Mode of Action of Diethyl Ether in Blocking Neuromuscular Transmission

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The action of diethyl ether has been studied on frog sciatic nerve-sartorius muscle preparations *in vitro* using modern single fiber techniques. At a concentration of ether which blocks the indirect twitch, the muscle fibers can be stimulated directly. They have normal resting potentials and the action potentials elicited show only minor changes in form. The neuromuscular block produced by ether is not effectively antagonized by edrophonium or succinylcholine. Ether causes reduction in the amplitude, and prolongation of the time course, of both the end-plate potential, and the miniature end-plate potential. Ether diminishes the sensitivity of the muscle postjunctional membrane to quaternary ammonium compounds such as carbamylcholine. The mechanism of the neuromuscular blocking action of ether differs from that of *d*-tubocurarine. It is suggested that ether causes changes in the behavior of the permeability controlling mechanisms which are engaged subsequent to receptor site activation.

MANY years ago, Auer and Meltzer [1] reported that diethyl ether has a "curare-like effect on the skeletal motor mechanism." Subsequently the neuromuscular blocking action of this anesthetic agent was confirmed by others. Gross and Cullen [2] showed that in dogs under ether anesthesia the tension output of the indirectly stimulated skeletal muscle is reduced, and that ether also reduces the contractile response initiated by an intra-arterial dose of acetylcholine. They also reported that the ether-produced block could be antagonized by neostigmine. From these results they concluded that ether blocks neuromuscular transmission, possibly by "depressing the receptor substance in the muscle cell." Naess [3] demonstrated *in vivo* that ether decreases the tension output of indirectly stimulated skeletal muscle before it causes diminution in the contractile response to direct stimulation. From results of later experiments on the antagonizing action of neostigmine on "ether block" and "curare block," Naess [4] argued that ether and curare do not have identical modes of action. Secher studied the action of ether on rat phrenic nerve-diaphragm preparations *in vitro*. He concluded that neuromuscular transmission was more easily blocked by ether than was axonal conduction but the difference in susceptibility was not great. The electrical excitability of muscle fibers was least sensitive to ether. Somjen and Gill [6] found that ether depresses synaptic transmission in the rat spinal cord *in vivo*, but under the experimental conditions, axonal conduction was unaffected. Yamaguchi [7] reported the effect of ether on directly stimulated isolated single frog muscle fibers. At high concentrations, ether blocked conduction with only a slight reduction in resting potential. This investigator did not study the effect of ether on neuromuscular transmission.

Because much of the available evidence on the neuromuscular blocking action of ether is indirect, the mechanism of the block is not firmly established. We have therefore studied the effect of ether at the neuromuscular junction and at nonjunctional sites utilizing modern single fiber techniques in order to obtain more definitive understanding of its action.

Methods

One of the problems which we have only partially solved, is that of controlling the concentration of ether applied to the nerve-muscle preparations. Ringer solution was equilibrated

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by bubbling a known ether-oxygen gas mixture through it. Various ether-oxygen mixtures were prepared using a Copper Kettle and calibrated flowmeters (Morris [8]). Four different gas mixtures were analyzed on a gas chromatograph and the concentrations found to be within 10 per cent of the expected values.

In order to minimize loss of ether from the equilibrated Ringer solution, the neuromuscular preparations were continuously perfused with fresh solution. Also when possible, as in the tension output experiments, the nerve-muscle chambers were almost entirely closed from the atmosphere.

In making intracellular recordings from single muscle fibers it was not feasible to enclose the muscle bath and for this reason the ether concentration in the bathing solution dropped below that of the equilibrated Ringer solution supplied. By comparing visual observations of the indirectly elicited twitch obtained in these electrophysiological experiments with results of the better controlled tension output experiments we estimate that the loss of ether concentration in the open bath did not exceed 40 per cent. Although we cannot more closely specify the ether concentration in the single fiber experiments, the data obtained from them, being powerful and direct, allowed us to draw valid general conclusions regarding the mechanism of action of ether.

All of our experiments were conducted on the isolated sciatic nerve-sartorius muscle preparation of the frog (Rana pipiens). Several different methods were used:

(A) *The effect of ether on the tension output of the indirectly stimulated frog sartorius muscle with and without the addition to the perfusing solutions of edrophonium (Tensilon), d-tubocurarine, and succinylcholine.*

The method used was that of Nastuk *et al.*[9] The sartorius muscle with its attached nerve was mounted on a myograph and the isometric tension output was displayed on an oscillograph and recorded photographically. Single rectangular nerve stimuli of 0.1 milliseconds duration and a strength three times that required to give maximal tension output were delivered at 1 minute intervals throughout the experiment. Temperature was not controlled and ranged between 18.5° C. and 25° C.; however, the difference between minimum and maximum values never exceeded 2° C. in each experiment. The control period was continued until a half hour interval was obtained during which the tension output of the preparation changed by no more than 10 per cent of the final value for this period. Previous experiments had shown that if these criteria are met, the decline of tension during the subsequent 2 hour period amounts to 5 per cent or less (Nastuk and Karis [10]). At the end of the control period, various test solutions were passed through the muscle chamber. Finally, the preparation was perfused with Ringer solution once again to determine whether irreversible changes had occurred.

(B) *Effect of diethyl ether on resting and action potentials at and distant from the end-plate of single muscle fibers.*

The method was basically as described by Nastuk [11] and Nastuk and Alexander.[12] Single muscle fibers were penetrated with microelectrodes filled with 3 *M* KCl. At and away from the junctional region, resting and action potentials were photographically recorded from an oscilloscope. The muscle fibers were indirectly stimulated through the attached nerve using supra-maximal stimuli of 0.1 milliseconds duration. Single muscle fibers were directly stimulated via an internal microelectrode; a second microelectrode placed about 50 μ away in the same fiber was used to record the initiated action potential (AP). Even though the impalements were carried out with care, the resting potential (RP) fell by a few millivolts when the second impalement was made. This loss is shown in the data. After the control values were taken, the preparation was perfused with Ringer solution which had been equilibrated with oxygen-ether.

(C) *The effect of diethyl ether on miniature end-plate potentials (mepp).*

The frog sartorius muscle preparation with its attached nerve was mounted in a dish. Low noise microelectrodes with a resistance between 6 and 12 megohms were used to measure the resting potential at the end-plate site. The mepp's were fed through a neutralized input capacitance amplifier (Type DS2C, Bioelectric Instruments, Inc.) into a Tektronix 564 Mod 08 storage oscilloscope and were photographically recorded. After a control

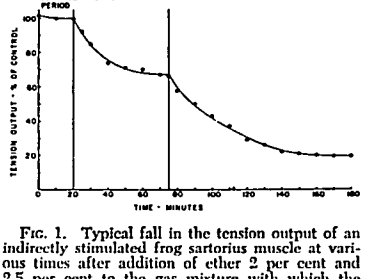

FIG. 1. Typical fall in the tension output of an indirectly stimulated frog sartorius muscle at various times after addition of ether 2 per cent and 2.5 per cent to the gas mixture with which the bathing solution was equilibrated.

record was obtained, a relatively large micropipette (tip 50 μ) containing Ringer solution equilibrated with 10 per cent ether in oxygen, was brought near the end-plate. Ether was allowed merely to diffuse from the micropipette since perfusion by pressure introduced mechanical and electrical artefacts. After the change in amplitude and duration of the miniature end-plate potentials (mepp's) were recorded, the nerve was electrically stimulated and the resulting end-plate or action potential was photographed on a second oscilloscope.

(D) Effect of diethyl ether on the chemosensitivity of the muscle postjunctional membrane.

The muscle was bathed in Ringer solution equilibrated with 5 per cent ether in O_2. At the end of 2 hours the bath was drained and filled with a similar gas-equilibrated Ringer solution which contained 27×10^{-6} *M* carbamylcholine. During the next 55 minutes resting potentials were measured at the end-plates of several muscle fibers. The bath then was drained and refilled with normal Ringer solution (50 minutes). Finally as a control, the solution was changed to Ringer solution plus carbamylcholine (27×10^{-6} *M*) without ether; during this time resting potentials were recorded at the end-plates of individual fibers.

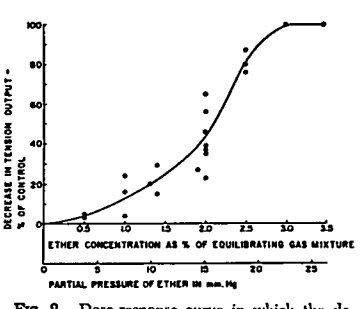

FIG. 2. Dose-response curve in which the decrease in tension output of indirectly stimulated frog sartorius muscles is plotted against the ether concentration in the equilibrating gas mixture.

Results

(A) The effect of ether on the tension output of the indirectly stimulated frog sartorius muscle and the interaction with edrophonium, d-tubocurarine (dTC) and succinylcholine.

Figure 1 shows a typical plot of the fall in tension output of 2 indirectly stimulated muscles after exposure to perfusing solutions containing 2 and 2½ per cent ether. From several such plots we constructed a dose response curve (fig. 2) which guided us in the choice of ether concentrations used in the subsequent experiments. For practical reasons it was not always feasible to prolong the application of ether to the point where the new steady state in tension output was fully established. To minimize this source of error, the only values

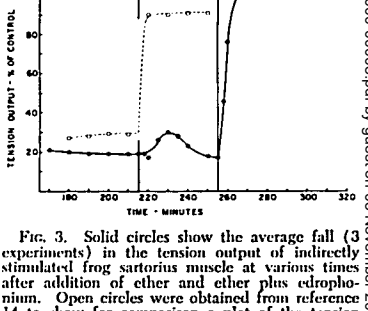

FIG. 3. Solid circles show the average fall (3 experiments) in the tension output of indirectly stimulated frog sartorius muscle at various times after addition of ether and ether plus edrophonium. Open circles were obtained from reference 14 to show for comparison a plot of the tension output after *d*TC and reversal of this block by addition of edrophonium in a lower concentration (10 μM).

used in figure 2 were those for which the last 15 minutes of the tension output curve showed an incremental change less than 3 per cent of the control tension output.

In all of these experiments an increase of tension output never occurred. This result suggests that if the ether-produced block originates at the end-plate site, it at least superficially resembles that produced by *d*TC (*i.e.*, nondepolarizing). To test this possible parallelism, edrophonium was added in a concentration where a maximum anticholinesterase effect could be expected. A *d*TC block of similar magnitude is readily reversed by edrophonium (Nastuk and Alving [9]). However, edrophonium produced only a weak transient increase in the tension output of the ether blocked preparation (fig. 3).

It has been shown (Nastuk and Karis [10]) that a neuromuscular block produced by *d*TC can be antagonized by succinylcholine. In contrast, succinylcholine (concentration 5×10^{-6} *M*) caused only a small transitory antagonism of an ether-produced block: such minor antagonism was followed by further deepening of the block (fig. 4). We also

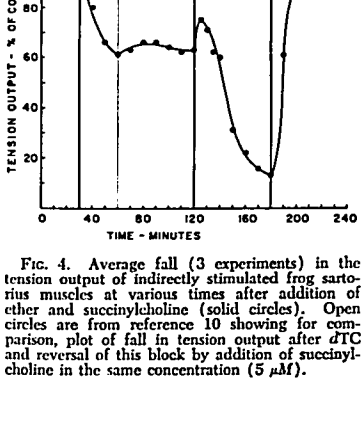

FIG. 4. Average fall (3 experiments) in the tension output of indirectly stimulated frog sartorius muscles at various times after addition of ether and succinylcholine (solid circles). Open circles are from reference 10 showing for comparison, plot of fall in tension output after *d*TC and reversal of this block by addition of succinylcholine in the same concentration (5 μM).

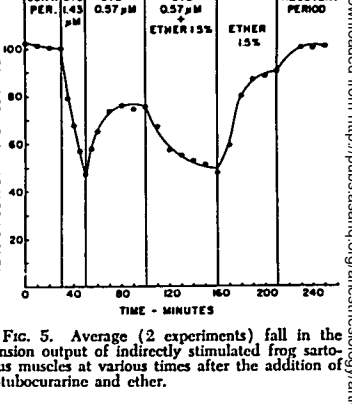

FIG. 5. Average (2 experiments) fall in the tension output of indirectly stimulated frog sartorius muscles at various times after the addition of *d*-tubocurarine and ether.

tested (not shown) the effect of succinylcholine at 2.5×10^{-6} *M* on an ether-produced block and the results obtained paralleled those seen with the higher concentration.

Finally we studied the interaction of *d*TC and ether in the production of neuromuscular block. Ether increased the depth of *d*TC-produced block and the blocking effect of these agents was roughly additive (fig. 5). Similar results were obtained if ether was first applied followed by *d*TC.

(B) The effect of diethyl ether on the resting and action potentials of single muscle fibers.

Table 1 shows the results obtained from one muscle-nerve preparation perfused with Ringer solution equilibrated with 2 per cent ether in O_2. Neuromuscular transmission was blocked in about ⅓ of the junctions studied. The end-plate potentials (epp) recorded at these blocked junctions showed a slower than normal rate of rise (average time from onset to peak was 1.7 msec.) and their amplitude was insufficient to reach the critical membrane potential at which an action potential is initiated. The outstanding change observed at these blocked junctions was that the end-plate potentials are greatly prolonged.

An intracellular recording from an ether-blocked end-plate is shown in traces A-D of figure 6. Note the unusual duration of the

TABLE 1. Effect of Ether on the Average Characteristics of Membrane Potentials Recorded from Single Muscle Fibers

	Blocked End-Plates		Nonblocked End-Plates		Data for Comparison (no ether)	
		n		*n*		*n*
Resting potential (RP) (mv.)	−94.3±1.4 (a)	8	−91.7±0.9 (a)	15	−94.6±0.8*	29
End-plate potential (epp) (mv.)	16.8±3.1	8				
Time from onset to peak (msec.)	1.7±0.1	7				
Time from peak to 1/2 decay (msec.)	>6 (b)	8			1.5† 3.1‡	7 6
Action potential (AP) (mv.)			124.4±1.3	14	120.9±1.5*	30
Potential difference across the active membrane at crest of AP (overshoot) (mv.).			32.9±1.0	14	25.9±1.3*	29
Maximum rate of rise of AP (v./sec.)			580±20.5	14	670±22*	29
Maximum rate of fall of AP (v./sec.)			112±5.6	14		

(a) Mean ± S.E.

(b) Some end-plate potentials were so much prolonged that the 1/2 decay time could not be measured at the recording speed used.

n Number of end-plates.

* From Nastuk (11).

† From Nastuk and Alexander (12). (Table 6-*d*TC 7.2 μM.)

‡ From Nastuk and Alexander (12). (Table 6-*d*TC 7.6 μM.)

epp. The nerve was stimulated during washout of ether and the recorded epp rapidly grew in amplitude until it became large enough to initiate a propagated action potential (trace E).

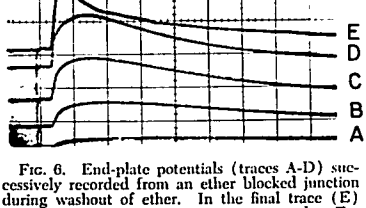

FIG. 6. End-plate potentials (traces A-D) successively recorded from an ether blocked junction during washout of ether. In the final trace (E) neuromuscular transmission was restored. For each successive trace the baseline was elevated. Calibration: large blocks—y axis = 20 mv., x axis = 5 msec.

The data in table 1 show that ether does not produce substantial changes in the muscle fiber resting potential recorded at the end-plate region. On the other hand, recordings of action potentials made at nonblocked end-plates show that in these ether treated preparations an increase in overshoot and a decrease in the maximum rate of rise occurred, both of which were statistically significant.

In the next experiment the action of ether on the electrical excitability of the muscle fibers was tested. The concentration of ether was raised to the point at which indirect stimulation produced no twitch. The cells were directly stimulated with one intracellular electrode; another intracellular electrode, placed nearby, was used for recording. A typical result is shown in figure 7 and the average results obtained are given in table 2. From the data in the uppermost line, one can

see that ether does not produce a statistically significant change in the resting potential. However, ether at this concentration caused statistically significant changes in the overshoot of the action potential, in the critical membrane potential at which an action potential is initiated, and in the negative after-potential.

The concentration of ether used in these experiments causes complete neuromuscular block but it leaves the muscle fibers electrically excitable even though some changes in the conduction mechanism of these fibers is produced. Thus one can see that the blocking effect of ether depends more on its action in depressing neuromuscular transmission than on its non-junctional action.

(C) The effect of diethyl ether on amplitude of miniature end-plate potentials.

In the next experiments we tried to determine whether ether reduces the sensitivity of the postjunctional membrane to acetylcholine (Ach). We approached this in two ways, one of which was to study miniature end-plate potentials (mepp's) at the neuromuscular junction.

The mepp's are randomly produced depolarizations of the resting postjunctional membrane. They reach 1.5 mv. in amplitude and the waveform resembles that of the epp's. Each mepp is produced by the release of a packet (quantum) of acetylcholine from the motor nerve terminals. When an action potential arrives at the telodendrites it causes release of about 200 quanta of Ach; these quanta, acting synchronously, give rise to the epp.

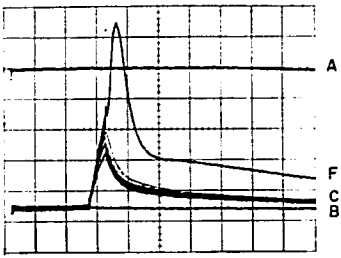

FIG. 7. Record taken under ether at a non-junctional site during application of cathodal stimuli. Trace A—with recording electrode outside fiber, trace B—with recording and stimulating electrodes inside fiber, traces C to E—with subliminal stimuli applied, trace F—with liminal stimulus applied initiating an AP. Calibration: large blocks—y axis = 20 mv., x axis = 1 msec.

TABLE 2. Average Characteristics of Membrane Potentials Recorded from Single Muscle Fibers Stimulated Through an Intracellular Electrode

	Control Values		Ether in Bathing Solution		After Ether Washout	
		n		*n*		*n*
Resting potential (RP) (mv.)	−91.1±0.6 (a)	9	−92.2±0.5	19	−94.6±0.4	17
RP (second electrode inserted) (mv.)	−81.7±1.7	9	−88.3±0.7	19	−90.6±0.7	17
Action potential (AP) (mv.)	119.9±1.8	9	120.0±0.9	19	128.7±1.0	17
Overshoot (mv.)	38.0±1.2	9	31.8±0.8	19	37.9±1.0	17
Critical membrane potential (mv.)	−47.6±1.1	9	−33.0±1.2	19	−37.7±1.2	17
Maximum rate of rise of AP (v./sec.)	467±21.3	6	420±10.4	19	438±6.1	17
Maximum rate of fall of AP (v./sec.)	168±6.3	9	156±2.8	19	167±2.1	17
Negative after-potential (value at knee) (mv.)	−70±1.0	9	−54.8±0.6	19	−68.6±0.1	17

(a) = Mean ± S.E.
n = Number of end-plates.

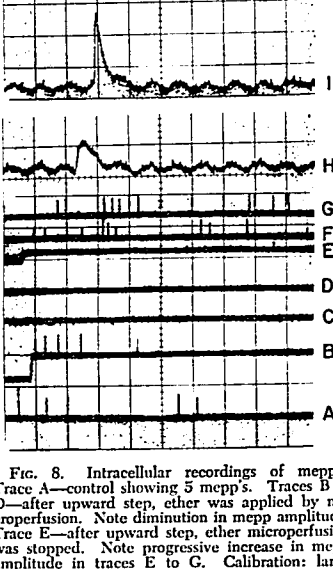

FIG. 8. Intracellular recordings of mepp's. Trace A—control showing 5 mepp's. Traces B to D—after upward step, ether was applied by microperfusion. Note diminution in mepp amplitude. Trace E—after upward step, ether microperfusion was stopped. Note progressive increase in mepp amplitude in traces E to G. Calibration: large blocks, y axis = 0.4 mv., x axis = 5 sec. Trace H —mepp during ether application. Trace I—control mepp. Calibration: large blocks—y axis = 0.2 mv., x axis = 20 msec.

Figure 8 shows a recording of mepp's before, during and after the perfusion of the end-plate with ether containing Ringer solution. It can be seen that after application of ether, the mepp's diminished in amplitude and then disappeared altogether. When the application of ether was discontinued, the mepp's returned and grew in amplitude. Similar results were obtained in several such experiments.

The time course of mepp's recorded from ether treated muscles was found to be prolonged (fig. 8, traces H, I). Both the rising and falling phases were slowed.

(D) Effect of diethyl ether on the chemosensitivity of the muscle postjunctional membrane.

In these experiments the action of ether on the response of the postjunctional membrane was tested directly by measuring the depolarization produced in the ether treated preparation when carbamylcholine was applied. Carbamylcholine (crab) rather than acetylcholine was used in these experiments because this compound is resistant to hydrolysis by acetylcholinesterase. After administration of ether (in a concentration sufficient to completely block the indirect twitch), application of carb 27×10^{-6} *M* caused less than 10 mv. depolarization of the postjunctional membrane (fig. 9). Following this, the ether and carb were removed (washout period) and then carb 27×10^{-6} *M* was applied in the absence of ether. Under these conditions the preparation twitched and the postjunctional membrane was depolarized more than 45 mv. The latter results are in agreement with those obtained by Nastuk and Gissen [13] (fig. 9, dotted line).

In order to be certain that an early more intense but *transient* depolarization did not occur at the moment carb was applied to the ether treated preparation, we measured the resting potential continuously in 5 fibers during microperfusion of the end-plate with carbamylcholine 27×10^{-6} *M*. The maximum depolarization measured in the presence of ether never exceeded 22 mv.

Discussion

Local and some nonvolatile anesthetic agents influence the mechanisms which regulate the ionic permeability of excitable cells (*c.g.*, Weidmann,[14] Shanes [15,16]). Such agents block neuromuscular transmission (*e.g.*, Thesleff [17] and synaptic transmission (Larrabee and Posternak,[18] Samjen and Gill [6]). Our interest in the present work was to learn more of the mechanism by which ether blocks neuromuscular transmission.

Our evidence shows that ether has a marked neuromuscular blocking action and that it also has some effect at nonjunctional regions of the muscle fiber. We may first consider the nonjunctional effects of ether.

Nonjunctional Effects of Ether. Recordings made at sites distant from the end-plate show that in the concentrations used, ether does not depolarize the resting muscle fiber. However action potentials recorded at this region show

a reduction in overshoot and the critical membrane potential lies closer to the zero potential level. Such changes, which are in agreement with those reported by Yamaguchi,[7] can be interpreted as indicating: (a) that ether diminishes the increase in sodium conductance which is associated with the action potential, (b) that ether *increases* the potassium conductance of the muscle fiber, (c) that both (a) and (b) occur. It appears unlikely that ether produced an *increase* in delayed potassium conductance because, during the muscle action potential, the maximum rate of repolarization is not increased (table 2). Also, the negative after-potential is greater in ether treated fibers which indicates that during the falling phase of the AP, the increased potassium conductance falls at a point where the repolarization is less complete than is normally the case. In addition we have found that the membrane conductance of resting muscle fibers is unaffected by ether in the concentration used in the present study (unreported experiments). We believe that our results are best explained by assuming that ether *decreases* both the rise of sodium conductance and the delayed rise in potassium conductance associated with the muscle action potential.

Junctional Effects of Ether. Concentrations of ether lower than those used in the above described experiments, produce an effective neuromuscular block (fig. 2). One cannot explain such a block on the basis of the above mentioned changes in electrical behavior of the nonjunctional membrane because these changes are small and a relatively high concentration of ether was needed to reveal them. Hence it can be concluded that ether has a relatively more important action on the neuromuscular transmission process.

After exposure to ether, the postjunctional membrane of the muscle fiber becomes less readily depolarized by carbamylcholine. This fact provides a basis for explaining the reduction in amplitude of epp and mepp and the increase in overshoot of action potentials neurally initiated at the end-plate. Apparently ether produces these changes in the behavior of the postjunctional membrane by a mechanism unlike that which underlies the action of *d*-tubocurarine (*i.e.*, competitive inhibition at receptor sites). There are two reasons for the

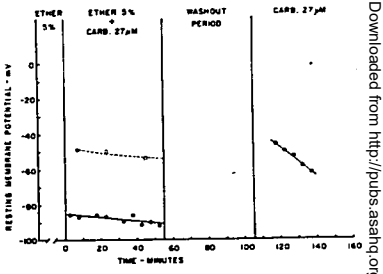

FIG. 9. Effect of carbamylcholine (27 μM) on the resting potential measured at the end-plate of muscle fibers, in the presence of ether (left), and with ether removed (right). Each solid circle represents the average of readings taken over a 5 minute period. Open circles (data of Nastuk and Gissen[12]) show the more intense depolarization produced by 27 μM carb in the absence of ether.

above statement: (a) ether causes a prolongation in the time course of the mepp's and especially of the epp's, (b) neuromuscular block produced by ether cannot be effectively antagonized by edrophonium or succinylcholine.

In addition to the demonstrated postjunctional effects, one might suppose that ether also acts prejunctionally on the telodendrites of the motor neuron. It could, for example, reduce the amount of acetylcholine released per nerve impulse (quantal content). This result would be obtained if ether sufficiently reduced the amplitude of the nerve action potential in the telodendrites (Katz[19]). However this mechanism would not explain why the time course of the epp is so greatly prolonged unless, as seems somewhat unlikely, ether also causes a great increase in the duration of the action potential in the telodendrites. Further, we may add another argument against low quantal content as an explanation of the ether-produced neuromuscular block, that is, such a block is not effectively antagonized by edrophonium (fig. 3). Finally we note that the diminution in amplitude of mepp's cannot be easily explained on a prejunctional basis. If the diminution in mepp amplitude were entirely prejunctional in origin, there would have to be a reduction in quantal size, *i.e.*, reduction in the amount of Ach per quantum re-

leased. We regard this explanation as unlikely because *all* of the mepp's recorded at a resting neuromuscular junction diminish a few seconds after ether is applied to the area. Thus one can argue that at least some of the quanta released would have been synthesized and stored prior to ether administration and hence these quanta should produce normal sized depolarizations of the postjunctional membrane, provided that the latter has normal sensitivity to Ach.

The prolongation of the epp seen in the etherized preparation is not readily explained by assuming that ether inhibits acetylcholinesterase. Prolongation of the epp after anticholinesterase inhibition (Nastuk and Alexander [12]) is generally not nearly so marked as that seen in the etherized neuromuscular junction. Furthermore there is no evidence that ether is an effective inhibitor of acetylcholinesterase.[20, 21]

The prolongation of the epp which occurs when ether is present might be explained on the basis that released Ach, which combines with receptor sites, dissociates from such combination more slowly than usual. Further, from evidence obtained in this study, we propose that ether causes a reduction in the sodium conductance produced during Ach activation of postjunctional membrane receptors. Thus combining these postulates we can make a summary statement that when ether is present, the increase in sodium conductance produced by Ach is less but more prolonged than is the case for the normal preparation. These speculations require experimental verification.

The postjunctional action of ether in blocking neuromuscular transmission poses some interesting theoretical questions. The receptor sites on this membrane are considered to be negatively charged and hence exert attraction on quaternary ammonium ions such as Ach or *d*TC. However ether is an uncharged molecule and if it interacts with the receptor sites, more than simple Coulombic forces must be involved. An alternative view is that ether does not act directly upon the postjunctional receptor sites, but causes changes in the behavior of the permeability controlling mechanisms which are engaged subsequent to receptor site activation. A mechanism such as this was postulated to explain the blocking action of the diquaternary drug, hexafluorenium.[18] Whatever the truth may be, it seems clear that ether and *d*TC do not block neuromuscular transmission by the same mechanism.

Summary

The action of diethyl ether has been studied on frog sciatic nerve-sartorius muscle preparations *in vitro*. The tension output of the indirectly stimulated muscle falls progressively as the partial pressure of ether in the perfusing fluid is increased. At approx. 15 mm. Hg ether, the tension output falls to 50 per cent of control and at approx. 23 mm. Hg ether the tension output is zero. At a concentration of ether which blocks the indirect twitch, the muscle fibers can be stimulated directly. They have normal resting potentials and the action potentials elicited show only minor changes in form. The neuromuscular block produced by ether is not effectively antagonized by edrophonium or succinylcholine. Ether causes reduction in the amplitude and prolongation of the time course of both the end-plate potential and the miniature end-plate potential. Ether diminishes the depolarizing action of carbamylcholine on the postjunctional membrane. The mechanism of the neuromuscular blocking action of ether differs from that of *d*-tubocurarine. Some suggested mechanisms of action of ether are discussed.

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HYPERCAPNIA Responses of vascular smooth muscle (VSM) to noradrenaline are depressed during respiratory acidosis, but it has not been reported whether the depression is effected at the adrenergic receptor or distal to it in the contractile mechanism. Hind limbs of dogs and cats were perfused and the responses of the VSM to intra-artrial doses of noradrenaline and pitressin were tested before and during hypercapnia. Maximum responses of VSM to noradrenaline were not depressed during the high carbon dioxide treatment. Therefore the depression probably is due to an influence on the drug-receptor or coupling reactions rather than the contractile mechanism. The depressive action was not confined to adrenergic drug-receptor reactions, as the responses to pitressin were also depressed by hypercapnia. Therefore, the depression probably was located between the drug-receptor reaction and the contractile mechanism in some coupling reaction common to the two drugs. (*Nash, C. W., Shivak, R. J., and Engelhardt, E. M.: Influence on Reserpine on Cardiovascular Responses to Noradrenaline, Tyramine and Pitressin During Respiratory Acidosis, Canad. J. Physiol. 43: 627 (July) 1965.*)