on the dissociation constant of the *d*-tubocurarine–receptor complex was examined. An altered dissociation constant would provide direct evidence for a change in the receptor.

Methods

The studies were done on isolated guinea pig lumbrical muscles suspended in Krebs' solution at 36 C and bubbled with 95 per cent oxygen–5 per cent carbon dioxide. Endplate depolarization caused by adding carbachol to the bath was recorded externally by Fatt's moving fluid electrode technique.[6] The voltage profile along the muscle was recorded by lowering the bath relative to the muscle so that the fluid level scanned the muscle surface. The potential between an electrode at the upper end of the muscle and one in the bath fluid was recorded on the Y-axis of an XY recorder, while the X-axis was driven by a voltage proportional to the position along the muscle surface. The muscle, which is equipotential in the absence of carbachol, shows an area of depolarization in the middle when the drug is added to the bath (*c.f.*, fig. 2, below). In order to minimize desensitization, carbachol responses were measured at 20-minute intervals.[5]

The study consisted of two parts.

* Assistant Professor of Anaesthesia.
† Associate Professor of Pharmacology.
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Address reprint requests to: Dr. B. E. Waud, Department of Anesthesia, Peter Bent Brigham Hospital, 721 Huntington Avenue, Boston, Massachusetts 02115.

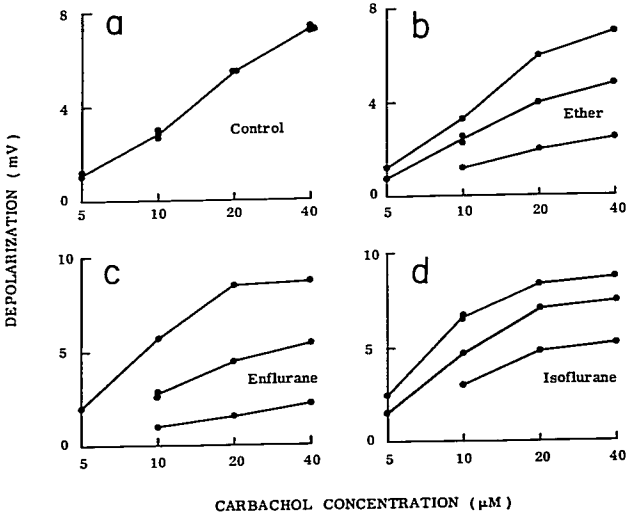

FIG. 1. The carbachol concentration–depolarization relationship. *Ordinates:* depolarization (mV). *Abscissae:* concentration of carbachol (μM). Each panel gives results from a representative muscle. *a*, control values for curves determined over a period of about four hours. There is no shift in the dose–response relationship with time. *b*, dose–response curves in the presence of diethyl ether, 0, 2 per cent, 9 per cent, from above downwards. *c*, dose–response curves in the presence of enflurane, 0, 2 per cent, 5.4 per cent. *d*, dose–response curves in the presence of isoflurane, 0, 1 per cent, 3 per cent.

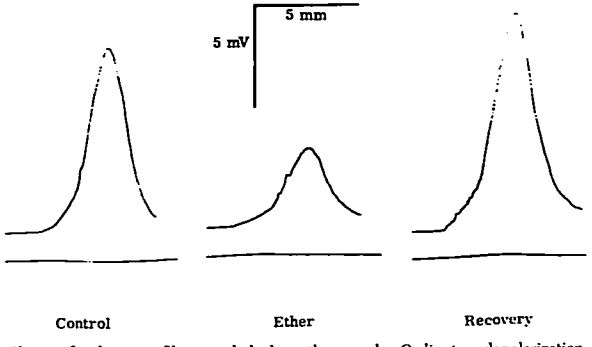

FIG. 2. Shape of voltage profile recorded along the muscle. *Ordinates:* depolarization. *Abscissae:* distance along the muscle; scales represent 5 mV and 5 mm, respectively. Bottom tracing in each panel is the control tracing before carbachol; the muscle surface is equipotential. Top tracing shows maximal response in the presence of carbachol, 40 μM: left panel before, middle panel during and right panel after recovery from diethyl ether, 11 per cent.

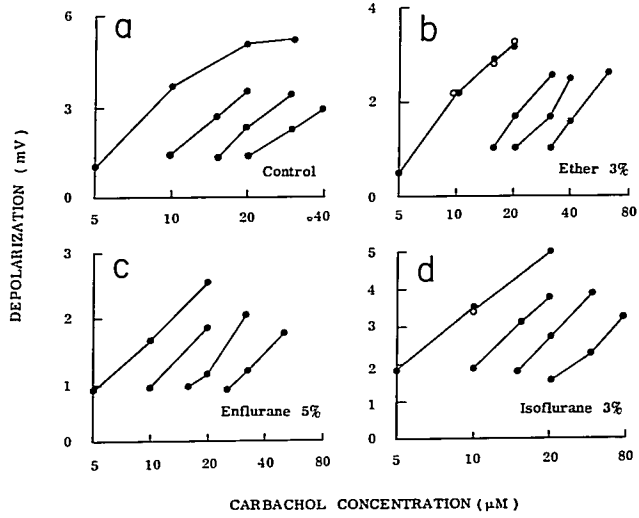

FIG. 3. Effects of anesthetics on the potency of *d*-tubocurarine. Dose–response curves as in figure 1. *Ordinates*: depolarization (mV). *Abscissae*: carbachol concentration (μM). Each panel gives results from a representative muscle. From left to right the concentrations of *d*-tubocurarine are 0, 0.1, 0.2, 0.4 μM. Open circles represent responses obtained after washout of *d*-tubocurarine. *d*-Tubocurarine shifts the dose–response curve to the right to the same extent in the presence and absence of the anesthetic agents.

1) ANESTHETIC–CARBACHOL INTERACTION

In each muscle studied, three carbachol dose–response curves were obtained as described above. Graded concentrations of carbachol were added to the muscle bath and peak depolarizations were used to construct a control dose–response curve. The muscle was then bathed in Krebs' solution which had been bubbled for at least one hour with 95 per cent oxygen–5 per cent carbon dioxide plus the concentration of anesthetic to be studied. To ensure equilibration, the anesthetic mixture was bubbled through the muscle bath for an additional 45 minutes before carbachol test doses were given and a second dose–response curve obtained. We felt that this exposure would guarantee a steady state, since anesthetic-induced shifts in the carbachol dose–response curves had stabilized within 20 minutes. The third dose–response curve was obtained after the muscle had been either allowed to equilibrate with a higher concentration of anesthetic or washed out with 95 per cent oxygen–5 per cent carbon dioxide for one hour. Anesthetic concentrations examined were in the ranges: diethyl ether, 1–10 per cent; enflurane, 1–5 per cent; isoflurane, 1–6 per cent.

2) ANESTHETIC–TUBOCURARINE INTERACTION

The dissociation constant of the *d*-tubocurarine was determined by the method of Arunlakshana and Schild,[7] as has been done recently by Lu,[8] Goldfine[9] and Waud, Cheng and Waud.[5] In each muscle, after a control dose–response curve was obtained, dose–response curves were elicited in the presence of several concentrations of *d*-tubocurarine. *d*-Tubocurarine was allowed to equilibrate for 30 minutes. (It has been determined that *d*-tubocurarine an-

tagonism reaches a steady state in 20 minutes.[5]) The estimate of the dissociation constant of *d*-tubocurarine was then obtained from the set of dose–response curves obtained with each muscle by the method described below. Corresponding estimates of the *d*-tubocurarine dissociation constant were then made in the presence of diethyl ether (2, 3, 6, 7, 8, or 10 per cent), enflurane (1, 2, 4, or 5 per cent), or isoflurane (1.5, 3, 4.5, or 6 per cent). In these experiments, the anesthetic was applied to the preparation throughout the whole experiment.

Since the anesthetics caused a considerable shift in the carbachol dose–response curves, it was important that the anesthetic concentration be carefully controlled. A special regulated vaporizing apparatus was designed to guarantee reliable and consistent anesthetic concentration. Concentrations of anesthetic in the delivered gas mixture were checked by gas chromatography before every dose of carbachol and were found to be consistent with the concentrations predicted from the settings of the needle valves and associated flowmeters.

Theory and Statistical Analysis

Estimates of the dissociation constants K_B of *d*-tubocurarine were obtained from analysis of the shifts of the dose–response curve to carbachol in the presence of *d*-tubocurarine. The statistical analysis, based on that of Waud and Parker,[10] is unfortunately rather complex. However, the following summary should give the interested reader the gist of the argument.

One starts with a model in which both carbachol (Drug A) and *d*-tubocurarine (B) react with the same receptor (R):

$$A + R \rightleftharpoons AR \qquad B + R \rightleftharpoons BR \qquad (1)$$

Like any such reaction, these can be characterized by dissociation constants K_A and K_B:

$$K_A = \frac{[A]\cdot[R]}{[AR]} \qquad K_B = \frac{[B]\cdot[R]}{[BR]} \qquad (2)$$

(It is K_B, one property of the receptor, that we shall seek to measure.)

Second, the receptor is either free or bound to carbachol or *d*-tubocurarine; thus, the total concentration of receptors is

$$[R_t] = [R] + [AR] + [BR] \qquad (3)$$

One can now solve (2) and (3) to get an expression for the fraction of receptors occupied by carbachol, $[AR]/[R_t]$:

$$\frac{[AR]}{[R_t]} = \frac{[A]}{[A] + K_A(1 + [B]/K_B)} \qquad (4)$$

In this equation, the term $K_A(1 + [B]/K_B)$ determines the scale of concentrations. In other words, to get any given [AR] (or ultimately, any given degree of depolarization), the carbachol concentration must be a particular ratio of $K_A(1 + [B]/K_B)$. If this parameter $K_A(1 + [B]/K_B)$ were doubled, for example, then twice as much carbachol would be required to get any given degree of depolarization. (This behavior is the reason a competitive agent like *d*-tubocurarine produces its characteristic "parallel shift to the right" of the carbachol dose–response curve.)

If we compare carbachol concentrations producing equal depolarizations in the presence and absence (B = 0) of *d*-tubocurarine, they will be in the ratio:

"dose-ratio"

$$= \frac{K_A(1 + [B]/K_B)}{K_A(1 + 0/K_B)} = 1 + [B]/K_B \qquad (5)$$

Thus, K_B can be estimated[7] from the dose-ratio as

$$K_B = [B]/(\text{dose-ratio} - 1) \qquad (6)$$

When dealing with experimental values, it is important to have an objective method for getting the actual estimate of K_B. This was done by fitting the observed dose–response results with a function of suitable shape and then obtaining the value of K_B as the maximum likelihood estimate of one of the parameters of the fitted curves. Specifically, the logistic function was used both for mathematical convenience, because it has generally been found to fit dose–response curves well, and because it can be viewed as a general form of equation (4). Thus, dose–response results (such as those in figure 3 below) were fitted to the equation

$$E = M\frac{A^P}{A^P + [K(1 + B^Q/K_B)]^P} \qquad (7)$$

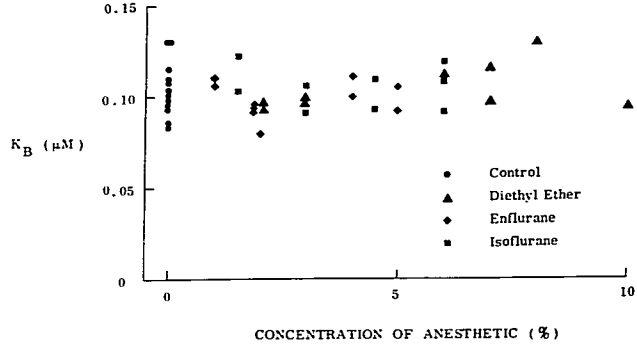

FIG. 4. Relation of estimate of *d*-tubocurarine–receptor dissociation constant to concentration of anesthetic. *Ordinates*: estimates of the dissociation constant K_B for *d*-tubocurarine (μM). *Abscissae*: per cent anesthetic administered. Each point represents the estimate from one muscle preparation. ● control, ▲ diethyl ether, ◆ enflurane, ■ isoflurane. The estimate of K_B is unaltered by the anesthetic agents.

where E is depolarization, M the maximal possible depolarization, A the concentration of carbachol, K the ED_{50}, P a parameter that determines the slope of the curve, while B and K_B are the concentration and dissociation constant of *d*-tubocurarine. The parameter Q should be unity if the model of equation (4) is correct. Curves were also fitted with separate values of P for each slope to test for parallelism of the dose–response curves. Thus, by determining Q and these separate slopes, it was possible to test whether the results were consistent with the experimental model (in all experiments, they were).

A computer program was written to give both the estimates of K, P, Q and K_B and their standard errors. In essence, the program started with trial values and refined them iteratively until those providing the closest (least-squares) fit to the observations were obtained.

Results

1) Anesthetic–Carbachol Interaction

All anesthetics studied shifted the carbachol dose–response curve downwards, and increasing concentrations caused increasing shifts (fig. 1). The extent of spreading of the voltage profile recorded along the muscle appeared unchanged (fig. 2). Normal sensitivity to carbachol could be obtained after washing out the anesthetic. Thus, the action of the anesthetics appears to be a reversible "scaling-down" of the depolarization produced by any given concentration of carbachol. Anesthetic concentrations that depressed the carbachol-response curves to about 20 per cent of their original values were about 10, 5, and 6 per cent for ether, enflurane, and isoflurane, respectively.

2) Anesthetic–*d*-Tubocurarine Interaction

None of the anesthetics affected the potency of *d*-tubocurarine. Figure 3*a* shows the effects of increasing concentrations of *d*-tubocurarine in the absence of an anesthetic. Figures 3*b*, 3*c*, and 3*d* show the effects of increasing concentrations of *d*-tubocurarine in the presence of 3 per cent diethyl ether, 5 per cent enflurane, and 3 per cent isoflurane, respectively. The shifts of the dose–response curves produced by *d*-tubocurarine were similar in all cases. In turn, as shown in figure 4, the calculated dissociation constant for *d*-tubocurarine was unchanged by the anesthetics (*i.e.*, when a straight line was fitted by least squares to the observations in

figure 4, the slope was not significantly different from zero).

Discussion

Diethyl ether, enflurane, and isoflurane block the effect of carbachol at the endplate region in a manner similar to that previously seen with halothane.[5] The downward shift of the carbachol dose–response curves in the presence of these anesthetics shows that the antagonism does not exhibit competitive kinetics (compare the effect of the anesthetics with the effect of the prototype competitive agent, *d*-tubocurarine; anesthetics depressed the slope of the carbachol dose–response curve (fig. 1), while *d*-tubocurarine shifted the curve to the right without a change in slope (fig. 3*a*)). However, although the anesthetics are not competitive antagonists, they might still alter the acetylcholine receptor in some way so as to prevent carbachol from having its usual effect. Thus, one might picture a receptor alteration such that carbachol binds less readily. However, one would then expect that the binding of *d*-tubocurarine to the receptor would also be altered. We have measured the ability of *d*-tubocurarine to combine with the receptor by determining the *d*-tubocurarine dissociation constant. Since the estimates of the *d*-tubocurarine dissociation constant were not changed by concentrations of the anesthetics sufficient to depress carbachol responses 70–80 per cent, the receptor appears to be unchanged. Therefore, these anesthetics must interfere with the depolarizing action of carbachol at some stage beyond the receptor. (For example, the anesthetics might interfere with ease of passage of ions through membrane channels. Such an effect might be studied by examining effects of anesthetics on carbachol-induced membrane voltage noise.) Furthermore, if the neuromuscular blocking effect of the anesthetics is postsynaptic, this, too, is not the result of a change in the receptor (the present experiments do not bear on any component of the anesthetic effect that might be presynaptic).

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