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Cooperative Interactions between General Anesthetics and QX-222 within the Pore of the Acetylcholine Receptor Ion Channel

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SUMMARY

To test the hypothesis that general anesthetics block nicotinic acetylcholine receptor channels by binding within the pore of the channel, we looked for competitive interactions between ether and QX-222 at the single channel current level. Experiments were performed on outside-out patches excised from BC3H-1 cells. QX-222 causes channels to flicker as it repeatedly binds within the pore of the channel and blocks the flow of current through the channel. Ether reduces the apparent unitary conductance of the channel. This effect of ether may be due to frequent, short-lived, unresolved, blockages of the channel. When both ether and QX-222 are applied, the effects of both drugs are seen on single channels. However, the duration of QX-222 blocking events are longer when ether is present; the duration of block is 0.89 ± 0.06 ms with 30 μM QX-222 alone and 2.23 ± 0.37 ms with 30 μM QX-222 + 20 mM ether (n = 5 ± S.D.; −100 mV). Similar results are obtained when butanol is used in place of ether. We conclude that ether and QX-222 do not compete for a common binding site. Conversely, ether decreases the dissociation rate of QX-222. The simplest interpretation of these data is that the binding sites for ether and the aromatic moiety of QX-222 are distinct but close to each other; when ether is bound to its site, the binding of QX-222 is stabilized. We cannot, however, discount the possibility that ether stabilizes QX-222 by binding to a remote site and allosterically modifying the pore of the channel.

The issue of whether general anesthetics exert their effects on membrane proteins by binding directly to the protein or by binding to the lipid bilayer and indirectly affecting the function of the protein is difficult to resolve. In contrast, the idea that cationic local anesthetics bind directly to proteins (in particular to the nicotinic AChR channel) is strongly supported. The binding site of QX-222, a quaternary amine derivative of the local anesthetic lidocaine, is thought to lie within the pore of the AChR channel, because it blocks open channels in a voltage-dependent manner (1, 2). In the presence of QX-222, channels exhibit a flickering behavior as molecules of QX-222 bind to and dissociate from the channel. Site-directed mutagenesis experiments reveal that the binding affinity of QX-222 is affected by specific amino acid substitutions made in the M2 membrane-spanning region of all five subunits of the AChR protein (3). The M2 region is thought to be an α-helical structure that forms the lining of the pore of the channel (4–6).

The action of some general anesthetics and alcohols on single AChR channels resembles that of QX-222; they cause the channels to flicker. However, this action is not adequately described by the open channel blocking model (7–9), and the effects of these uncharged drugs are not strongly voltage dependent. The actions of general anesthetics are well described by a model in which drug molecules have access to a binding (blocking or inhibitory) site when the gate of the channel is either open or closed (8, 10, 11). If the inhibitory binding site of nonpolar general anesthetics is the same as that of QX-222, general anesthetics will decrease the binding affinity of QX-222. In this paper, we describe experiments designed to test for competition between general anesthetics and QX-222 by making patch clamp recordings from AChR channels in excised patches from BC3H-1 cells.

Materials and Methods

We studied nicotinic AChRs expressed by the clonal BC3H-1 cell line (13). These receptors have the $\alpha_2\beta\gamma\delta$ subunit composition characteristic of the embryonic form of the muscle type AChR. Before starting patch clamp experiments, we replaced the culture medium with an "extracellular solution" containing (in mM): NaCl (150), KCl (5.6), $CaCl_2$ (1.8), $MgCl_2$ (1.0), and HEPES pH 7.3 (10). Patch pipettes were filled with a solution consisting of KCl (140), EGTA (5), $MgCl_2$ (5), and HEPES pH 7.3 (10) and had resistances of 4–6 MΩ. An outside-out patch (seal resistance ≥ 10 GΩ) was obtained (13) and moved into position at the outflow limb of the perfusion system.

For single channel experiments, the perfusion system consisted of

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ABBREVIATIONS: ACh, acetylcholine; AChR, acetylcholine receptor; QX-222, *N'*-(trimethyl-amino-methyl)-2',6'-xylidide.

four reservoirs (plastic intravenous drip bags) containing 200 nM ACh in extracellular solution, a manual switching valve, and plastic (ethyl vinyl acetate) tubing, one end of which was immersed in the culture dish. Three of the reservoirs also contained drug 1, drug 2, or (drug 1 + drug 2); the other reservoir is considered to be the "control" solution. Drug 1 was 5–25 mM ether (diethylether) or 5–25 mM *n*-butanol; drug 2 was 30 μM QX-222. The reservoirs were tightly sealed to avoid evaporation of volatile drugs. Single channel currents were measured with a patch clamp amplifier, filtered at 3 kHz (−3 db frequency, 8-pole Bessel filter), digitized at 50 μs/point, and stored on the hard disk of a laboratory computer.

The patch was held at −100 mV unless otherwise noted. We recorded four 5-s segments of single channel activity with control solution flowing past the excised patch. Data collection was then repeated during perfusion of the other three solutions. Finally, another set of data was recorded during perfusion of control solution. Our method of data analysis was the same as described previously (10) with the exception that event durations were binned on a logarithmic time axis and the histograms were fitted to a 2- or 3-exponential probability density function using a Simplex algorithm (14). The resolution was 100 μs for openings and 50 μs for closures. For each experimental condition, between 200 and 4000 events were accumulated for histogram analysis of event durations.

For rapid perfusion experiments, the perfusion system consisted of eight reservoirs, a solenoid-driven pinch valve, and a V-shaped piece of tubing immersed in the culture dish. This system is capable of making solution changes within 100 μs (15). Four of the reservoirs contained extracellular solutions of the drug combinations listed above with no ACh (normal flows), and four contained the same drug combinations with 300 μM ACh (test flows). 300 μM ACh saturates the agonist binding sites (16) but has only a small (10%) channel-blocking effect at −100 mV (17). The excised patch was perfused with a normal solution, and transient applications of test solution (the test solution containing the same drugs as the normal solution) were made.

The patch was held at −100 mV during applications of test solution. An ensemble of 20 applications of test solution, each 35 ms in duration and separated by 3 s, was obtained for each pair of normal and test solution. We obtained an ensemble of responses to the drug-free pair of solutions (control) before and after each of the other pairs of solutions. These experiments required extremely stable patches for which there was little rundown of channel activity during the 20 min needed to complete a full series of solution applications. The resulting macroscopic currents were measured with a patch clamp amplifier, filtered at 5 kHz, digitized at 10 μs/point, and stored on the computer. The ensemble mean of the current responses was calculated. We accepted data only if the ensemble means of the two controls in each trio of ensemble means (control, drug, control) differed by no more than 10%. We then calculated the ratio of the drug ensemble mean current to the control ensemble mean current (see Fig. 6).

Experiments were performed at room temperature (20–25°). Average values presented in the text and shown on figures include standard deviations.

Results

Fig. 1 shows examples of bursts obtained from a single patch exposed to control solution, 30 μM QX-222, 20 mM ether, and a mixture of 30 μM QX-222 + 20 mM ether. Channels are activated by 200 nM ACh. Under control conditions (for which two bursts are shown), channels appear as isolated openings infrequently interrupted by brief closures (no brief closures are seen in these examples). 30 μM QX-222 has no effect on the current amplitude, but it causes the channels to flicker. Events occur in bursts of briefer than normal openings. In the presence of 20 mM ether, channels have a smaller, noisier current amplitude. Some brief gaps can be resolved, so we consider this effect of ether to be an *apparent* reduction in amplitude due to incomplete resolution of fast flickering (18). When the patch is exposed to both QX-222 and ether, channels exhibit features of both drugs: the flickery behavior induced by QX-222 and the amplitude reduction due to ether.

A quantitative analysis of single channel properties of several hundred events obtained under each experimental condition is given in Table 1. The complexity of channel kinetics in the presence of two drugs makes it difficult to determine whether ether and QX-222 modify each other's actions. For example, it appears that ether reduces the number of openings/burst induced by QX-222 (the observed number of openings/burst is 3.5 for QX-222 alone versus 2.4 for both drugs together); however, this may simply be a consequence of ether reducing the burst duration. And, although QX-222 attenuates the amplitude reduction caused by ether (64% for ether alone versus 73% for both drugs together), the combined results of experiments on five patches indicate that this is not statistically significant (the ratio of channel amplitude in the presence of both drugs to channel amplitude in the presence of 20 mM ether alone is 1.0 ± 0.1). A conservative interpretation of the data in Table 1 would be that QX-222 and ether affect channels independently.

Clear evidence that QX-222 and ether *do* interact can be seen in the duration of gaps within bursts. Fig. 2 shows the closed time histograms obtained for each of the four experimental conditions. In each of the histograms, the long duration component represents the closed interval between bursts; it depends on the number of channels in the patch and on the concentration of ether (19) and will not be considered further in this paper. In the control histogram, the small, brief closed component near 0.1 ms represents the occasional brief gaps due to repeated openings of a single channel. The dominant component of the closed time histogram for QX-222 occurs at 0.9 ms; this component corresponds to the flickering gaps induced by QX-222. The brief component in the histogram for ether is at about 0.07 ms; it represents partially resolved fast flickering

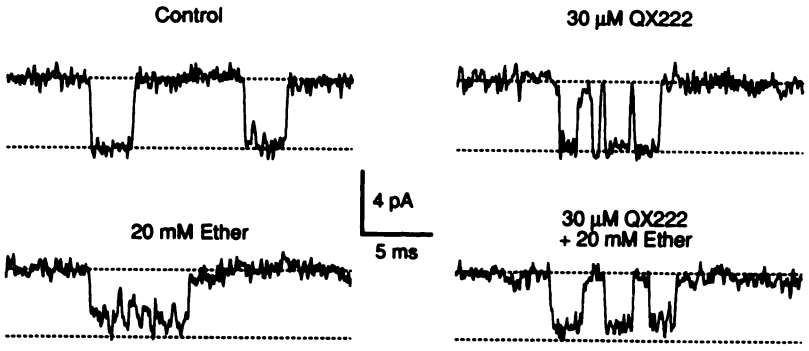


Fig. 1. Examples of single channels activated by 200 nM ACh under control conditions and during exposure to 30 μM QX-222, 20 mM ether, and both drugs. The applied potential was −100 mV. The *dashed line* indicates the baseline and current amplitude levels in the control recordings.

TABLE 1

Some kinetic and conductance properties of single AChR channels obtained in the presence of four different perfusion solutions

Data are from same patch as shown in Figs. 1 and 2. τ_{open} is the mean open time, τ_{burst} is the mean burst duration, $N_{O/B}$ is the number of openings/burst, and $N_{openings}$ is the number of openings considered in these calculations.

Condition	τ_{open}	Amplitude	τ_{burst}	$N_{O/B}$	$N_{openings}$
	ms	*pA*	*ms*		
Control	4.0	−3.9	4.1	1.0	205
30 μM QX-222	0.91	−3.8	5.5	3.5	329
20 mM ether	1.1	−2.5	2.1	1.7	793
Both	0.63	−2.8	3.9	2.4	558

induced by ether. The closed time histogram for the combination of drugs exhibits two brief components, one corresponding to each drug. The fastest component, due to rapid flickering by ether, is again only partially resolved. The other component, due to flickering by QX-222, is clearly resolved and has a time constant of 2.6 ms. Thus, in the presence of 20 mM ether, the flickering gap duration of QX-222 is nearly 3-fold longer than with QX-222 alone.

The increase in QX-222 gap duration by ether is dependent on the concentration of ether (Fig. 3). 15 mM ether increases the gap duration by a factor of 2. The increase in the gap duration is correlated with the apparent decrease in current amplitude by ether (Fig. 4, *closed symbols*). This correlation implies that both effects have the same dependence on the concentration of ether, so that both effects may be due to the same action of ether.

The effect of *n*-butanol on AChR channels is similar to the effect of ether; butanol causes an apparent decrease in current amplitude over a similar concentration range as ether (9). The effect of butanol on the duration of blocking gaps produced by QX-222 is the same as the effect of ether on gaps. And, just as with ether, the increase in the gap duration is correlated with the apparent decrease in current amplitude by butanol (Fig. 4, *open symbols*).

Block by QX-222 is voltage dependent; the blocking gaps have a longer duration at negative potentials (1). Fig. 5*A* shows the gap duration as a function of voltage in the absence and presence of 20 mM butanol. Butanol increases the gap duration by a factor of 2.3 ± 0.4 at all potentials. As a result, when 20 mM butanol is present, QX-222 blocks the channel as if the patch potential were shifted in the hyperpolarizing direction by 50 mV. 20 mM butanol also decreases the apparent single channel current amplitude by a constant factor of 0.76 ± 0.05 over this potential range (Fig. 5*B*). This implies that butanol decreases the apparent conductance of the channel and is not consistent with an actual change in electrical potential across the channel.

We also found evidence for interactions between hexanol and QX-222; 125 μM hexanol increased the QX-222 gap duration by a factor of 1.35 ± 0.20 (n = 4). The flickering induced by longer chain length alcohols and more potent general anesthetics occurs on a similar time scale to QX-222 gaps, so we did not look for interactions between these combinations of drugs. However, procaine induces longer gaps than does QX-222. Octanol (20 μM) increases the gap duration of procaine (10 μM) from 2.5 to 4.0 ms.

The conventional pharmacological approach to assay for interactions between drugs is to construct a concentration-effect curve for drug 1 alone and for drug 1 plus a fixed concentration of drug 2. Such experiments can be done on AChR channels by recording the macroscopic current response to rapid perfusion of ACh to a patch containing many channels. A control current response to rapid application of 300 μM ACh is shown in the *left panel* of Fig. 6. The current onset occurs with a time constant of 0.13 ms (limited by the rate of agonist binding and channel opening (15)) and reaches a level of −75 pA corresponding to the activation of about 19 channels. The slight decay in the current trace is due to fast desensitization occurring on the 50 ms time scale (20). The *right panel* of Fig. 6 shows a macroscopic current response to ACh in the continuous presence of 20 mM butanol. Butanol reduces the amplitude of the response (the trace is scaled by a factor of 1.55), but this response resembles the control response in every other way.

The trace labeled *QX* in the *left panel* of Fig. 6 was recorded

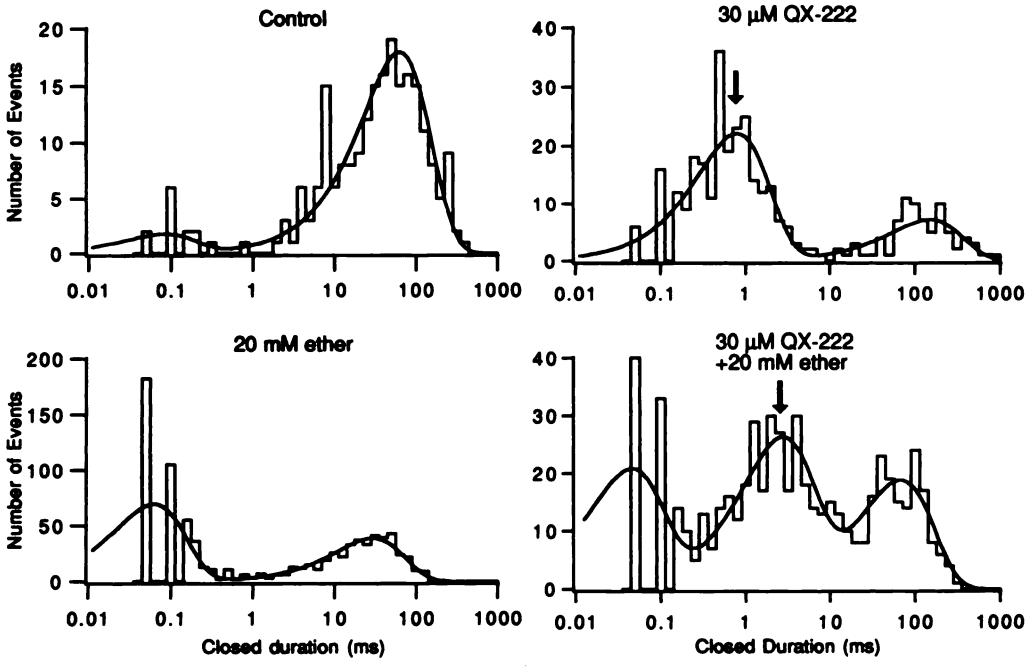


Fig. 2. Closed time histograms compiled from 300–500 events recorded from a single patch during exposure to indicated drugs. Histograms are presented with a log-binned time axis (14) and are fitted with a 2 (*left*) or 3 (*right*) exponential probability density function. *Arrows* point to the mean duration of gaps induced by QX-222. The longest component in each histogram represents the time between independent openings or bursts of channels.

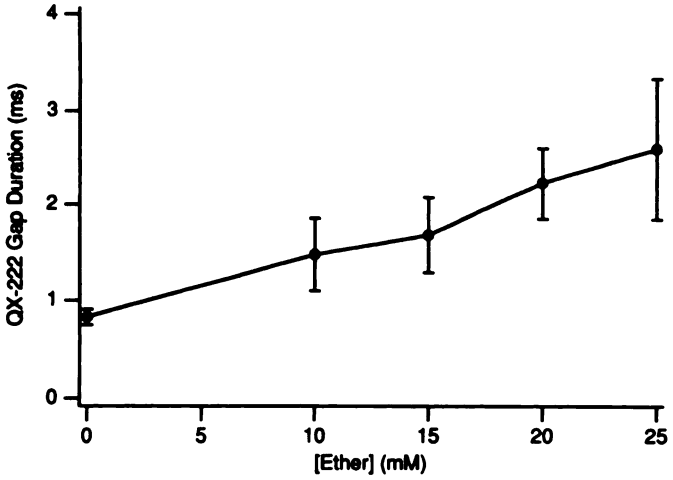


Fig. 3. Ether increases the duration of gaps induced by 30 μM QX-222. The data are presented as the means and S.D. of measurements on four or five patches at each concentration. Data points are joined by line segments.

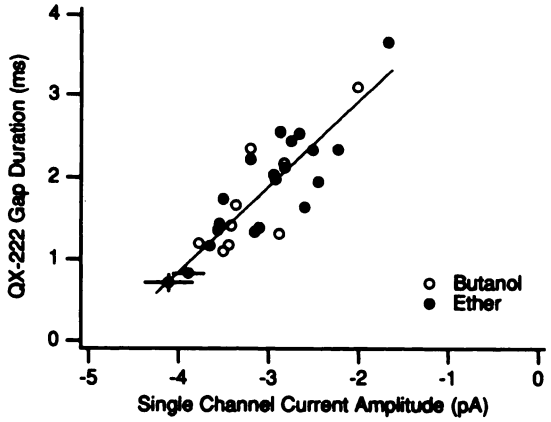


Fig. 4. The effect of ether and butanol on single channel current amplitude is correlated to the effect of these drugs on the gap duration induced by QX-222. There were 17 patches with ether (5–25 mM) and 10 patches with butanol (10–40 mM). The points with *error bars* are the means and S.D. of values obtained with QX-222 alone (separated according to the drug subsequently used on the patch). The *line* is the best fit of all the data by linear regression analysis ($r^2 = 0.76$; $p < 0.0001$; slope = 1.0 ms/pA; intercept = 5.0 ms).

while the patch was continuously exposed to 30 μM QX-222 and rapidly perfused with ACh. This trace exhibits a fast decay (0.43 ms) arising from the block of channels by QX-222 after the channels are opened by ACh. After the decay, the current amplitude is reduced to 42% of control. The smaller trace in the *right panel* of Fig. 6 was obtained while the patch was exposed to both 20 mM butanol and 30 μM QX-222. A decay (0.93 ms) is seen as QX-222 equilibrates with open channels. After the decay, the current amplitude is reduced to 25% of the response in the presence of 20 mM butanol alone. Comparison of the QX and QX + butanol responses shows that QX-222 is a more potent blocker when the channels are exposed to butanol.

The results of the macroscopic current experiments are summarized in the concentration-effect curves of Fig. 7. The current responses in the presence of 6, 20, and 55 mM butanol alone (normalized to the control response) are shown as *open symbols*. 30 μM QX-222 alone reduced the current to 0.45 ± 0.08 ($n = 8$) of control. The current responses in the presence of both butanol and 30 μM QX-222 (normalized to the response with

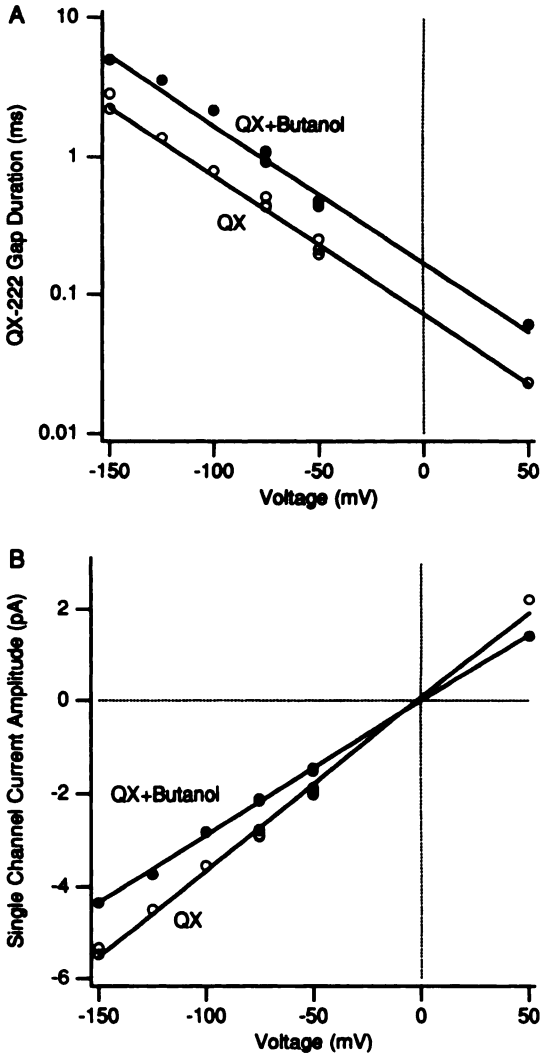


Fig. 5. *A*, the voltage dependence of the QX-222 gap duration in the presence (●) and absence (○) of 20 mM butanol. Note that a logarithmic scale is used for gap duration. The *lines* are the best fits of the data by linear regression analysis. For both data sets, the slope gives an *e*-fold change in gap duration with a 42 mV change in potential. *B*, the voltage dependence of the single channel current amplitude in the presence (●) and absence (○) of 20 mM butanol (30 μM QX-222 was present for both data sets). The *lines* are the best fits of the data by linear regression analysis. The slope of the line for QX-222 alone is 37 pS; the slope of the line for both QX-222 and 20 mM butanol is 29 pS.

30 μM QX-222 alone) are shown as *closed symbols*. The effect of QX-222 is to shift the butanol concentration-effect curve to the left; 30 μM QX-222 reduces the half-effective concentration of butanol from about 30 mM to 20 mM.

Discussion

The possibility that local anesthetics inhibit the AChR channel by physically plugging the pore of the channel was first proposed in 1977 (21, 22) on the basis of endplate current measurements. Single channel recording provided visual (channel flickering) and quantitative support for open channel block as the mechanism of action for the local anesthetic QX-222 (1, 2). And, little doubt remained after site-directed mutagenesis experiments indicated that QX-222 (3) and chlorpromazine (6) bind to specific amino acids within M2. In 1981, a similar mechanism for the inhibition of AChR channels by general

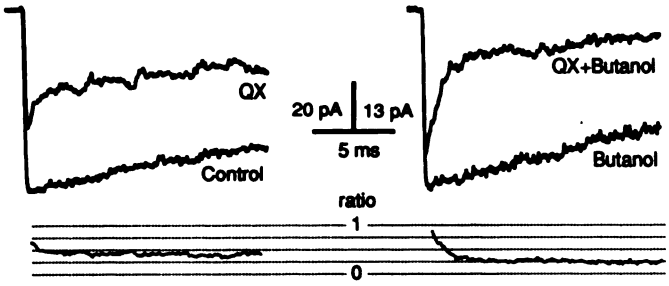


Fig. 6. Macroscopic current responses to rapid perfusion of 300 μM ACh to a patch containing about 19 channels. Each trace is the ensemble mean of 20 responses. The *left panel* shows the response when no drug was present (*Control*) and when 30 μM QX-222 was present. The *right panel* shows the response when 20 mM butanol was present and both 30 μM QX-222 and 20 mM butanol were present. In each case, the drug(s) was present both before and during exposure to ACh. The *dotted traces* at the *bottom* of the figure are the current ratios QX:control (*left*) and QX + butanol:butanol (*right*). The combination of drugs is more potent than would be expected if they acted independently. See text for details.

anesthetics was considered (23). This model would include drug binding to both open and closed channels. Subsequently, end-plate current measurements (24) and patch clamp recording showed that such a model was adequate to explain many of the observed effects (8–11, 18). However, there is no direct evidence that general anesthetics bind within the pore of channel; binding at an allosteric, inhibitory site remains a viable alternative.

The experiments described here were designed to test the hypothesis that nonpolar drugs such as ether, butanol, and hexanol compete with QX-222 for binding to a site with the M2 region lining the pore of the channel. Competition would have been manifested as an apparent decrease in the affinity of one drug when the second drug is also present. We found no evidence for such competition. On the contrary, we found that ether and butanol stabilize the binding of QX-222 in the pore. In single channel experiments, stabilization of QX-222 binding by ether is seen as an increase in QX-222 gap duration (Figs. 2 and 3). An increase in the gap duration corresponds to a decrease in the dissociation rate constant for QX-222. If a general anesthetic causes QX-222 to bind more tightly, then it must also be true that QX-222 causes the general anesthetic to bind more tightly. In macroscopic current recording, this is seen as a leftward shift in the butanol concentration-effect curve by QX-222 (Fig. 7). (This effect is not detected in single channel events, because no current flows while QX-222 is bound, making it impossible to assess the amount of binding by general anesthetic.)

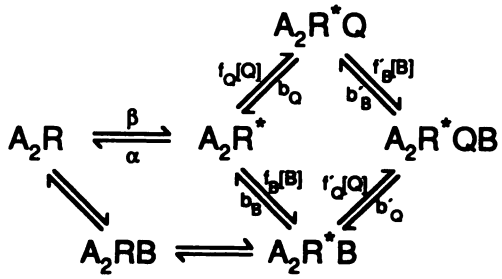
It is possible that the process by which ether and butanol stabilize the binding of QX-222 is distinct from the process by which ether and butanol inhibit current flow through the channel. Channel inhibition by these drugs is characterized by a decrease in the apparent conductance of the channel and an increase in open channel noise. This probably arises from frequent, brief (≈10 μs) but incompletely resolved binding events that completely interrupt the flow of ions through the channel (18). Fig. 4 shows that the two processes, channel inhibition and QX-222 stabilization, have the same dependence on the concentration of butanol and ether. Either both processes are due to the same effect of the drug or the two processes are distinct but have the same concentration dependence by chance.

Our results rule out the idea that ether and butanol compete with QX-222 for a single binding site within the pore of the channel. However, ether and butanol might bind elsewhere within the pore. One possibility is that these drugs stabilize the binding of QX-222 by binding close to the binding site of the nonpolar xylidine ring of QX-222. The xylidine ring binds to nonpolar amino acids close to the center of the pore, for example, serine 252 on the α-subunit and alanine 261 on the γ-subunit (3). AChR mutants containing a single amino acid substitution that decreases the polarity of the pore in this region (for example, substitution of alanine for serine on the α-subunit) increase the gap duration of QX-222 by a factor of two (3). We found that 15 mM butanol also causes a 2-fold increase in QX-222 gap duration (Fig. 3). Thus, the simplest interpretation of our data is that butanol stabilizes the binding of QX-222 by binding close to the xylidine ring binding site and decreasing the local polarity. When anesthetic is bound to this site, dissociation of QX-222 from the channel is decreased. Of course, we cannot discount the possibility that general anesthetics accomplish this by binding at a remote site to allosterically alter the binding affinity of QX-222.

The ability of hydrophobic amino acids in M2 to serve as binding sites for nonpolar substances is underscored by other recent experiments. The nonpolar, noncompetitive antagonist, 3-(trifluoromethyl)-3-*m*-(iodophenyl)-diazirine, specifically photolabels amino acid residues in M2 in the β- and δ-subunits of wild-type *Torpedo* AChR (25). In BC3H-1 cells, the unblocking rates for a series of cationic channel blockers is related to the length of the hydrocarbon chain of the compounds. The unblocking rate decreases with increasing chain length, suggesting a hydrophobic binding site within the channel (26).

Forman (27) found no evidence for interactions between procaine and octanol in *Torpedo* AChRs. This might appear to contradict our results. However, those experiments measured ion flux through receptor-containing vesicles on a 10-s time resolution, in contrast to our submillisecond current measurements. On the 10-s time scale, AChR channel kinetics are dominated by desensitization processes and may not reflect what happens under nondesensitizing conditions.

Scheme I is a partial model for the interaction of QX-222 (Q) and butanol or ether (B) with the AChR channel. Only the doubly liganded states of the AChR are considered, because 300 μM saturates nearly all of the agonist binding sites (16). We assume that QX-222 binds only with the open state of the channel (A_2R^*) but that butanol can bind (within the pore or elsewhere on the AChR protein) with both the open and closed (A_2R) states. Association rate constants are denoted by f_Q and f_B; dissociation rate constants are denoted by b_Q and b_B. The binding of QX-222 may be different when butanol is already bound (and vice versa) as indicated by the primed rate constants (microscopic reversibility demands that only seven of the eight rate constants in the "open channel" loop are independent).



SCHEME 1.

We wish to identify several qualitative relationships predicted by Scheme I. When there is no butanol present, there are only three states (A_2R, A_2R^*, and A_2R^*Q), and Scheme I provides a simple expression for the QX-222 gap duration, $1/b_Q$. Our measurements give $b_Q = 1100/s$. When butanol is present, the expression for the gap duration (the longest closed time constant) is complicated. Thus, the measured gap durations do not simply reflect, b'_Q, the rate for QX-222 dissociation from the A_2R^*QB state. However, it is clear that $b'_Q < b_Q$. The mean open duration is determined by the rate constants leading from the open state A_2R^*. With QX-222 alone, this is $1/(\alpha + f_Q[Q])$. Since $\alpha = 0.25/ms$, our results for 30 μM QX-222 lead to $f_Q = 3 \times 10^7/M/s$. The mean open duration in the presence of both drugs is $1/(\alpha + f_Q[Q] + f_B[B])$, which is shorter than the mean open duration in the presence of QX-222 alone. Although the measured open durations in the presence of butanol are overestimated due to unresolved brief blocking events, our measurements qualitatively exhibit the predicted reduction in open duration (Table 1).

In macroscopic current measurements, Scheme I predicts that the decay time constant in the absence of butanol is given by $1/(f_Q[Q] + b_Q)$ (Scheme I predicts a two-component decay; this is the slower, dominant component) and the ratio of equilibrium current to control is $b_Q/(f_Q[Q] + b_Q)$. For 30 μM QX-222, we measured a time constant of 0.43 ms and an equilibrium current ratio of 0.45. This determines $f_Q = 4 \times 10^7/M/s$ and $b_Q = 1000/s$, in good agreement with the results of single channel experiments described above. Again, the addition of butanol complicates the kinetics, but both f'_Q and b'_Q are important factors in determining the current decay time constant.

Neher (2) found that block by QX-222, at concentrations less than 40 μM, is well described by an open channel blocking model. This issue is also addressed by our macroscopic current experiments. In the trace labeled QX in Fig. 6, the current decay after addition of agonist represents the kinetics of QX-222 block of open channels. The onset of the current response partially obscures this decay. If we extrapolate the current (time constant 0.43 ms) back to time zero (when the current onset begins), we obtain a peak current of −65 pA. Because the control peak current is −74 pA, 30 μM QX-222 blocks no more than 12% of the closed channels. In order to obtain better time resolution in this type of experiment, we would have to use higher concentrations of ACh to reduce the time constant of current onset (15). Such experiments would be difficult to interpret, because of the large channel-blocking effect produced by >300 μM ACh (15, 17). Extrapolation of the current trace labeled QX + butanol in Fig. 6 is more reliable, because the decay time constant is longer. This gives an estimated peak current of −47 pA; nearly indistinguishable from the peak of the 20 mM butanol control trace. This suggests that when butanol is present, 30 μM QX-222 does not significantly bind to closed channels.

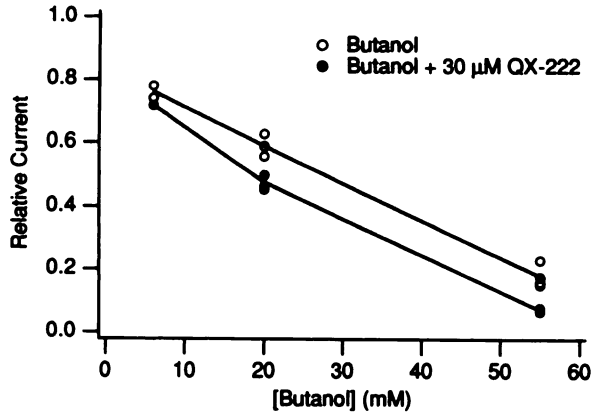


Fig. 7. Normalized macroscopic currents activated by rapid perfusion of 300 μM ACh as a function of butanol concentration. The *open circles* represent the macroscopic currents obtained in the presence of butanol relative to control (no drug). The *closed circles* represent the macroscopic currents obtained in the presence of butanol + QX-222 relative to QX-222 alone. The butanol concentration-effect curve is shifted to the left by QX-222.

The results presented here demonstrate that nonpolar molecules such as ether and butanol exhibit a cooperative interaction with QX-222, a drug that binds specifically within the pore of the AChR channel. The macroscopic current experiments show this interaction as a leftward shift in the butanol concentration-effect relationship. The single channel experiments allow us to isolate the inhibitory effect as a stabilization of QX-222 bound to the pore of the channel. These results and other measurements (7–11, 18) are consistent with the idea that alcohols and anesthetics bind within the pore of the channel. However, these experiments measure channel function, and they cannot be used to make definitive statements about structure; that is, the location of the binding site for butanol and ether. Only structural experiments will provide a clear distinction between drug binding sites within the channel pore and drug binding sites on other regions of the channel protein.

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References

1. Neher, E., and J. H. Steinbach. Local anesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol.* **277**:153–176 (1978).
2. Neher, E. The charge carried by single-channel currents of rat cultured muscle cells in the presence of local anaesthetics. *J. Physiol.* **339**:663–678 (1983).
3. Charnet, P., C. Labarca, R. J. Leonard, N. J. Vogelaar, L. Czyzyk, A. Gouin, N. Davidson, and H. A. Lester. An open-channel blocker interacts with adjacent turns of α-helices in the nicotinic acetylcholine receptor. *Neuron* **2**:87–95 (1990).
4. Hucho F., W. Oberthür, and F. Lottspeich. The ion channel of the nicotinic acetylcholine receptor is formed by the homologous helices M II of the receptor subunits. *FEBS Lett.* **205**:137–142 (1986).
5. Imoto K., C. Methfessel, B. Sakmann, M. Mishina, Y. Mori, T. Konno, K. Fukuda, M. Kurasaki, H. Bujo, Y. Fujita, and S. Numa. Location of a δ-subunit region determining ion transport through the acetylcholine receptor channel. *Nature* **324**:670–676 (1986).
6. Revah, F., J. L. Galzi, J. Giraudat, P. Y. Haumont, F. Lederer, and J-P Changeux. The noncompetitive blocker [^{3}H]chlorpromazine labels 3 amino acids of the acetylcholine receptor γ-subunit: implications for the α-helical organization of regions MII and for the structure of the ion channel. *Proc. Natl. Acad. Sci. U. S. A.* **87**:4675–4679 (1990).
7. Brett, R. S., J. P. Dilger, and K. F. Yland. Isoflurane causes "flickering" of the acetylcholine receptor channel: observations using the patch clamp. *Anesthesiology* **69**:161–170 (1988).
8. Murrell, R. D., M. S. Braun, and D. A. Haydon. Actions of *n*-alcohols on nicotinic acetylcholine receptor channels in cultured rat myotubes. *J. Physiol.* **437**:431–448 (1991).
9. Dilger, J. P., and R. S. Brett. Actions of volatile anesthetics and alcohols on cholinergic receptor channels. *Ann. N. Y. Acad. Sci.* **625**:616–627 (1991).
10. Dilger, J. P., R. S. Brett, and L. A. Lesko. Effects of isoflurane on acetylcholine receptor channels. 1. Single-channel currents. *Mol. Pharmacol.* **41**:127–133 (1992).
11. Dilger, J. P., R. S. Brett, and H. I. Mody. Effects of isoflurane on acetylcholine receptor channels. 2. Currents elicited by rapid perfusion of acetylcholine. *Mol. Pharmacol.* **44**:1056–1063 (1993).
12. Schubert, D., A. J. Harris, C. E. Devine, and S. F. Heinemann. Characterization of a unique muscle cell line. *J. Cell Biol.* **61**:398–413 (1974).
13. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved

patch-clamp techniques for high-resolution current recording from cells and cell free membrane patches. *Pflügers Arch.* **391**:85–100 (1981).
14. Sigworth, F. J., and S. M. Sine. Data transformations for improved display and fitting of single channel dwell time histograms. *Biophys. J.* **52**:1047–1054 (1987).
15. Liu Y., and J. P. Dilger. Opening rate of acetylcholine receptor channels. *Biophys. J.* **60**:424–432 (1991).
16. Dilger, J. P., and R. S. Brett. Direct measurement of the concentration- and time-dependent open probability of the nicotinic acetylcholine receptor channel. *Biophys. J.* **57**:723–731 (1990).
17. Sine, S. M., and J. H. Steinbach. Agonists block currents through acetylcholine receptor channels. *Biophys. J.* **46**:277–284 (1984).
18. Dilger, J. P., A. M. Vidal, H. I. Mody, and Y. Liu. Evidence for direct actions of general anesthetics on an ion channel protein: a new look at a unified mechanism of action. *Anesthesiology*, in press
19. Liu, Y., J. P. Dilger, and A. M. Vidal. Effects of alcohols and volatile anesthetics on the activation of nicotinic acetylcholine receptor channels. *Mol. Pharmacol.* **45**:1235–1241 (1994).
20. Dilger, J. P., and Y. Liu. Desensitization of acetylcholine receptors in BC3H-1 cells. *Pflügers Arch.* **420**:479–485 (1992).
21. Ruff, R. L. A quantitative analysis of local anaesthetic alteration of miniature end-plate currents and end-plate current fluctuations. *J. Physiol.* **264**:89–124 (1977).
22. Adams, P. R. Voltage jump analysis of procaine action at frog end-plate. *J. Physiol.* **268**:291–318 (1977).
23. Gage, P. W., and O. P. Hamill. Effects of anesthetics on ion channels in synapses, in *International Review of Physiology* (R. Porter, ed.). University Park Press, Baltimore, Vol. 25. 1–45 (1981).
24. McLarnon, J. G., P. Pennefather, D. M. J. Quastel. Mechanisms of nicotinic channel blockade by anesthetics, in *Molecular and Cellular Mechanisms of Anesthetics* (S. H. Roth and K. W. Miller, eds.). Plenum Press, New York, 155–164 (1986).
25. White B. H., and J. B. Cohen. Agonist-induced changes in the structure of the acetylcholine receptor M2 regions revealed by photoincorporation of an uncharged nicotinic noncompetitive antagonist. *J. Biol. Chem.* **267**:15770–15783 (1992).
26. Carter, A. A., and R. E. Oswald. Channel blocking properties of a series of nicotinic cholinergic agonists. *Biophys. J.* **65**:840–851 (1993).
27. Forman, S. A. Inhibition of cation channel function at the nicotinic acetylcholine receptor from *Torpedo*: agonist self inhibition and anesthetic drugs. Ph. D. thesis, Harvard University, Cambridge, MA (1989).

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