

Modification of Sodium and Potassium Channel Gating Kinetics by Ether and Halothane

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ABSTRACT The effects of ether and halothane on the kinetics of sodium and potassium currents were investigated in the crayfish giant axon. Both general anesthetics produced a reversible, dose-dependent speeding up of sodium current inactivation at all membrane potentials, with no change in the rising phase of the currents. Double-pulse inactivation experiments with ether also showed faster inactivation, but the rate of recovery from inactivation at negative potentials was not affected. Ether shifted the midpoint of the steady-state fast inactivation curve in the hyperpolarizing direction and made the curve steeper. The activation of potassium currents was faster with ether present, with no change in the voltage dependence of steady-state potassium currents. Ether and halothane are known to perturb the structure of lipid bilayer membranes; the alterations in sodium and potassium channel gating kinetics are consistent with the hypothesis that the rates of the gating processes of the channels can be affected by the state of the lipids surrounding the channels, but a direct effect of ether and halothane on the protein part of the channels cannot be ruled out. Ether did not affect the capacitance of the axon membrane.

INTRODUCTION

The action potential in nerves is produced by voltage- and time-dependent changes in the ion permeability of the nerve membrane (Hodgkin et al., 1952). These permeability changes are almost certainly due to pores (Armstrong, 1975) composed at least partially of protein (Armstrong et al., 1973; Shrager, 1974 and 1975).

The gating mechanisms governing the opening and closing of the ionic channels are not well understood. One question that must be asked is to what extent the lipid milieu surrounding the ionic channels might influence their gating properties. Campbell and Hille (1976) suggested that the differences in the rates of gating seen between sodium channels in frog nerve and those in frog muscle might be due to different membrane material surrounding the channels, which otherwise have nearly identical properties. Discontinuities in the temperature dependence of sodium channel gating kinetics have been observed (Chiu et al., 1979; Schwarz, 1979), and it has been suggested that they represent the effect of phase transitions of the membrane lipids.

Gaseous or volatile-liquid general anesthetics are capable of perturbing the lipid structure of bilayer membranes. Most of the changes observed can be lumped together as reflecting an increase in the "fluidity" of the membrane with general anesthetics present: the order estimated by the average tilt of nitroxide spin labels on fatty-acid chains is decreased (Trudell et al., 1973; Boggs et al., 1976; Mastrangelo et al., 1978); the rotational and lateral diffusion rates of various molecules are increased (Vanderkooi et al., 1977); native and carrier-mediated ionic permeabilities are increased (Johnson and Miller, 1970; Johnson et al., 1973; Pang et al., 1979).

The work described here is an attempt to use general anesthetics as a tool to perturb the lipid matrix of a nerve membrane. There are two apparent effects of the general anesthetics ether and halothane on the kinetics of the sodium and potassium currents in the crayfish giant axon membrane: sodium current inactivation and potassium current activation are faster with anesthetic present. The increased rate of sodium current inactivation might represent either a time-dependent blocking of open sodium channels or an increase in the rate of the normal inactivation gating process, but it seems likely that the effect on potassium current kinetics is a true increase in the rate of the gating process.

A preliminary report of this work has been published (Bean et al., 1979).

METHODS

Voltage Clamp

Giant axons from the crayfish *Procambarus clarkii* were dissected, cleaned, and cannulated with a piggy-back voltage-clamp electrode as described by Shrager (1974).

The voltage-clamp currents were measured in two different ways in different experiments. In the early experiments, the current signal was obtained from the current flowing to a central 2-mm length of platinized platinum foil flanked by 5-mm guard regions. In later experiments, current density was measured with a differential electrode consisting of two closely spaced platinum wires, a technique devised by Cole and Moore (1960) for measuring currents from a smaller length of axon that is described in greater detail elsewhere.¹ Series resistance compensation (Hodgkin et al., 1952) for 3–13 ohm-cm² was included in the voltage-clamp circuit; the current signal was filtered at 12 kHz before being fed back into the circuit to allow more complete compensation (Sigworth, 1979).

Recording and Analysis of Currents

Currents were amplified, digitized, and temporarily stored in an IMSAI 8080 microcomputer (IMS Associates, San Leandro, Calif.); later, the digital data were transferred to a PDP 8/E minicomputer (Digital Equipment Corp., Marlboro, Mass.) for analysis.

In most sodium and potassium current experiments, leak and capacitive currents were subtracted from records of total current by using linearly scaled currents in response to hyperpolarizations. In some sodium current experiments, leak and ca-

¹ Bean, B. P. Sodium channel inactivation in the crayfish giant axon. Must channels open before inactivating? Submitted for publication.

pacitative currents were obtained by preceding the test pulse by a prepulse long and large enough to totally inactivate sodium current.

Time constants for sodium current inactivation were obtained by least-squares fits (using weighted residuals) to the falling phase of the currents. Usually, the fit was made between the times at which the current had decayed to 75 and 20% of its peak value.

Solutions

Axons were dissected and prepared in van Harreveld's (1936) solution: 205 mM NaCl, 5.4 mM KCl, 13.5 mM CaCl₂, 2.6 mM MgCl₂, 2.3 mM NaHCO₃, pH 7.5–7.6. Potassium currents were measured in van Harreveld's solution with 100 nM tetrodotoxin (TTX) added. Sodium current experiments were performed in modified van Harreveld's solution containing 1 mM 4-aminopyridine (4-AP) to block potassium current (Meves and Pichon, 1975), with *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES) buffer (2.3 mM) replacing bicarbonate. In most sodium current experiments, lowered sodium and potassium concentrations were used, with NaCl and KCl partially replaced by isoosmotic amounts of tetramethylammonium chloride. Solutions were 433–438 mosM before the addition of anesthetic.

Temperature Control

The nerve chamber was cooled by two Peltier devices and by precooling the bathing solution with ice-water coils. Temperature was measured with a small thermistor mounted within a few hundred microns of the axon. 95 ms after each current sweep, the temperature signal was digitized and stored together with the current sweep; it was also converted to degrees and displayed at the computer teletype.

Preparation and Delivery of Anesthetic Solutions

Ether (2-9239, J. T. Baker, Phillipsburg, N. J.) was redistilled and stored in wood-corked glass flasks. Solutions were made up by pipetting ether through a small hole in the cork of a flask of swirling saline. Solutions both with and without ether flowed through the chamber (~10-ml capacity) at rates of from 10 to 40 ml/min. In control experiments, the constancy of the aqueous ether concentrations in the chamber was checked using ultraviolet absorption spectroscopy; ether concentrations in the chamber were constant to within 3% of the nominal values, with flow rates as low as 5 ml/min.

Halothane (Ayerst Laboratories, N. Y.) was redistilled to remove the preservative thymol and was stored in light-tight glass containers. Halothane solutions were made by dilution of a solution of saline saturated with halothane at room temperature, and concentrations were calculated using a value of 32 mM for this saturated solution (Secher, 1971).

The nerve chamber and associated apparatus were mounted on a vibration-damped platform inside a chemical fume hood.

RESULTS

Faster Decay of Sodium Currents

The inactivation phase of sodium currents becomes faster when ether is applied to an axon. Fig. 1 *a* and *b* shows sodium currents at -20 and $+65$ mV before, during, and after exposure to 100 mM ether. The falling phases of the

currents are shown superimposed with least-squares fits to an exponential decline to zero; it is clear that the rate of decay of the current is reversibly increased by ether at both potentials. The fit to a single exponential is excellent at -20 mV both with and without ether, but at $+65$ mV there is a biphasic decay in both cases. Biphasic decay for currents at potentials greater than about $+50$ mV was seen in all axons, but the slower component is usually quite small, as in Fig. 1 *b*, and it was ignored in the analysis.

The change in kinetics produced by ether appears as fast as the solution is exchanged (a few minutes). The reversal is complete or nearly complete after

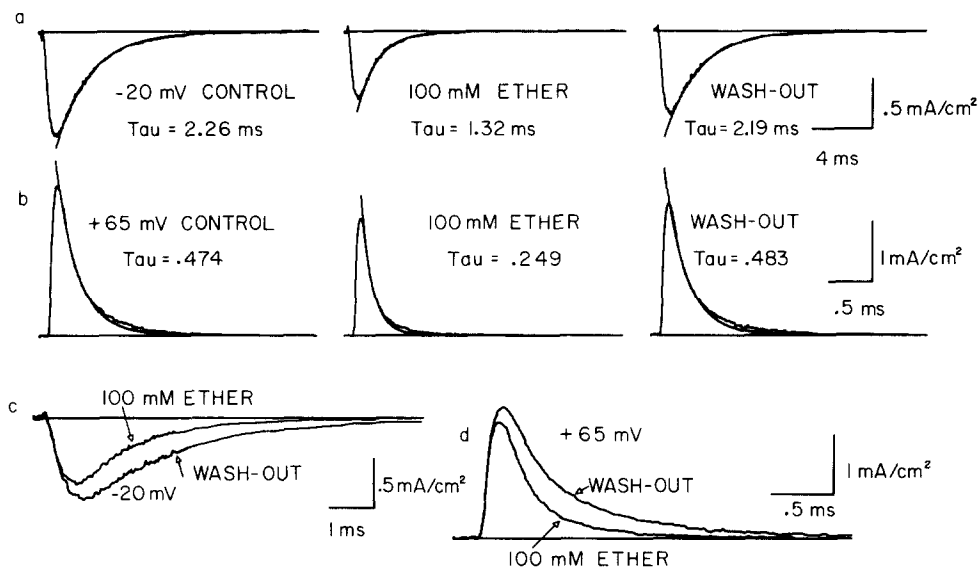


FIGURE 1. Effect of ether on sodium current kinetics. (a) Application and wash-out of 100 mM ether at -20 mV. Currents are superimposed with exponential fits to falling phases. (b) Same, for outward currents at $+65$ mV. (c) Superimposed currents from *a*, with ether and after wash-out. (d) Superimposed currents from *b*, with ether and after wash-out. *a* and *b* are from the same application of ether. $\frac{1}{4}$ Na, $\frac{1}{2}$ K, 1 mM 4-AP. Differential current electrode. Holding potential, -80 mV. Temperatures: (a) 3.2°C for control, ether, wash-out. (b) 3.1°C for control, 3.8°C for ether, 3.6°C for wash-out.

15–20 min of rinsing with ether-free saline. Rinsing produces complete reversal of the effects on kinetics for ether concentrations of up to 200 mM.

The time constants for inactivation over the range of membrane potentials from -40 to 95 mV are shown in Fig. 2 for the same application of 100 mM ether from which the records in Fig. 1 were taken. The inactivation time constants are reduced by ether over the entire potential range. The percent decrease in time constant in most experiments was slightly larger at large depolarizations (53% at $+75$ mV, 59% at $+95$ mV) than at small or moderate depolarizations (46% at -40 mV, 34% at 0 mV).

The dependence on ether concentration of the decrease in the inactivation

time constant is shown in Fig. 3. Each point is a mean value obtained from applications of ether to a number of axons. Experiments were performed with a variety of external sodium and potassium concentrations and with holding potentials from -72 to -105 mV; there were no obvious differences in effects

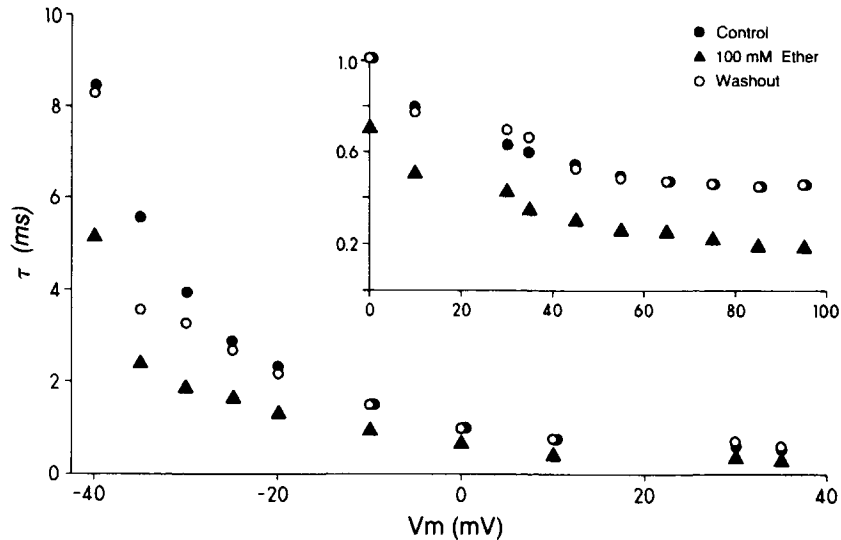


FIGURE 2. Inactivation time constant as a function of membrane potential before, during, and after 100 mM ether. Ether was applied for 9 min and then rinsed for 18 min. $\frac{1}{4}$ Na, $\frac{1}{2}$ K, 1 mM 4-AP. Differential current electrode. Holding potential, -80 mV. Temperatures: control, 3.1 – 3.5°C ; 100 mM ether, 3.0 – 3.8°C ; wash-out, 3.3 – 3.6°C . At most potentials, control, ether, and wash-out temperatures are matched to within 0.2 – 0.3°C . (*Inset*) Data for 0 to $+100$ mV on expanded scales.

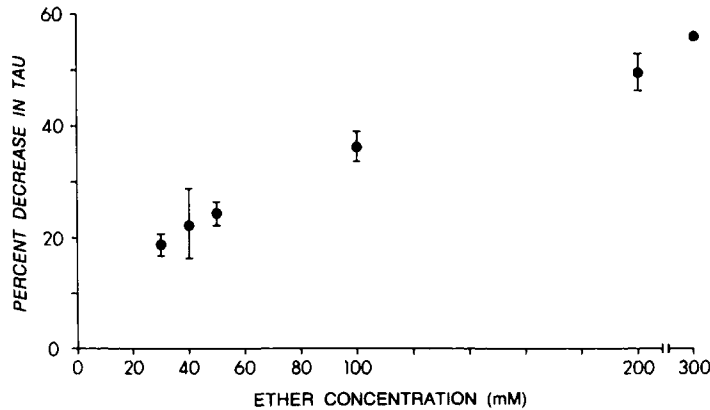


FIGURE 3. Concentration dependence of the decrease in inactivation time constant. Each point is the mean \pm SEM for applications of ether to a number of axons (five for 30 mM, four for 40 mM, four for 50 mM, seven for 100 mM, three for 200 mM, one for 300 mM). The value for each axon was determined as the mean for a number of membrane potentials between -20 and $+90$ mV.

with changes in any of these parameters. Most experiments were carried out in the temperature range -1° to 8°C ; a few experiments at 25°C showed changes in kinetics of about the same magnitude, but this point was not investigated in detail.

The experiments summarized in Fig. 3 were all performed with 1 mM 4-AP to block potassium currents, but the presence of 4-AP was not required for the effect of ether on sodium channel kinetics. In one experiment, sodium currents were obtained by a TTX-subtraction procedure with no 4-AP present: 50 mM ether was applied and rinsed, 100 nM TTX was applied to block sodium currents, and 50 mM ether was applied again. The reversibility of the effect of ether on the potassium currents allowed accurate determinations of sodium current time-course by subtracting the TTX-insensitive currents both with and without ether. The time constant for sodium current decay at -15 mV was decreased by 25% with ether present, which is in good quantitative agreement with the results summarized in Fig. 3 for 50 mM ether with 4-AP.

Effects of Ether on Sodium Current Amplitude

In addition to speeding up the inactivation phase of sodium currents, ether changes the amplitude of the currents. There are two irreversible effects on amplitude, which occur only during the first application of ether to an axon, and a third effect, which is reversible with repeated applications and wash-outs. First, there is an irreversible loss of 10–20% of the sodium current during the first application of ether: compare, for example, the control and wash-out currents in Fig. 1. Second, the first application of ether in a concentration greater than about 60 mM results in an irreversible shift, in the depolarizing direction, of the voltage dependence of a slow inactivation process that governs the availability of sodium channels. The slow inactivation process in the crayfish axon has been described by Shrager (1977), and Starkus and Shrager (1978) found that internal treatment of the axon with trypsin irreversibly shifted the voltage dependence of the process in the depolarizing direction. Application and removal of large concentrations of ether or halothane produces similar, but smaller, shifts of 5–8 mV. This irreversible shift is specific to slow inactivation and not an expression of a generalized shift such as might be given by a change in membrane surface charge, because the effects of ether on fast inactivation and potassium channel activation kinetics are entirely reversible, and because ether has no effects on the positions of the current-voltage relations for sodium channel or potassium channel activation. A consequence of the shift in slow inactivation is that, when ether or halothane is applied to an axon at the normal resting potential of -75 mV, the currents following wash-out of the anesthetic are invariably greater than the control currents (see Fig. 4). To avoid these changes in current amplitude, axons were usually held at more negative potentials, where slow inactivation is not present.

The third effect of ether on sodium current amplitude, which is reversible with repeated applications and rinsings, is a small decrease in peak current sizes caused by the faster inactivation. Thus, when currents with and without

ether are compared under conditions under which there is no irreversible change in amplitude, there is no difference in the initial rising phases of the currents; the current with ether present is smaller at the peak and at later times due to the more rapid decay. In Fig. 1 *c* and *d*, the currents in 100 mM ether are compared with those after rinsing. The holding potential is negative enough that there is no change in amplitude due to the shift in slow inactivation, and the irreversible loss of current as a result of the initial application of ether is already complete. The rising phases of the currents with and without ether match perfectly, at both potentials. Comparisons of this sort in many axons always showed no effect of ether on the rising phase of the current. Thus, although ether speeds up the inactivation kinetics of sodium currents, the kinetics of activation are not affected.

Effect of Halothane on Inactivation Kinetics

Halothane is an inhalation anesthetic with basic pharmacological effects similar to those of ether. Halothane's effects on sodium current kinetics in the crayfish axon appear to be essentially identical to those of ether. Fig. 4 shows sodium currents at -2 mV before, during, and after exposure to 32 mM halothane. The exponential fits to the decay phases of the currents are shown in *b*, *c*, and *d*; as with ether, the decay time constant is considerably decreased by halothane, and time constants are reduced at all membrane potentials, for both inward and outward currents.

Halothane solutions change the kinetics of sodium currents with a time-course of many minutes, in contrast to the rapid action of ether. The reversal on wash-out is also slow. After the 9-min exposure in the application of halothane shown in Fig. 4, the decay time constant was still decreasing, and after 30 min of rinsing with halothane-free solution, recovery was still not complete. It seems likely that halothane's action and reversal are slower than ether's because of the difference in water:membrane partition coefficient between the two compounds. The oil:water partition coefficient is 285 for halothane and 4 for ether (olive oil, 25°C ; Regan and Eger, 1967). The membranes of the Schwann cell layer surrounding the axon may act to buffer the halothane concentration near the axon membrane.

Because of the slow onset and recovery of halothane's effects, further experiments, aimed at understanding in more detail how inactivation is modified by general anesthetics, were carried out with ether.

Double-Pulse Experiments

The faster decay of sodium currents with ether present is reminiscent of the faster decay seen with internal pancuronium ion (Yeh and Narahashi, 1977) and quaternary strychnine derivatives (Shapiro, 1977; Cahalan and Almers, 1979). In the case of these compounds, the increased rate of decay appears to be due to time-dependent block of open sodium channels by drug molecules rather than to a speeding up of the normal inactivation mechanism. One experiment that led to this conclusion in the case of pancuronium was one in which the rate of inactivation was determined by the double-pulse method for

small depolarizations, where there is very little opening of sodium channels. In this case, the time constant and steady-state level of inactivation were not affected by pancuronium (Yeh and Narashashi, 1977). It seemed to us important to test this point with ether.

Double-pulse experiments show that the time constant for inactivation is decreased by ether at small depolarizations and to a degree similar to the change in decay time constant for the single-pulse currents at larger potentials. The results of one such experiment are shown in Fig. 5. The membrane was depolarized from its resting potential of -75 mV to -50 mV for a variable length of time and, after a brief return to rest, the level of inactivation reached

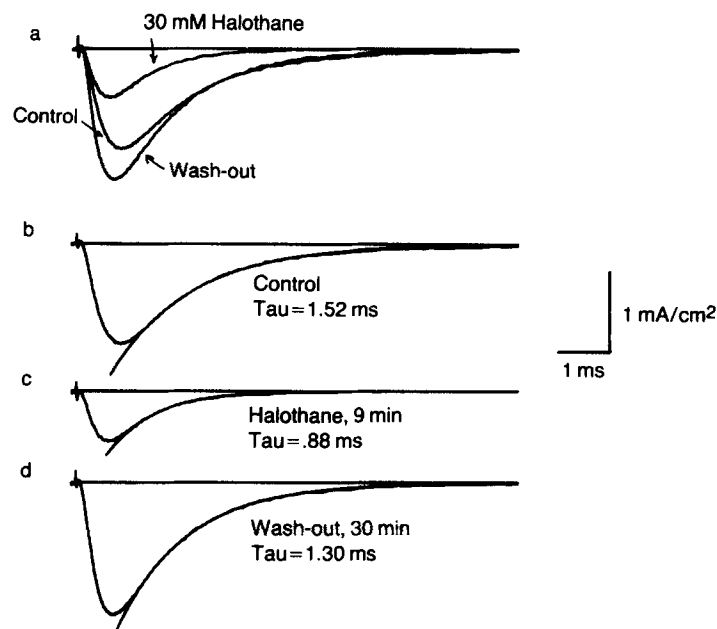


FIGURE 4. Effect of halothane on sodium current kinetics. Depolarization from -72 to -12 mV. (a) Control, 30 mM halothane, wash-out currents superimposed. (b, c, d) Currents superimposed with exponential fits to falling phases. Full Na, Full K, 1 mM 4-AP. Virtual ground current electrode. Temperatures: control, 2.5°C ; ether, 2.5°C ; wash-out, 2.6°C .

during the prepulse was assayed by the peak sodium current flowing during the test pulse to $+5$ mV. For the example shown in Fig. 5, the time-course of inactivation measured in this way is quite well described, both with and without ether, by a single exponential decay to a nonzero asymptote. With 100 mM ether present, the time constant for the decay is reduced $\sim 31\%$ and the asymptotic value is smaller; in the same axon, 100 mM ether decreased the decay time constant for currents near 0 mV by 25–30%. Time constants and steady-state values for double-pulse inactivation were smaller with ether in all axons examined. Results of double-pulse experiments are given in Table I.

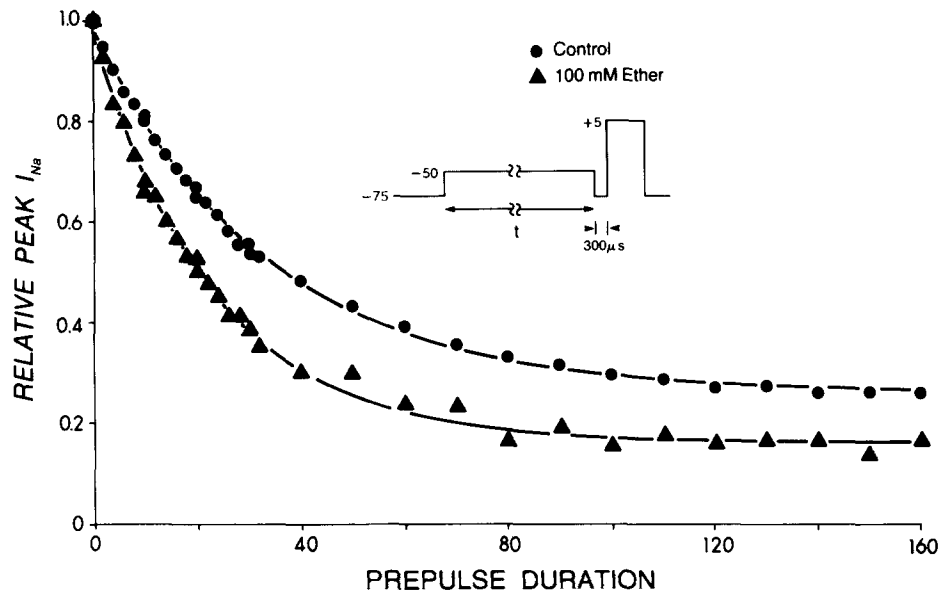


FIGURE 5. Effect of ether on the time-course of inactivation determined by the double-pulse method. Peak current during the test pulse is plotted as a function of the prepulse duration. Points are fit with an exponential decay to an asymptote. Control: $y = 0.73 \exp(-t/33.2 \text{ ms}) + 0.26$; Ether: $y = 0.82 \exp(-t/22.9 \text{ ms}) + 0.16$. Full Na, Full K, 1 mM 4-AP. Virtual ground current electrode. Holding potential, -75 mV . Temperatures: control, $1.9\text{--}2.0^\circ\text{C}$; ether, $1.8\text{--}2.1^\circ\text{C}$.

TABLE I
EFFECT OF ETHER ON DOUBLE-PULSE INACTIVATION

Axon	Membrane potential	Control		100 mM Ether		Wash-out		Temperature
		τ	Asymp.	τ	Asymp.	τ	Asymp.	
I-1	-50 mV	33 ms	0.26	23	0.16			$1.2\text{--}2.0$
I-2	-55	47	0.56	23	0.13			$2.3\text{--}3.0$
	-45	28	0.10	9.1	0.04			$2.9\text{--}3.3$
	-35	8.6	0.06	3.4	0.03			$3.5\text{--}3.9$
I-3	-61	59	0.68	46	0.47	54	0.66	$4.0\text{--}4.4$
	-46	19	0.22	11	0.04	19	0.23	$4.1\text{--}5.0$
	-36	7.1	0.04	3.6	0.04	6.6	0	$3.8\text{--}4.6$

Steady-State Fast Inactivation

It is evident from the data in Fig. 5 and Table I that inactivation at small depolarizations is more complete with ether present. This effect of ether was examined in greater detail by measuring "steady-state" fast inactivation over an appropriate range of membrane potentials. A long (50- to 150-ms) prepulse to a variable membrane potential is followed by a test pulse of fixed height

(Hodgkin and Huxley, 1952); the peak current during the test pulse is taken as an index of the level of inactivation achieved during the prepulse. In principle, the prepulse is long enough for an asymptotic value of inactivation to be reached. In Fig. 6 are typical steady-state inactivation curves determined in this manner and obtained with and without 100 mM ether. There are two changes produced by ether: first, the midpoint of the curve is shifted to the left along the membrane potential axis, in the hyperpolarizing direction; second, the curve is significantly steeper with ether present. It is convenient to describe these changes by fitting an analytical expression to the data points;

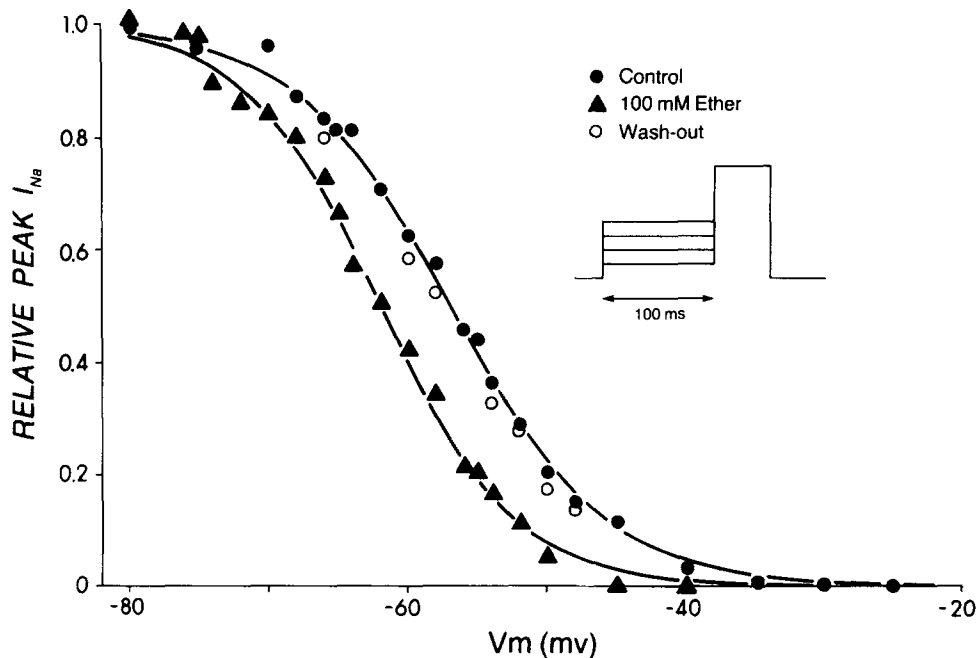


FIGURE 6. "Steady-state" fast inactivation vs. membrane potential, with and without ether. Relative peak sodium current during the test pulse is plotted as a function of the membrane potential during the 100-ms prepulse. Holding potential, -80 mV; test potential, $+50$ mV. Reference potential for normalization, -80 mV. $\frac{1}{2}$ Na, $\frac{2}{3}$ K, 1 mM 4-AP. Differential current electrode. Temperatures: control, 1.7 – 2.1°C ; ether, 0.5 – 1.9°C ; wash-out, 1.9 – 2.4°C .

the smooth curves in Fig. 6 are drawn according to the expression (Hodgkin and Huxley, 1952) $1/[1 + \exp \{(V - V_h)/K\}]$, where V_h is the value, in millivolts, of the membrane potential at which inactivation is one-half complete, and K is a parameter determining the steepness of the curve. In the example shown in Fig. 6, the control V_h value of -57 mV was shifted to -62 mV by ether, and K declined from 5.6 to 4.8. These changes were almost completely reversed when the ether was washed out.

One difficulty in defining and measuring steady-state fast inactivation should be mentioned. This is a slow inactivation process with a potential

dependence that overlaps fast inactivation (Shrager, 1977; Starkus and Shrager, 1978). Fast inactivation has time constants of 20–60 ms in the range from –50 to –70 mV (3°C), so that a prepulse duration of at least 100 ms is required for a reasonable approximation to steady-state levels. Removal of slow inactivation proceeds with a time constant of ~150 ms at –115 mV (8°C) (Shrager, 1977) so that, in an axon with substantial slow inactivation present at the holding potential, much of the increase in the test-pulse currents after hyperpolarizing prepulses may be due to the removal of slow inactivation rather than to the removal of fast inactivation. Contamination from slow inactivation can be minimized by confining measurements of fast inactivation to membrane potentials more positive than the holding potential; the onset of slow inactivation has a time constant of >1 s in the range –75 to –55 mV (8°C) (Shrager, 1977). This was confirmed by the double-pulse experiments summarized in Table I; in all cases the time-course of inactivation was fit well by a single exponential decay (except for a small initial lag), and there was no evidence of a second slow component.

TABLE II
EFFECT OF ETHER ON STEADY-STATE FAST INACTIVATION

Axon	Holding potential <i>mV</i>	Prepulse duration <i>ms</i>	Control			100 mM Ether			Wash-out		
			<i>C</i>	<i>V_h</i>	<i>K</i>	<i>C</i>	<i>V_h</i>	<i>K</i>	<i>C</i>	<i>V_h</i>	<i>K</i>
			<i>mV</i>			<i>mV</i>			<i>mV</i>		
II-1	–73	50	1.1	–59	6.4	1.1	–62	5.5			
II-2	–80	100	1.0	–57	5.4	1.0	–62	4.8	1.0	–58	5.5
II-3	–97	150	0.97	–61	5.8	0.98	–67	5.5	0.98	–63	6.0

The results presented in Fig. 6 were obtained from an axon held at –80 mV in a low-potassium solution; the test-pulse currents were normalized with respect to the current with no prepulse, and the fits to $1/\{1 + \exp [(V - V_h)/K]\}$ were made by eye. A more accurate fitting procedure, which recognizes that removal of fast inactivation may be incomplete at the holding potential, was employed to give the results summarized in Table II for this and two other axons. The test-pulse currents were normalized relative to the test-pulse current with no prepulse, and the data were fit by $C/\{1 + \exp [(V - V_h)/K]\}$; the best least-squares fit allowing *C*, *V_h*, and *K* to vary was obtained by a computer program using the Patternsearch algorithm (Colquhoun, 1971). (The parameters calculated for axon II-2, which are the data shown in Fig. 6, are only slightly different from those fit by eye.) In all three axons, *V_h* was shifted to a more negative value by ether, and the curve was made steeper. The results from axons II-2, II-3, and I-3 demonstrate that these changes were reversed when the ether was rinsed out.

Thus, ether affects both the rate and steady-state value of inactivation at small depolarizations. The contrast between these results and those obtained with pancuronium (Yeh and Narahashi, 1977) make it tempting to conclude that ether acts by affecting normal inactivation rather than by a pancuronium-like blocking mechanism. However, firm support for this conclusion would

require more knowledge about the normal inactivation mechanism than is now available. If activation and inactivation are independent processes, as in the Hodgkin-Huxley model for sodium channel kinetics (Hodgkin and Huxley, 1952), channels can inactivate at small depolarizations without first opening, and acceleration of inactivation at these potentials could not be due to time-dependent block of open channels. However, if inactivation were strictly coupled to the prior activation of channels instead of being an independent process, block of open channels by exogenous molecules would affect the rate and steady-state value of inactivation at small as well as large depolarizations. In this case, the failure of pancuronium to affect inactivation at small depolarizations may be due to the voltage dependence of its association and

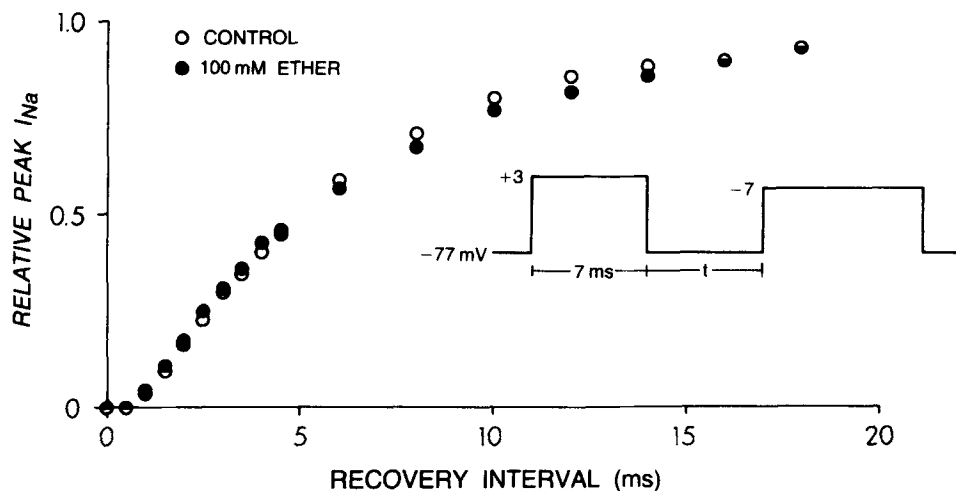


FIGURE 7. Recovery from inactivation. The peak current for the test pulse to -7 mV is plotted vs. the recovery interval at -77 mV; the currents are normalized with respect to the current for a pulse from -77 to -7 mV with no conditioning pulse. $\frac{1}{2}$ Na, $\frac{1}{2}$ K, 1 mM 4-AP. Differential electrode. Temperatures: control, 4.1–4.2°C; 100 mM ether, 4.1–4.3°C.

dissociation rate constants rather than to failure of channels to open. In any case, as it is still uncertain whether channels open before inactivating at small depolarizations (see Bezanilla and Armstrong [1977]), it is not possible to use ether's effects on inactivation at small depolarizations as an argument against a time-dependent blocking mechanism.

Recovery from Inactivation

At negative membrane potentials, at which inactivation is removed in the steady state, there is no opening of channels in either independent or coupled models of inactivation. If ether affected the rate of recovery from inactivation at negative potentials, this would be clear evidence of an effect on the normal gating mechanism. The results of an experiment testing this point are shown in Fig. 7. The membrane was depolarized from the holding potential of -77 mV to $+3$ mV for 7 ms, long enough to produce complete inactivation, and,

after a variable recovery interval at -77 mV, the extent of recovery from inactivation was assayed by a test pulse to -7 mV. In the control experiment, and with 100 mM ether present, the recovery from inactivation shows complex kinetics, with an initial lag and then a recovery phase that shows at least two time constants after the lag. However, ether produces no significant change in the time-course. This application of ether had the usual effect on the devel-

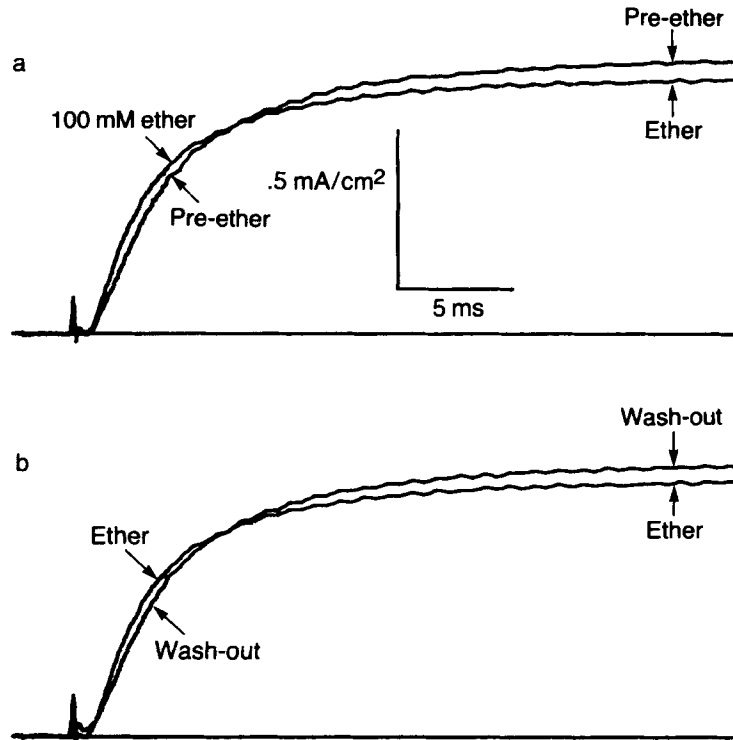


FIGURE 8. Change in potassium current kinetics with 100 mM ether. Depolarization from -70 to $+120$ mV. 100 mM ether had previously been applied to the axon and rinsed out. (a) Current before ether and current after 10 min of 100 mM ether. (b) Current with ether and current after 20 min of rinsing with ether-free saline. The spikes at the beginning of the currents are due to imperfect subtraction of capacitive transients. Full Na, Full K, 100 nM TTX. Virtual ground current electrode. Temperatures: pre-ether, 4.3°C ; ether, 3.7°C ; wash-out, 3.8°C .

opment of inactivation; the time constant for the decay of the test-pulse currents at -7 mV decreased from a control value of 1.1 to 0.62 ms with ether present. The lack of effect of 100 mM ether on the kinetics of recovery from inactivation was found at potentials from -77 to -92 mV in three axons.

Potassium Current Kinetics

In most of the tested axons, the kinetics of potassium current activation were faster when ether was applied. An example is shown in Fig. 8. Potassium

currents in response to a depolarization from -70 to $+120$ mV are shown before, during, and after an application of 100 mM ether. Although the potassium current in ether reaches a smaller steady-state value, it is actually larger than the current with no ether for the first 6 ms or so. The kinetics of the currents before ether and after rinsing are nearly identical.

The effect of ether on potassium current kinetics appears, and is reversed, with a time-course similar to that for the effect on sodium current decay. As with the effect of ether on sodium currents, the first application of ether to an axon results in an irreversible loss of 10–20% of the potassium current. Subsequent applications produce a small, reversible decrease in steady-state current, an $\sim 10\%$ decrease for 100 mM ether. This is seen in Fig. 8; this axon had previously been exposed to 100 mM ether, so that the irreversible loss of

TABLE III
EFFECT OF ETHER ON POTASSIUM CURRENT KINETICS

Axon	Ether concentration <i>mM</i>	Crossover	Percent decrease in time to half-maximum	
			Compared with control	Compared with wash-out
1	50	YES	20	30
	50	YES	25	
2	60	YES		23
3	50	NO		
4	40	YES	25	
5	50	NO		
6	40	NO	2	
	80	YES	17	
	160	YES	24	32
7	100	YES	17	22
	300	YES	20	25
8	80	YES	13	
9	160	YES	28	34
	480	YES	40	
10	100	YES	26	23

current had already occurred. Ether produces no change in the voltage dependence of steady-state potassium current, and the activation kinetics are faster at all membrane potentials.

The production of faster potassium channel kinetics by ether was a fairly consistent finding. 10 axons were used for experiments on potassium currents. Eight of the axons showed clear changes in kinetics, with “cross-over” between control and ether currents, such as that seen in Fig. 8, with ether concentrations from 40 to 480 mM. The results are summarized in Table III. The effect on activation kinetics is expressed quantitatively as the percent decrease in the time to half-maximum current; the numbers given for each axon represent averages of values for currents at several potentials between 0 and $+130$ mV. The currents were not analyzed in terms of Hodgkin-Huxley kinetics because of the problem of loading of the periaxonal space with potassium during the

course of the currents; n^4 or n^5 kinetics often did not fit very well. In most of the axons, the time to half-maximum current after wash-out of the ether was slightly longer than in the control. This may be because the currents after rinsing were usually smaller than the control currents; the small differences in current kinetics could be due to changes in the time-course of loading of the periaxonal space. In axon 10 (from which Fig. 8 is taken), in which there was only a reversible decrease in current sizes, the effect of ether on current kinetics was nearly perfectly reversible.

Membrane Capacitance

It is possible that the changes in sodium and potassium current kinetics produced by the general anesthetics are due to the demonstrated ability of these agents to alter the lipid structure of membranes. Changes in the fluidity or viscosity of the axon membrane would be difficult to demonstrate using electrophysiological techniques. However, if adsorption of the general anesthetics changed either the thickness or the dielectric constant of the membrane, the specific capacitance of the membrane would be changed. Changes in membrane capacitance by other hydrophobic molecules have been reported: both alkanes and benzyl alcohol decrease the capacitance of artificial lipid bilayers (Haydon et al., 1977 *a* and 1977 *b*; Ashcroft et al., 1977 *a* and 1977 *b*) and pentane has been reported to decrease the capacitance of the squid axon membrane (Haydon et al., 1979).

However, ether appears to have no effect on the capacitance of the crayfish axon membrane. Ether had no effect on the capacitative transient produced by a voltage step under voltage clamp; when the capacitative currents were recorded on an oscilloscope using a fast time base, the transients with and without ether superimposed to within the width of the trace. To make measurements of capacitance that could conveniently be digitized for analysis, a triangular wave form was applied under voltage clamp. The method and its results are illustrated in Fig. 9. Fig. 9 *a* shows the command voltage form, a 14.3-mV, peak-to-peak, 1.2-Hz triangular wave applied at a holding potential of -81 mV. The current signal in response to this wave form is shown in *b*; it is close to the response expected from a resistor and capacitor in parallel. The values of membrane resistance (380 ohm-cm²) and capacitance (0.89 μ F/cm²) are estimated by fitting the data with the theoretical response of an *RC* combination. In Fig. 9 *d*, the current in 100 mM ether is shown; it is essentially identical to the control current, as is emphasized by *e*, where the currents with and without ether are superimposed. A change in capacitance $>5\%$ would probably have been detected in this experiment.

DISCUSSION

The principal results of this investigation are that ether and halothane increase the rate of sodium current inactivation and that potassium current activation kinetics are also faster with ether present. A key question is, are these changes in current kinetics caused by true changes in the rate constants of the normal gating processes of the channels?

It has been found that general anesthetics cause acetylcholine-activated

endplate channels to close more rapidly (Gage and Hamill, 1976 *a* and 1976 *b*; Gage et al., 1979). This effect may be due to a change in the rate constant of the normal gating process. However, another possibility, which was also found to be consistent with the results, is that the general anesthetics act through a mechanism whereby free anesthetic molecules bind to and block open channels, so that an open channel may become nonconducting either by the normal gating process or by being blocked by an anesthetic molecule.

The mechanism by which ether and halothane speed up sodium current inactivation is similarly ambiguous. As discussed in Results, the effects of ether on inactivation—the speeding up of inactivation at small as well as large depolarizations, the shift of the steady-state inactivation curve, and the lack

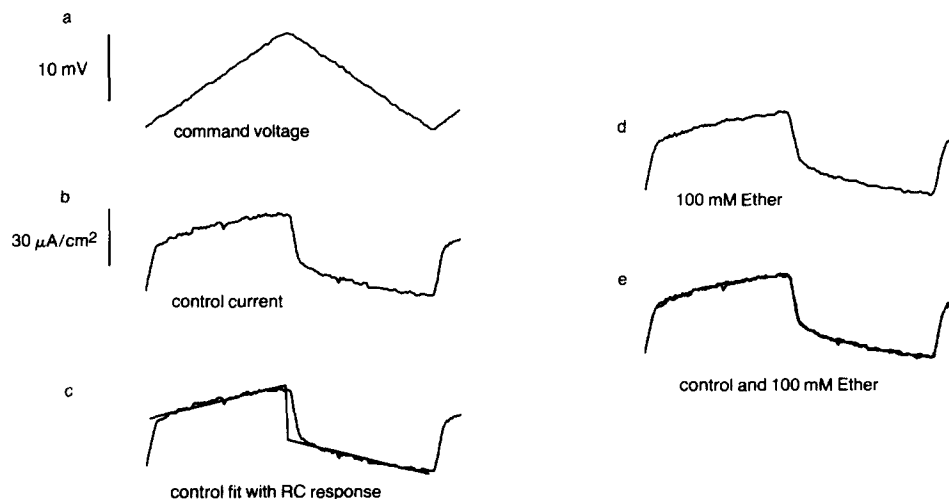


FIGURE 9. Lack of effect of 100 mM ether on membrane capacitance. (a) Voltage wave form. (b) Membrane current. (c) Fit using expected response of a parallel RC combination. Fit was made by eye. (d) Same as *b* but with 100 mM ether. (e) Currents with and without ether superposed. $\frac{1}{4}$ Na, $\frac{1}{2}$ K, 1 mM 4-AP, 100 nM TTX. Virtual ground current electrode. Holding potential, -81 mV. Temperature: 3.7°C .

of effect on recovery from inactivation at negative potentials—could be consistent either with a time-dependent blocking mechanism or with a change in the rate constants of the normal gating process. The decrease in inactivation time constant as a function of ether concentration (Fig. 3) is another result which in principle might have distinguished between the two mechanisms but which in practice had an outcome consistent with both possibilities. If ether acted by a blocking mechanism, the rate of blocking of open channels would be proportional to the ether concentration. This relation can be used in conjunction with models of the normal activation and inactivation kinetics to predict the form of the $\Delta\tau_h$ vs. [ether] relation. Such kinetic modelling showed that the data in Fig. 3 is consistent with a time-dependent blocking mechanism.

The possibility that a time-dependent blocking mechanism could account for the effects of general anesthetics on the kinetics of both endplate currents and sodium currents makes the results with potassium currents especially valuable: the speeding up of potassium current activation can be unambiguously interpreted as a speeding up of a gating process. Because the currents in ether are actually larger than the control currents at short times (Fig. 8), it is not possible to describe the effect of ether as a time-dependent blocking of open channels. The fact that the effect of ether on potassium currents represents a genuine change in gating kinetics gives more weight to the possibility that the effects on endplate and sodium current kinetics also represent increases in the rate constants of the native gating processes. It seems possible that the ability of general anesthetics to increase the fluidity of lipid membranes might underly all three effects. Thus, as with other membrane proteins, the operating characteristics of membrane ionic channels may be determined, in part, by the lipid environment of the protein portion of the channels. Further support for this proposition will depend on convergent results obtained using other methods to alter lipid composition or viscosity, such as fusion of lipid vesicles. In particular, it will be interesting to see whether other methods show selective changes in potassium channel activation and sodium channel inactivation but not sodium channel activation kinetics.

Comparison with Previous Results and Other Agents

This is the first report of general anesthetic-induced changes in sodium and potassium current kinetics, but the shift in the voltage dependence of the inactivation curve (Fig. 6) has been noted in other nerves with ether (Kendig et al., 1979) and trichloroethylene (Shrivastav et al., 1976). The ability of other small, neutral molecules to change sodium and potassium current kinetics has been reported. Octanol decreases the time constant of sodium channel inactivation in the squid axon, probably by affecting the normal inactivation mechanism (Oxford and Swenson, 1979) and *n*-pentane is reported to speed both sodium channel activation and inactivation, as well as potassium channel activation (Haydon et al., 1979). It seems likely that these effects are closely related to those of ether and halothane.

It is striking how little the amplitude of sodium currents is affected by even large concentrations of ether. Except for an irreversible 10–20% reduction on the first application, up to 200 mM ether reduces peak sodium currents only by speeding up the inactivation phase, as is shown in Fig. 1. Thus, ether does not decrease the number of operating sodium channels. This result offers a counterexample to the notion that all hydrophobic molecules can act as nonspecific blockers of the sodium channel and suggests that hydrophobic molecules that are potent blockers of the sodium channel, like neutral local anesthetics, probably act by binding to specific receptors (see Hille [1977]).

Pharmacological Significance

The results reported here show that clinical concentrations of general anesthetics almost certainly do not act by blocking sodium channels. Blood concentrations of ether during anesthesia correspond to 15–24 mM in saline

solution (Ronconi, 1923; Haggard, 1924; Steward et al., 1973). The smallest ether concentration studied here was 30 mM, which decreased the time constant for sodium current inactivation by ~20% (Fig. 3) and otherwise had little effect on sodium current amplitude. Furthermore, even concentrations of ether in the range 30–80 mM, which do significantly block conduction in some nerves (Larrabee and Posternak, 1952; Richards et al., 1975; Richards and White, 1975), had relatively little effect on sodium current amplitude, acting mainly by speeding up inactivation. This suggests that nerve block by high concentrations of general anesthetics, which could occur during respiratory arrest, might be due to depolarization of nerve membranes and the resultant inactivation of sodium channels rather than to direct block of the channels. Although neither ether nor halothane affected the resting potential of the crayfish giant axon at low temperatures, depolarization of other nerves by ether has been reported (Wright, 1947; Lorente de No, 1947).

Although effects of general anesthetics on sodium and potassium channels are unlikely to be of primary importance in producing clinical anesthesia, they may contribute to second-order pharmacological effects. Because both the partitioning of ether into hydrophobic phases and its potency as a narcotic increase with increasing temperature (Regan and Eger, 1967; Cherkin and Catchpool, 1964), it is possible that the kinetics of sodium and potassium channels in the vertebrate central nervous system at 37°C are affected by clinical concentrations. Ether has been observed to produce changes in repetitive firing patterns of central neurons (Somjen and Gill, 1963; Richards et al., 1975) and to enhance accommodation to small depolarizations (Somjen and Gill, 1963). These effects could be consistent with changes in ionic channel kinetics of the type reported here.

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