times were significantly reduced after exposure to halothane. Pooled data, including *m*- and *j*-channel open time values from attached and cell-free patches, are plotted in Fig. 2, which shows a concentration-dependent reduction of channel open times by halothane.

DISCUSSION

Anesthetic potency, which is best predicted by its lipid solubility, is essentially independent of the size, shape, and chemical nature of general anesthetics (8). This suggests that the mechanism of anesthetic action is not directly related to a specific ligand-to-binding site interaction but, rather, may depend on a more general effect on membrane structure. General anesthetics are thought to exert their effects by disordering (or fluidizing) the lipids in which receptor proteins are embedded (9, 10). Such fluidization is predicted to permit a faster channel closure, resulting in a reduction of mean channel open time (10). Our direct measurements of receptor channel properties show that the channel amplitudes are unaffected but their open times are significantly reduced, in a concentration-dependent manner, following exposure to halothane.

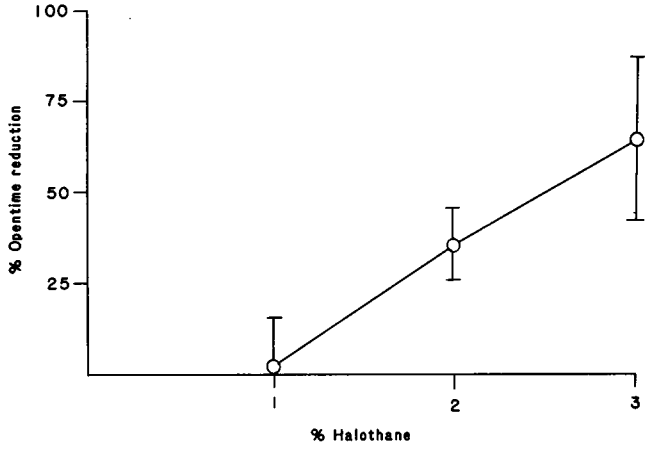

FIGURE 2 Effect of halothane on the mean open time of channel events from six patches. Intact and excised patches as well as *m* and *j* channels were combined for a given concentration of halothane. Paired-student *t*-tests were done (four at each concentration) to determine significance. At higher halothane concentrations (2%, 3%), experimental points were significantly different ($p < 0.001$) from matched control values.

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DEUTERIUM OXIDE EFFECTS ON FROG ENDPLATE CHANNELS

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Experiments have been performed studying the effects of deuterium oxide (D_2O) on acetylcholine-activated (ACh) channels at the frog neuromuscular junction. The results indicate that the particular solvent can affect all three

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measured properties of the channels: the reversal potential, the single channel conductance, and the mean channel lifetime.

METHODS

A monolayer of muscle fibers from the cutaneous pectoris muscle from northern *Rana pipiens* was studied using a two-microelectrode voltage clamp. A third microelectrode was used to deliver acetylcholine-chloride (ACh) to the endplate region by iontophoresis.

The solutions tested were normal Na Ringers (115 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 4 mM HEPES, pH 7.4) with either H_2O or D_2O (Sigma Chemical Co., St. Louis, Missouri) as the solvent.

The reversal potential was determined by interpolation of the ACh-induced endplate channel (epc) vs. holding potential relationship. The single channel conductance (γ) and the mean channel lifetime (τ) were calculated from fluctuation analysis of the steady-state ACh-induced epc current. See Lewis (1) for further information about the methods and procedures.

RESULTS

The mean reversal potential values measured in the two solutions at temperatures ranging from 8° to 20°C are shown in Table I. Temperatures over the range of 12° to 20°C appeared to have little effect on the reversal potential, so these values in D_2O or H_2O were pooled. The net result is that the reversal potential is slightly hyperpolarized in D_2O Ringers compared with H_2O Ringers (-7.9 ± 0.1 mV vs. -5.2 ± 0.6 mV). This is significant because the difference in reversal potential indicates that the solvent can influence the selectivity of the channel. The permeability ratio P_K/P_{NA} can be calculated, assuming internal activities for Na of 7 mM, 80 mM for K (1), and assuming that the Ca contribution to the reversal potential is negligible. Under these assumptions, the GHK potential equation is

$$\frac{P_K}{P_{Na}} = \frac{[Na]_o - [Na]_i \exp(FV_o/RT)}{[K]_i \exp(FV_o/RT) - [K]_o}$$

where V_o is the reversal potential; the subscripts i and o refer to intracellular and extracellular activites; and F, R, and T have their usual thermodynamic meanings. In D_2O Ringers, the channel is more permeable to potassium ions relative to sodium ions than it is in H_2O Ringers, with a calculated value of P_K/P_{Na} of 1.48 compared to 1.31 in H_2O.

TABLE I
REVERSAL POTENTIAL (*MV*) FOR THE SOLVENTS H_2O AND D_2O AT DIFFERENT TEMPERATURES

	H_2O	D_2O
8°C	-4.25 ± 0.9* ($n = 8$)‡	-3.0 ± 0.8 ($n = 5$)
12	-6.2 ± 0.5 ($n = 12$)	-7.7 ± 0.6 ($n = 9$)
16	-5.1 ± 0.7 ($n = 7$)	-8.0 ± 0.9 ($n = 8$)
20	-4.3 ± 1.3 ($n = 5$)	-8.0 ± 0.9 ($n = 3$)

*± SEM.
‡Number of determinations.

The single-channel conductance is also influenced by the solvent. The mean single-channel conductance values for a temperature of 12°C and a holding potential of -70 mV are 38.4 ± 1.3 pS ($n = 6$) for H_2O Ringers and 25.7 ± 1.0 pS ($n = 7$) for D_2O Ringers. Table II lists the ratios of the single channel conductances in D_2O Ringers relative to the value in H_2O Ringers for the different temperatures. The single channel conductance decreases in D_2O Ringers as a function of temperature. The fact that the single channel conductance decreases in D_2O Ringers is to be expected because the viscosity of D_2O solutions is higher than for H_2O. Table II also lists the ratio of inverse viscosities of D_2O relative to H_2O at various temperatures. (The viscosity values are interpolated from Table II in Hardy and Cottington, reference 2.) At most temperatures, the single channel conductance is decreased more in D_2O Ringers than can be explained by the increase in viscosity of D_2O solutions. Consequently, D_2O must be influencing barrier heights and/or well depths experienced by ions in the channel (3).

The mean channel lifetime, τ, is also influenced by the solvent. The lifetime is shortened in D_2O compared to H_2O, and this is true for every temperature (8°–20°C). For a temperature of 12°C and a holding potential of -70 mV, the mean channel lifetime is 3.55 ± 0.11 ms ($n = 6$) for H_2O Ringers and 3.01 ± 0.08 ms ($n = 7$) for D_2O Ringers. Table II also lists the ratios of the mean channel lifetime in D_2O Ringers relative to the value in H_2O Ringers for the various temperatures tested. These ratios are not significantly different from the ratios of the inverse viscosities of the two solutions.

At high temperatures D_2O is more like H_2O in its physical behavior (e.g., viscosity) and in its three dimensional structure. Consequently, D_2O effects on channel parameters would be expected to be large at low temperatures and smaller at high temperatures. The ratio τ_{D_2O}/τ_{H_2O} does not show significant temperature dependence. In contrast, the ratio $\gamma_{D_2O}/\gamma_{H_2O}$ is 0.67 ± 0.07 (± SD) for a temperature of 12°C, and the ratio increases to 0.80 ± 0.10 for 8°C and decreases to 0.61 ± 0.11 for 20°C. However, given the errors associated with these ratios, it is not possible to conclude much except that the γ ratios may

TABLE II
COMPARISON OF RATIOS IN D_2O COMPARED TO H_2O AT A HOLDING POTENTIAL OF -70 MV

	$\gamma_{D_2O}/\gamma_{H_2O}$	τ_{D_2O}/τ_{H_2O}	$(1/\eta_{D_2O})/(1/\eta_{H_2O})$*
8	0.80 ± 0.10‡	0.71 ± 0.10‡	0.77
12	0.67 ± 0.07	0.85 ± 0.08	0.78
16	0.64 ± 0.10	0.77 ± 0.13	0.79
20	0.61 ± 0.11	0.85 ± 0.05	0.80

*Viscosity values, η, interpolated from Hardy and Cottington (1949), Table 2.
‡± SD.

exhibit an apparent temperature dependence which is the opposite of that expected.

CONCLUSION

In deuterium oxide solvent the channel closing rate is speeded up so that the mean channel lifetime is smaller. A possible explanation is that the pK's of some groups involved in channel gating change in D_2O, making the open state less stable.

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ACTIVATION AND INACTIVATION KINETICS OF *TORPEDO CALIFORNICA* ACETYLCHOLINE RECEPTOR IN RECONSTITUTED MEMBRANES

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The function of nicotinic acetylcholine receptors (AChR) in fish electric organs and at vertebrate neuromuscular junctions can be conveniently divided into four subfunctions: *a*, specific ligand binding; *b*, coupling of binding to cation permeability increases; *c*, ion permeation; and *d*, desensitization, a process by which ion channels are inactivated in the prolonged presence of activating ligands (1). Two recent advances in AChR research provide a unique opportunity to characterize both the biochemical and ion permeability properties of the AChR in a single, well-defined membrane environment. Reliable methods for reconstitution of AChR ion permeability control properties in lipid vesicles have been developed (1, 2), and quench-flow techniques to measure the initial rates of ion fluxes across membrane vesicles in the millisecond time domain have been successfully applied to AChR-containing membranes (3, 4).

We provide here quantitative measurements of both activation and inactivation kinetics of AChR-mediated cation flux in reconstituted vesicles containing purified *Torpedo californica* AChR. We also show that treatment of AChR with the disulfide reducing agent dithiothreitol (DTT) alters the ligand affinity of the AChR without altering channel properties. These results, together with previous work from our laboratory (5, 6), show that the purified AChR protein retains four important functional characteristics: *a*, a high efficiency of cation translocation; *b*, a fast inactivation process in the subsecond time domain; *c*, a slower inactivation process in the seconds time domain; and *d*, a sensitivity to specific chemical modifications.

MATERIALS AND METHODS

Methods for isolation of membranes from *Torpedo californica electroplax*, extraction and purification of AChR in cholate by affinity chromatography, and reconstitution of AChR with soybean lipids (Asolectin, Associated Concentrates, Woodside, NY) were as described by Walker et al. (5). Ion flux was measured using the quench-flow techniques developed by Cash and Hess (4) as modified by Walker et al. (5). Trapped cations were detected following ion-exchange chromatography (2, 5). The activating ligand was carbamylcholine chloride (Carb), the quenching reagent was d-tubocurarine chloride (Curare), and the radioactive tracer cation was 86-Rb^+.

RESULTS AND DISCUSSION

Reconstituted membranes containing 1 mg/ml purified AChR (6–8 nm α-bungarotoxin binding sites/mg of protein) and 20 mg/ml soybean lipids were tested for ion flux using the quench-flow technique as described by Walker et al. (5). A typical value for J_a, the maximum flux rate constant at saturating Carb concentrations, was 100 s^{-1}. Such a value corresponds to the movement of 10^4 ions/AChR channel/s as expected for fully functional AChR under the ionic conditions of the assay.

The effects of DTT reduction on the initial flux rates

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