EFFECTS OF HALOTHANE ON THE ACETYLCHOLINE RECEPTOR CHANNEL IN CULTURED *XENOPUS* MYOCYTES

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General anesthetics, regardless of their molecular structure, cause disordering of membrane lipids and are believed to increase membrane fluidity (1, 2). General anesthetics also reduce the amplitude and time course of nerve-evoked endplate depolarization (3). The latter effect results from an increase in the decay rate of endplate currents, presumably due to the shortening of AChR channel open times. We have used the patch clamp technique (4) to determine what effects the volatile anesthestic, halothane, may have on the properties of single acetylcholine receptor (AChR) channels.

MATERIALS AND METHODS

Uninnervated muscle cells from *Xenopus laevis* embryos (stages 19–22) were grown in culture as before (5). Cultures were continuously superfused, at room temperature, with recording medium (120 mM NaCl, 1.6 mM KCl, 1 mM $CaCl_2$, 8 mM HEPES, pH 7.4; for cell-free patches, EGTA 5 was added and $CaCl_2$ omitted) bubbled with air or air with vaporized (Forreger vaporizer, Forreger, Smithtown, NY) thymol-free halothane. Electrodes were pulled from 50-μl glass pipettes (Drummond Scientific Co., Broomall, PA) to resistances of 3–10 MΩ according to Hamill et al. (4), and filled with recording medium (diluted 10% with distilled water) containing 0.2 μM ACh.

Single channel currents were stored on FM tape (Store 4DS, Racal Recorders, Inc., Rockville, MD). A 3–5 min control record was obtained just prior to switching to halothane. The effects of halothane were monitored during the first five minutes of exposure to the anesthetic.

Our analysis is based on three cell-attached and three cell-free patches. Event amplitudes and durations were obtained from digitized records (100-μs intervals). Open-time histograms were made using 100-μs bins (open-time closures of <1 ms were ignored). The mean channel open time was estimated from the time constant of the fitted exponential $N(t) = N_T \alpha \exp[-\alpha t]$, using a nonlinear least-squares method where $N(t)$ is the number of events at time t, N_T is the total number of events, and α is the time constant. Events <0.6 ms were excluded due to the circuit's time constant (120 μs).

RESULTS

Insets in Fig. 1 show typical oscilloscopic traces from a cell-attached patch just prior to *A* and during exposure to 2% halothane *B*. The two event types seen in inset *A*, based on current amplitude, represent two populations of channels also observed by others (6, 4, 7). Amplitude histograms were assembled (not shown) and used to identify these populations. The smaller events are termed *m* channels, and the larger ones are called *j* channels. Exposure to halothane affected neither the mean amplitude of either population nor the amplitude of patches exposed to higher concentrations of halothane.

The control mean, *m*, channel open time, in this patch, was 2.2 ms; it was 1.0 ms for the *j* channels. On exposure to 2% halothane, *m*- and *j*-channel open times were significantly reduced to 1.2 ms ($p < 0.01$) and 0.7 ms ($p < 0.005$). Inside-out cell-free patches (holding potential −120 mV) also had two populations of events whose open

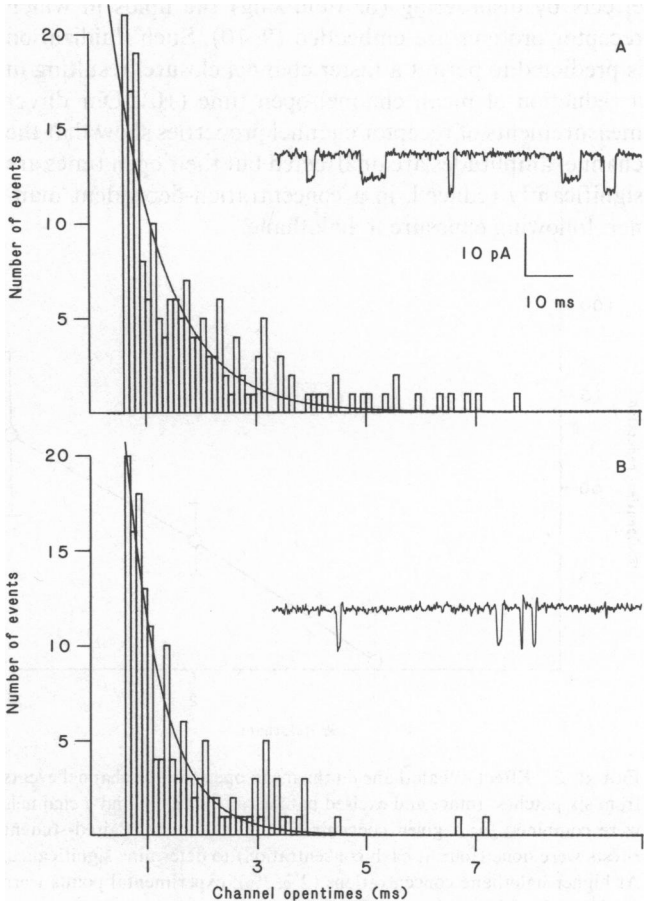

FIGURE 1 Control single channel current record (upper *inset*) from a cell-attached patch. The holding potential was −60 mV from resting potential. Two channel populations (see text) can be seen. The mean amplitude (± SD) of the small, *m* channels is 6.8 ± 1.0 pA; that of the larger, *j* channels is 10.7 ± 1.3 pA. (A separation amplitude of 8.2 pA was chosen by eye.) The control *j*-channel open-time histogram (*A*) has been fitted with an exponential curve whose time constant is 1.06 ms^{-1}, corresponding to a mean open time of 1.0 ms. Similarly, the *m* channels (not shown) have an open time of 2.2 ms. In *B*, a current record (lower *inset*) from the same patch after exposure to 2% halothane shows a predominance of short-duration events. The *j*-channel open-time histogram has a time constant of 1.53 ms^{-1}, corresponding to a reduced mean open time of 0.7 ms. The *m*-channel mean open time was reduced to 1.2 ms.

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.

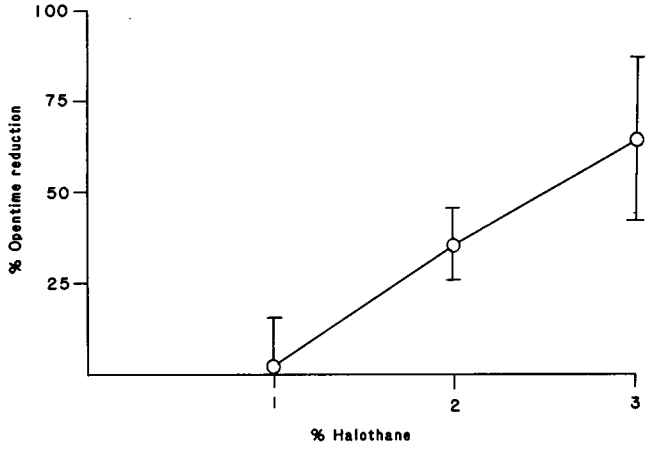

FIGURE 2 Effect of halothane on the mean open time of channel events from six patches. Intact and excised patches as well as *m* and *j* channels were combined for a given concentration of halothane. Paired-student *t*-tests were done (four at each concentration) to determine significance. At higher 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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