The Effect of Volatile Anesthetic Agents on Neuromuscular Transmission

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It is commonly observed that general anesthesia produced by the administration of volatile anesthetic agents is accompanied by muscular relaxation. This raises the question as to the site of action and the mechanism by which such muscle relaxation is produced. As early as 1914 Auer and Meltzer [1] reported that ether possesses a curare-like action. More recently Watland [2] in 1957, Ngai [3] 1965 and Katz [4] 1966 have shown that neuromuscular transmission *in vivo* can be blocked by volatile anesthetics but only if extremely high concentrations of the anesthetics are administered. Thus, from these studies one could conclude that the muscular relaxation produced during general anesthesia is more attributable to the central action of the anesthetic than to a peripheral action at the neuromuscular junction. Nonetheless the peripheral action of these anesthetic agents on the neuromuscular system is by no means negligible. For example, during light ether anesthesia complete neuromuscular block can be produced by the administration of relatively small doses of curare that would be ineffective in producing neuromuscular block in the absence of ether.

In order to obtain more direct evidence concerning the site and mode of actions of various volatile anesthetics on the neuromuscular system we have studied the action of several of these agents on the frog sartorius muscle sciatic nerve preparation *in vitro*. By this means we have been able to design and carry out definitive experiments employing single fiber techniques. Such experiments cannot easily be conducted in the intact mammalian organism.

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The first series of experiments was designed to determine the range of concentrations of anesthetics which would depress the tension output of the indirectly stimulated muscle. Figure 1 shows the experimental arrangement with the sartorius muscle mounted in an isometric myograph. The nerve was stimulated once a minute with a supramaximal stimulus and the evoked tension output was measured with a mechanoelectric transducer. The resulting signal was fed into an oscilloscope and photographically recorded. A major technical difficulty in this investigation was the control of the concentration of anesthetic present in the bathing solution. We chose to equilibrate the Ringer's solution by saturating it with an accurately determined mixture of oxygen and anesthetic vapor at a known partial pressure, delivered by an anesthetic apparatus.

Figure 2 shows the results obtained when an indirectly stimulated muscle was exposed to 2 and 2.5 per cent ether. After a stable control period was achieved, addition of 2 per cent ether decreased tension output to approximately 60 per cent of the initial value. At 2.5 per cent ether the tension output decreased to approximately 20 per cent of the control. Complete block was obtained with approximately 3 per cent ether in the equilibrating mixture. Perhaps it is of interest to mention here that when cyclopropane up to approximately 20 per cent was applied, the tension output increased to above the control value; but at higher concentrations of cyclopropane, complete block developed. A similar but much smaller initial potentiation of tension output amounting to 2 or 3 per cent of the control value was seen with halothane.

It is evident from these experiments that these anesthetics depress the tension output of indirectly stimulated muscle. The question

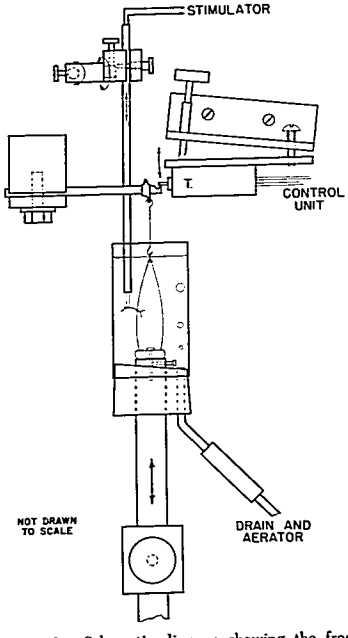

FIG. 1. Schematic diagram showing the frog sartorius muscle mounted in an isometric myograph. The nerve is stimulated once a minute. The bathing Ringer's solution is bubbled with a mixture of oxygen and anesthetic agent.

then arose, do these agents exert their blocking effect on nerve, the neuromuscular junction or the muscle fiber itself?

We found, in general, agreement with the earlier work of Secher,[5] that axonal conduction in the isolated frog sciatic nerve trunk was not affected when these trunks were exposed to ether or halothane in concentrations which completely blocked the indirectly stimulated frog nerve-muscle preparation. Furthermore, at these blocking concentrations of ether, we found that muscle fibers can be directly stimulated through an intracellular microelectrode and that the action potential which is initiated produces contraction of the muscle fiber.[6] Similar results have been obtained with halothane.[7]

We conclude from these experiments that it is the *neuromuscular transmission* process which is most susceptible to blockade by these volatile anesthetics. We therefore tried to determine whether the site of action was prejunctional, postjunctional or both.

In the next series of experiments we explored the *postjunctional* locus by studying the chemosensitivity of the postjunctional membrane. Resting potentials were measured intracellularly with a microelectrode introduced at endplate sites. Exposure of a muscle preparation to ether at a concentration sufficient to block completely the indirect twitch did not cause membrane depolarization. Resting potentials measured during a two-hour period averaged 94 mv., a value equal to that of the

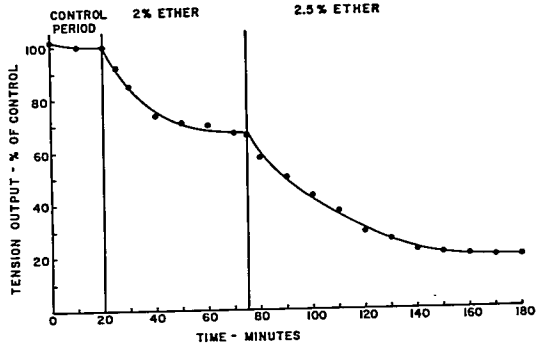

FIG. 2. Fall in tension output of the indirectly stimulated frog sartorius muscle at various times after addition of ether 2 per cent and 2.5 per cent to the gas mixture with which the bathing solution was equilibrated.

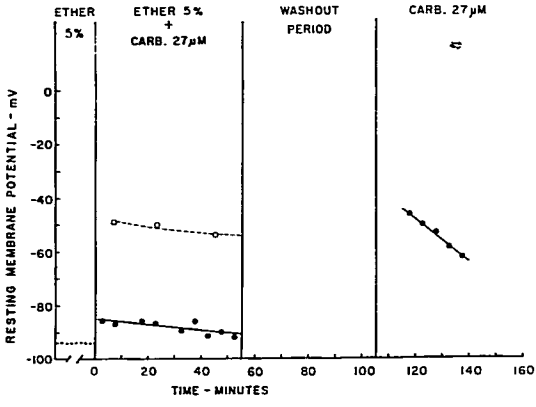

FIG. 3. Effect of carbamylcholine (27 μM) on the resting potential measured at the endplate of muscle fibers in the presence of ether (left) and with ether removed (right). Each solid circle represents the average of readings from individual fibers taken over a 5 minute period. Open circles (data of Nastuk and Gissen[8]) show the more intense depolarization produced by 27 μM carbamylcholine in the absence of ether. During the 2 hour period of pretreatment with ether the resting potential averaged 94 mv. (dotted line).

controls (figure 3, dotted line). Thereafter, carbamylcholine (27 μM concentration) was added. Carbamylcholine rather than acetylcholine was used in these experiments because this compound is resistant to hydrolysis by acetylcholinesterase. As can be seen in figure 3, the average of individual measurements taken over a 5 minute period (solid dots) showed that carbamylcholine in the presence of ether produced relatively slight membrane depolarization. It is interesting to compare this result with that obtained when carbamylcholine was added directly to control preparations or to preparations from which the ether was removed. In the latter, carbamylcholine caused the preparations to twitch and produced postjunctional membrane depolarization exceeding 45 mv. The above effects of ether are also produced by halothane.[7] One can conclude from these experiments that during exposure to ether or halothane the response of the postjunctional membrane to depolarizing agents such as carbamylcholine is markedly reduced.

In a third group of experiments we tried to compare possible *prejunctional* effects of these anesthetics with their postjunctional action. The first experiment was concerned with the effect of volatile anesthetics on the amplitude of miniature endplate potentials.

When a microelectrode is introduced at the endplate site and high amplification is used, randomly produced depolarizations of about 0.5 mv. can be measured. These so-called miniature endplate potentials are believed to be produced by the release of small packets (quanta) of acetylcholine from the nerve terminal. When the neuromuscular junction was exposed to Ringer's solution containing a low concentration of ether or halothane, the amplitude of these miniature endplate potentials decreased to imperceptible levels (fig. 4). After discontinuation of the anesthetic, the miniature endplate potentials progressively rose in amplitude. At present, it is accepted that an appreciable number of preformed acetylcholine quanta are stored in the nerve terminals and that these quanta are approximately equal in size. Thus, a *progressive* decrease in the amplitude of the miniature endplate potential is most easily explained on the basis that ether causes a progressive diminution in the sensitivity of the postjunctional membrane to acetylcholine. As we have demonstrated directly, loss of postjunctional membrane sensitivity is indeed produced by ether and halothane and the extent of this change is sufficient to explain the decrease in amplitude of these miniature endplate potentials.

Although our next 2 experiments are taken from work in progress, the results obtained are

FIG. 4. Intracellular recordings of mepp's. Trace A—control showing 5 mepp's. Traces B to D—ether was applied by microperfusion. Trace E—after upward step ether microperfusion was stopped. Note the progressive increase in mepp amplitude in traces E to G. Calibration: large blocks, y axis = 0.4 mv., x axis = 5 sec.

direct and seem interesting enough to be reported here.

In 1963 Hubbard and Schmidt[9] showed that when a recording microelectrode was carefully placed external to a motor nerve terminal, stimulation of the nerve results in a recording that shows successively: the stimulus artefact; the action potential of the nerve ending; and, the endplate potential produced at the postjunctional membrane. In our experiment the muscle preparation was exposed to Ringer's solution containing curare in a concentration just sufficient to prevent contraction. An endplate potential and a presynaptic action potential were demonstrable as shown in figure 5. If, under such experimental conditions, the preparation was microperfused at the endplate with Ringer's solution plus *d*-tubocurarine and 0.1 per cent v/v halothane, the endplate potential produced at the postjunctional membrane disappeared, but this occurred without affecting the magnitude of the nerve action potential. The most likely explanation for the decrease in the amplitude of the endplate potential is that the sensitivity of the postjunctional membrane is decreased by halothane at a concentration which does not significantly affect the nerve ending.

Our final preliminary report concerns a comparison of the action of halothane on depolarization caused by both neurally released and externally applied acetylcholine. A schematic

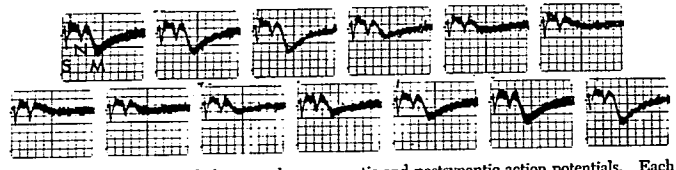

FIG. 5. The effect of halothane on the presynaptic and postsynaptic action potentials. Each oscillogram represents several superimposed sweeps each of which is a record made externally at the neuromuscular junction. Left upper frame shows the recording made at a curarized preparation. S = stimulus artefact; N = action potential in nerve terminal; M = endplate potential. Microperfusion with halothane was started between the first and second frame of the upper row. Note the progressive decrease in the amplitude of the endplate potential in the third and sixth frame. The lower row represents recovery following discontinuation of the microperfusion with halothane. Calibration: Blocks, y axis = 0.1 mv., x axis = 1 msec.

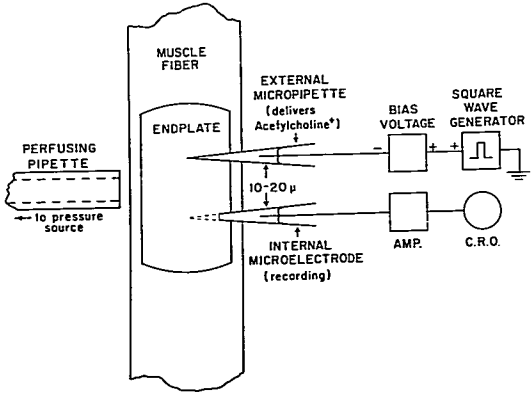

FIG. 6. Schematic diagram of experimental setup for recording of depolarizations produced by nerve stimulation and externally applied acetylcholine. The perfusing pipette contained 0.1 per cent v/v halothane.

diagram of the test is shown in figure 6; this preparation was also curarized to prevent movement. The membrane potential was recorded with an intracellular microelectrode. Acetylcholine was applied to a sensitive spot at the postjunctional membrane by electrophoretically ejecting this compound from a pipette loaded with acetylcholine. Following nerve stimulation, acetylcholine is released from the nerve terminals giving rise to an endplate potential. By alternatively stimulating the nerve and pulsing the acetylcholine pipette, we can measure postjunctional membrane potential changes caused by either means.

We found, in this experiment, that upon perfusion with halothane containing Ringer's solution, the endplate potentials as well as the depolarizations caused by the acetylcholine pulses decreased in amplitude at approximately the same rate and to the same degree.[7] Since the neurally initiated response of the postjunctional membrane (endplate potential) is a measure of both acetylcholine released by nerve and postjunctional sensitivity, while depolarization of the postjunctional membrane produced by the acetylcholine pulse is a measure *only* of postjunctional membrane sensitivity, one can conclude that the effect of halothane is mainly on the postjunctional membrane.

In summary, we conclude from the foregoing experiments that anesthetics such as ether and halothane block neuromuscular transmission *in vitro* principally because they reduce the sensitivity of the postjunctional membrane to acetylcholine. During general anesthesia, the muscular relaxation produced by volatile anesthetics such as ether and halothane is probably mainly attributable to their central rather than to their peripheral action. However, even under such conditions, the peripheral action which involves a reduction in the sensitivity of the postjunctional membrane to acetylcholine is not negligible and could be significant when neuromuscular transmission is impaired by the presence of curare-like agents or neurological diseases.

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DISCUSSION

Sir John Eccles: You have observed an amazing effect with complete suppression both of the miniature endplate potential and of the EPP's. You do not have to qualify your statement, this is an immense effect and all the neuromuscular block could be explained by it.

Dr. Karis: Several investigators have shown that volatile anesthetics can produce a neuromuscular block. To my knowledge, no studies have been reported concerning the mechanisms of action of these agents at the neuromuscular junction. In the last two experiments (figs. 5 and 6) the Ringer's solution used to microperfuse the endplate site contained halothane 0.1 per cent v/v. With this high concentration of halothane it was possible merely to let this solution slowly leak out of the pipette onto the endplate and thereby avoid artifacts of movement. The advantage of this approach was that we were able to study the progressive changes of the electrical events occurring at the postjunctional membrane and the nerve terminal. As I mentioned before, halothane at a much lower concentration (1.5 per cent in the equilibrating gas) produced a complete neuromuscular block.

Dr. Ngai: To clarify this, when you say 3 per cent or 1 per cent, you are referring to percentage of an atmosphere, that is, the partial pressure in the gas bubbling through the Ringer's solution; but 0.1 per cent halothane is volume/volume of liquid, a much higher concentration.

Sir John Eccles: I suppose that one doesn't get a blood concentration as high as 0.1 per cent halothane when it is used as an anesthetic.

Dr. Karis: Indeed, for general anesthesia that would be much too high. However, it is unlikely that we ever approached this high concentration during any of our experiments. Although the perfusion pipette contained halothane at 0.1 per cent v/v, this solution must have been rapidly diluted, since the rate of perfusion was kept very low.

Sir John Eccles: The other point is, of course, that with those results you always have an initial curarization before you start. What happens if you do not have this initial curarization? You can still do the experiment, but it is a bit more difficult.

Dr. Karis: You cannot externally record an action potential in the nerve terminal without complete mechanical quiescence of the preparation. A curare-block is essential.

Sir John Eccles: You can block neuromuscular transmission with magnesium.

Dr. Karis: We have attempted experiments in preparations blocked with magnesium. The few that were successful gave similar results.

Sir John Eccles: Still this is such a tremendous effect that you should be able to test it without complications from other drugs.

Dr. Karis: The effect of anesthetics on endplate potentials and on miniature endplate potentials was obtained on preparations which were not exposed to other drugs. We used curare only when it was essential to prevent movement, so that we were able to take sequential recordings at different anesthetic concentrations at the same endplate.

Sir John Eccles: You could do it with the whole frog sartorius and external electrodes lying across the muscle, as Katz did originally, and get the excitatory potentials with the muscle still contracting.

Dr. de Jong: This is a magnificent piece of work, but I am concerned by the concentrations of anesthetic drugs used. Drs. Ngai and Katz found both in animals and in man that high concentrations of anesthetics are needed before an effect on neuromuscular transmission is observed. Dr. Freund and I at Washington have also found that at high anesthetic concentrations where profound muscular relaxation is observed, little if any effect on the neuromuscular junction is present, both electrically and mechanically; so we are concerned by the marked differences between your

work on single fibers and our work on intact muscle *in vivo*.

Dr. Karis: I agree that in the clinical production of muscular relaxation by volatile anesthetics the central action is more important than peripheral neuromuscular blockade. However, even under clinical conditions, the peripheral action of these agents is not negligible. Beyond these practical matters, I wish to stress the point that we undertook this work in an attempt to obtain an understanding of the basic action of volatile anesthetics in the most simple preparation available. If we are able to understand how these agents work in this simple amphibian preparation, we may be able to get a better understanding of the pharmacological action of these agents in animals and in man, *in vivo*.

Dr. Ngai: It remains true that in man or in an intact cat or decerebrate cat, you do need up to 40 per cent of ether in the inspired air to have appreciable neuromuscular block. I cannot explain the difference.

Dr. Salmoiraghi: Have you done any measurements on the effect of anesthetics on threshold?

Dr. Karis: We have not studied threshold currents. Inoue and Frank reported in 1965, that threshold currents decreased somewhat with 0.5 per cent ether, with essentially no change in threshold potential. At 1 per cent ether both threshold current and threshold depolarization were increased. (Inoue, F., and Frank, G. B.: Canad. J. Physiol. Pharm. 43: 751, 1965.)

Sir John Eccles: When you used concentrations of anesthetics which did not depress the miniature endplate potentials to zero, did you find that the concentration of anesthetic altered the frequency of miniature firing? This is a more subtle test of a change in the presynaptic mechanism, and one would like to know also about the number of quanta released per nerve impulse under these conditions? This would be more subtle than the complete block that you have demonstrated, and should be done with lower doses. Finally, I would suggest that the rat diaphragm preparation might be a favorable one, when moving into the mammalian field.

Dr. Karis: I agree completely with the comments you have made. The experiments you suggest involve technical difficulties. The chamber we used for microelectrode recordings is open to air, so that in order to control the concentration of a volatile anesthetic agent, a constant rather vigorous flow of freshly equilibrated Ringer's solution has to be provided. This can generate many artifacts so that the low voltage recordings of miniature endplate potentials, already depressed by the anesthetic, would get lost in the noise. In our perfusion experiments, however, the amplitude of the miniature endplate potentials decreased under the influence of the anesthetic before we were able to recognize any changes in frequency.

Dr. Ngai: Perhaps you could repeat your miniature endplate studies with a lower concentration of halothane, instead of 0.1 per cent volume/volume.

Sir John Eccles: That was just my point. Lower the concentration, get a steady level, and then see what happens to the frequency.

Dr. Karis: The difficulty is to get a steady level at a low concentration of the volatile anesthetic, without causing artifacts which will make the record unreadable.

Dr. Somjen: I should like to see confirmation of your results by a count of spontaneous miniatures at a steady low concentration. If you cannot do it with halothane, why not do it with barbiturates, which apparently have a very similar action as far as the postsynaptic junction is concerned?

Dr. Karis: One would not have many difficulties in counting the spontaneous miniature endplate potentials during the study of nonvolatile agents like barbiturates. Our main interest, however, was the study of the action of the volatile anesthetic agents.

Dr. Salmoiraghi: Why do you use volatile anesthetics which are very difficult to control, when barbiturates would seem better suited for your purposes?

Dr. Karis: Volatile anesthetics, contrary to the barbiturates, (both used in clinical concentrations) can produce profound muscular relaxation. This is of interest to the anesthesiologist and we wished to get more understanding of the mechanism of action of these agents.

Questioner: In regard to the effect of apparently different concentrations of anesthetic, what was the temperature of the bathing solution? The solubility of halothane is altered at lower temperatures and this might be a contributing factor.

Dr. Karis: The experiments were done at room temperature.