0026-895X/94/061235-07$03.00/0
Copyright © by The American Society for Pharmacology and Experimental Therapeutics
All rights of reproduction in any form reserved.
MOLECULAR PHARMACOLOGY, 45:1235–1241, 1994.

MOL PHARM

Effects of Alcohols and Volatile Anesthetics on the Activation of Nicotinic Acetylcholine Receptor Channels

YI LIU,[1] JAMES P. DILGER, and ANA MARIA VIDAL

Departments of Anesthesiology (Y.L., J.P.D., A.M.V.) and Physiology and Biophysics (J.P.D.), University at Stony Brook, Stony Brook, New York 11794–8480

Received December 9, 1993; Accepted March 23, 1994

SUMMARY

The *n*-alcohols butanol through nonanol and the volatile anesthetic ether increase the frequency of bursts of nicotinic acetylcholine (ACh) receptor channels induced by low concentrations of agonists. For example, 10 mM butanol increases the burst frequency induced by 0.2 μM ACh (a full agonist) and 1 μM decamethonium (a partial agonist) by 1.6-fold and 2.7-fold, respectively. An increase in burst frequency could arise from effects of the drug on agonist binding, channel gating, or desensitization. To distinguish among these alternatives, we measured the current response to rapid application of saturating concentrations of agonists. We found that 10 mM butanol increases the peak current induced by 100 μM decamethonium by 2-fold. In addition, 20 mM butanol and 3 mM pentanol both decrease the onset time of the current response to 10 mM ACh by about 40%. In contrast, ether does not increase the current response to 100 μM decamethonium and does not significantly change the onset time for 10 mM ACh. Neither ether nor butanol changes the degree of steady state desensitization induced by 0.2 μM ACh. We conclude that butanol and pentanol increase burst frequency by increasing the channel opening rate, whereas ether does so by increasing the agonist binding affinity of the ACh receptor.

Short-chain *n*-alcohols, such as ethanol, have an excitatory effect on the nicotinic ACh receptor. They prolong miniature end-plate currents (1–3), decrease the frequency of the endplate noise power spectrum (4), and increase the apparent affinity of the ACh receptor for ACh (4–9). Results from single-channel recording show that ethanol increases both the frequency and duration of bursts of openings induced by low concentrations of ACh (9). In contrast, longer-chain alcohols and many other general anesthetics inhibit neuromuscular transmission by decreasing the duration of miniature end-plate currents (2, 10, 11). Single-channel recording (12, 13) reveals that intermediate- and long-chain *n*-alcohols, as well as volatile anesthetics such as isoflurane, decrease the duration of bursts of openings induced by low concentrations of ACh. Intermediate-chain *n*-alcohols also either reduce the apparent single-channel current amplitude or cause channel openings to flicker. These inhibitory effects of *n*-alcohols and volatile anesthetics on ACh receptor channels can be described in terms of a kinetic model in which both open and closed channels can be blocked by alcohol or anesthetic (12, 13).

Decanol and isoflurane have also been shown to increase the frequency of bursts induced by low concentrations of ACh (13, 14). This suggests that excitatory effects on ACh receptor channel activation may not be unique to short-chain alcohols but, rather, may be common to *n*-alcohols and perhaps also to a variety of other general anesthetics.

An increase in single-channel burst activity by alcohols and volatile anesthetics can be attributed to one or more of the following: (a) an increase in the affinity of the receptor for agonist, (b) an increase in the efficacy of an agonist, and (c) a decrease in the degree of steady state desensitization induced by continuous exposure to low concentrations of agonist. The major effect of ethanol is to increase the efficacy of agonists for the ACh receptor by both increasing the channel opening rate and decreasing the channel closing rate (9). The major effect of isoflurane is to increase the ACh binding affinity (15). Here, we extend our study to the alcohols butanol through nonanol and the volatile anesthetic ether. We use the techniques of equilibrium single-channel recording and rapid perfusion of agonists and drugs. We show that, although ether and the *n*-alcohols all increase the frequency of bursts, they do not share the same mechanism of action on the ACh receptor. Butanol and pentanol increase the opening rate of the channel. Ether, like isoflurane, increases agonist binding affinity. We were unable to determine the mechanism of increasing frequency of bursts for the alcohols hexanol to nonanol.

This research was supported by a grant from the National Institutes of Health (GM42095).

[1] Present address: Laboratory of Molecular Physiology, Massachusetts General Hospital, Wellman 340A, 50 Blossom Street, Boston, MA 02114.

ABBREVIATIONS: ACh, acetylcholine; Deca, decamethonium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012

MOL PHARM

MOLECULAR PHARMACOLOGY

Materials and Methods

Clonal BC3H-1 cells were cultured and prepared for patch-clamp recording as described previously (16). At the time of experiment, culture medium was exchanged with an extracellular solution containing (in mM) 150 NaCl, 5.6 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, pH 7.2–7.4. The pipette solution contained (in mM) 140 KCl, 5 EGTA, 1 $MgCl_2$, and 10 HEPES, pH 7.3. Upon excision from the cell, the outside-out patch (17) was moved into position for perfusion with agonists and drugs.

Three different perfusion protocols were used in the experiments reported here. The first involved single-channel recording during equilibrium exposure to agonist. Data were recorded while the patch was continuously exposed to a low concentration of agonist (0.2 μM ACh or 1 μM Deca). The patch was then continuously perfused with a solution containing the same agonist plus the indicated concentration of a drug, and more data were recorded. A third set of data was recorded upon return to the original solution. The second protocol involved single-channel recording during transient exposure to agonist. This differed from the aforementioned protocol in that agonist- and/or drug-containing solutions were applied to the patch for 120-msec time periods. Between agonist applications, the patch was perfused with agonist-free, but same drug-containing, solution for 1 sec. The third protocol involved macroscopic current recording during transient exposure to agonist (18). Data were recorded as the patch was rapidly exposed to a saturating concentration of agonist (10 mM ACh or 100 μM Deca) for 20 or 100–170 msec, respectively. Between agonist applications, the patch was perfused with agonist-free solution for 3 sec. Similar recordings were acquired as agonist- and drug-containing solution was applied to the patch during either transient or continuous exposure to a drug. A third set of data was recorded upon return to the original (drug-free) solutions.

Meaningful data are obtained only when there is minimal rundown of channel activity in the patch for about 10–30 min. The single-channel data reported here are from patches in which the longer component in the closed-duration histogram in controls after drug exposure is no more than twice as long as that before drug exposure. In most cases, the two time constants are within 50% of each other. The peak macroscopic currents in controls before and after drug exposure differ by no more than 20%. The average activities of the two controls were used for calculating the effect of the drug.

Currents flowing through the patch were measured with a List EPC-7 patch-clamp amplifier, filtered with an eight-pole Bessel filter at 3, 10, and 3 kHz, and digitized at 20, 320, and 10 kHz for single-channel recording, onset measurements with 10 mM ACh, and macroscopic measurements with 100 μM Deca, respectively. Data were stored on the hard disk of a PDP-11 minicomputer. Experiments were performed with −100 mV applied potential (except onset experiments, which were performed at +50 mV), at room temperature (20–24°). Data were analyzed off-line with user-written computer programs.

Analysis of the single-channel data was performed as described previously (14). The only difference was that events were logarithmically binned in the duration histograms before being fitted. Burst-duration histograms of channels activated by 0.2 μM ACh were fitted with a two-exponential probability density function. The major long component was used in the calculation of the frequency of bursts. The number of long bursts was divided by the total recording time to obtain the burst frequency. Burst-duration histograms of channels activated by 1 μM Deca were fitted with a single-exponential probability density function. The fraction of multiple openings was generally small (<5–10%).

The ensemble mean of 10–40 current responses was calculated for each macroscopic current measurement. For onset experiments, individual traces were aligned at the midpoint of the onset phase before the mean was calculated. The onset time was defined as the 20–80% current rise time (18).

Results are expressed in the form of mean ± standard deviation. The significance of differences between two values was determined with a two-tailed t test.

Results

The n-alcohols increase the frequency of bursts of nicotinic ACh receptor channels induced by low concentrations of ACh. This is illustrated in Fig. 1, A and B, which compares single-channel activity induced by equilibrium exposure to 0.2 μM ACh alone (control) (Fig. 1A) and 0.2 μM ACh plus 10 mM butanol (Fig. 1B). Most bursts consist of a single resolved opening under our recording conditions, both in the absence and in the presence of 10 mM butanol. An increase in burst frequency by 10 mM butanol is also seen when channels are activated by the partial agonist Deca (Fig. 1, C and D). Under control conditions, the frequency of bursts ranges from 2 to 40 sec^{-1}, depending upon the number of channels in the patch. Bursts of openings occur 2–3 times more frequently in the presence of 10 mM butanol than in controls. Ether and the n-alcohols from pentanol to nonanol also increase the burst frequency induced by 0.2 μM ACh (see Fig. 4).

Butanol (10 mM) has little effect on the mean burst duration induced by 0.2 μM ACh. However, it decreases the burst duration induced by 1 μM Deca (see Fig. 3). The apparent single-channel current induced by either 0.2 μM ACh or 1 μM Deca is reduced by about 10% when 10 mM butanol is present. These changes in burst duration and apparent single-channel current are characteristic of the inhibitory effects of butanol (13).

Fig. 2 shows histograms of closed-time durations of channels activated by 1 μM Deca in the absence (Fig. 2A) and presence (Fig. 2B) of 10 mM butanol. The closed time histogram of control has two components. The larger component (92%), with a time constant of 190 msec, corresponds to the time between independent channel activations and varies from patch to patch, depending on the number of channels in a patch. The smaller component, with a time constant of about 300 μsec (8%), represents brief gaps within bursts, which occur infrequently. The major effect of 10 mM butanol is a 3-fold decrease in the time constant of the long component (to 65 msec). This corresponds to an increase in burst frequency from 5 sec^{-1} to 15 sec^{-1} (before correction for the fraction of long bursts; see Materials and Methods).

Histograms of open-time durations of channels activated by 1 μM Deca both in the absence and in the presence of 10 mM butanol can be described by a single-exponential probability density function (Fig. 3). Butanol reduces the time constant from the control value of 1.6 msec (Fig. 3A) to 0.9 msec (Fig. 3B). When averaged over six patches, the time constant is reduced to 51 ± 8% (mean ± standard deviation) of the control. When channels are activated with 0.2 μM ACh, a two-exponential probability density function is needed to fit the open-duration histograms (data not shown). The time constant of the major long component is decreased slightly, to 91 ± 8% (n = 6) of the control value. In control experiments, the brief component has a time constant of 200–300 μsec and generally accounts for 20–30% of the total openings (14). These values are not significantly different when butanol is present. We did not study this component further. Because the number of resolved openings per burst is very close to 1 (<1.05) both in the absence and in the presence of butanol and for both ACh and Deca, the histograms of burst durations closely follow those of the corresponding open-time durations.

Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012

aspet

MOL PHARM

MOLECULAR PHARMACOLOGY

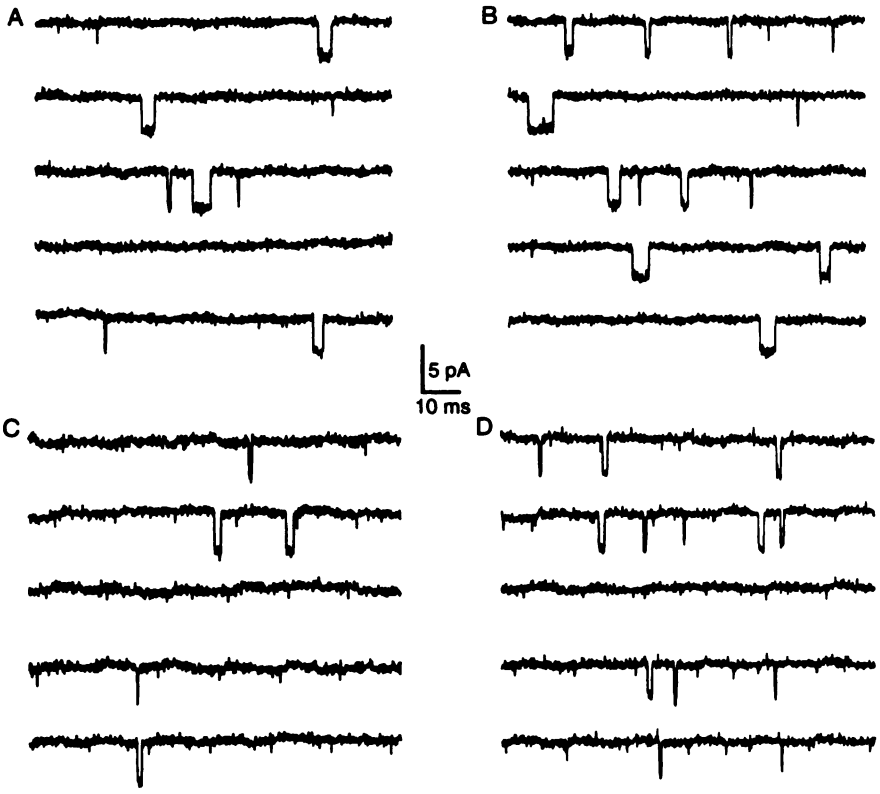


Fig. 1. Single-channel recordings (500 msec, continuous traces) at low concentrations of agonist in the absence (A and C) and presence (B and D) of 10 mM butanol. The patch potential was −100 mV. A and B are from a single patch exposed to 0.2 μM ACh. C and D are from another patch exposed to 1 μM Deca.

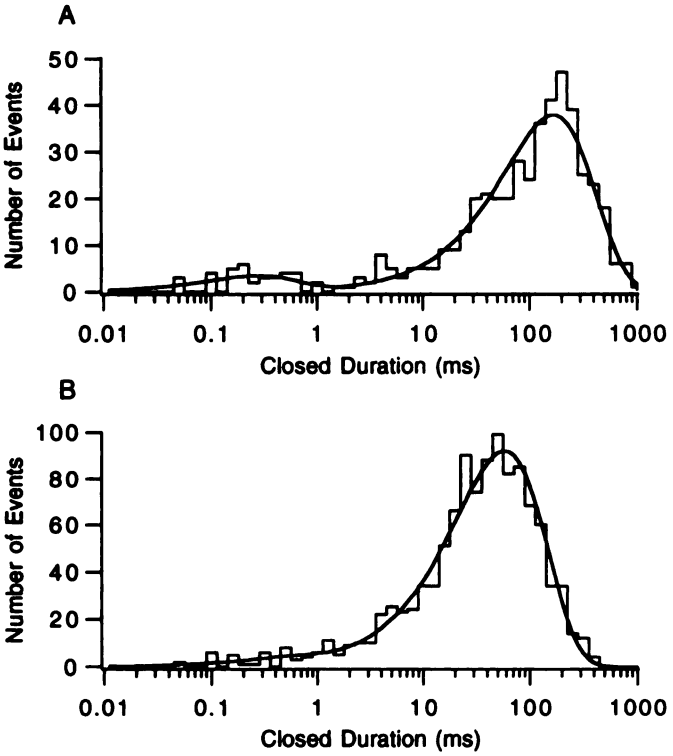


Fig. 2. Histograms of closed-time durations induced by 1 μM Deca in the absence (A) (total events n_t = 480) and presence (B) (n_t = 1110) of 10 mM butanol. Same patch as for Fig. 1, C and D. Both histograms can be fitted well with a two-exponential function. The long and short closed durations are τ_{lc} = 187 msec (92%) and τ_{sc} = 0.3 msec (8%) (A) and τ_{lc} = 65 msec (97.5%) and τ_{sc} = 0.45 msec (2.5%) (B).

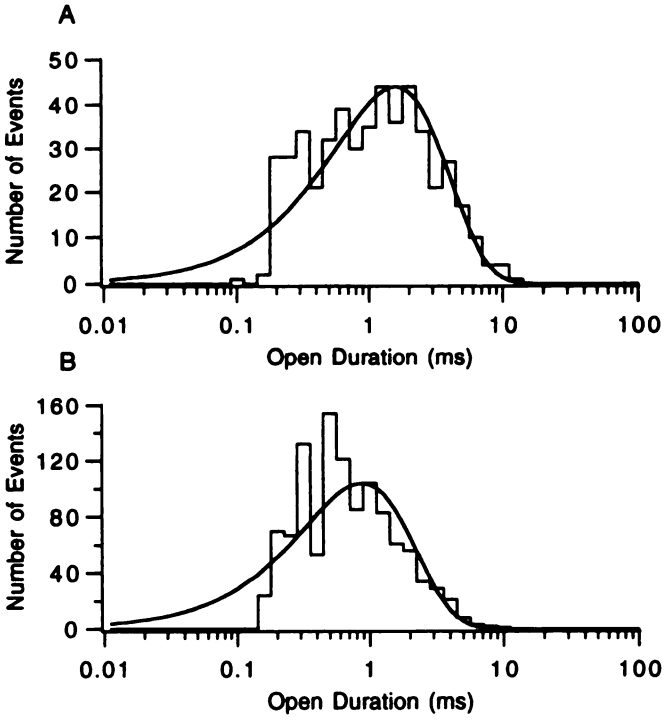


Fig. 3. Histograms of open-time durations induced by 1 μM Deca in the absence (A) (n_t = 492) and presence (B) (n_t = 1108) of 10 mM butanol. Same patch as for Fig. 1, C and D. Both histograms can be described by a single-exponential function. The time constants are 1.6 msec (A) and 0.9 msec (B).

Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012

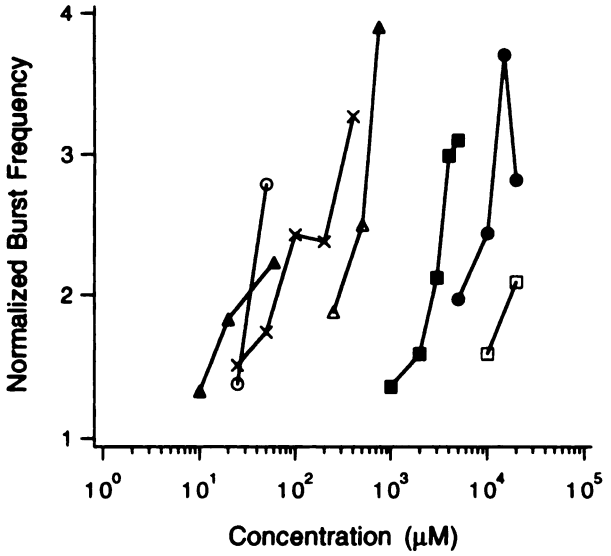


Fig. 4. Effects of anesthetics on the frequency of bursts induced by 0.2 μM ACh. The anesthetics used were ether (●), butanol (□), pentanol (■), hexanol (△), heptanol (×), octanol (○), and nonanol (▲). Data points involving the same anesthetic are connected with *straight lines*. The number of patches varied from one to eight for the different anesthetics studied. All data were recorded at −100 mV and filtered at 3 kHz, except for the data for 1, 3, and 4 mM pentanol, which were recorded at −120 mV and filtered at 6 kHz.

Fig. 4 summarizes the effects of the *n*-alcohols (butanol through nonanol) and ether on burst frequency induced by 0.2 μM ACh. The alcohols and ether all increase the burst frequency in a concentration-dependent fashion. The potency of the alcohols to increase burst frequency increases with the hydrocarbon chain length up to eight carbons. A 2-fold increase in burst frequency is achieved by (in mM) 20 butanol, 3 pentanol, 0.3 hexanol, 0.08 heptanol, 0.03 octanol, and 0.03 nonanol.

In control experiments (no drug present), the frequency of bursts is higher when agonist is applied transiently than when it is applied continuously. There are about twice as many bursts when 0.2 μM ACh is applied transiently as when it is applied continuously.[2] Constant exposure to 0.2 μM ACh desensitizes about half of the channels within a few seconds.[2] It has been shown that *n*-alcohols and other volatile anesthetics alter steady state desensitization (19). To test the possibility that ether and the *n*-alcohols increase the frequency of bursts by decreasing the degree of desensitization induced by equilibrium exposure of agonist, we recorded single-channel currents during transient exposure to low concentrations of agonists. Table 1 compares effects of 10 mM butanol and 15 mM ether on burst frequency using two protocols, i.e., transient and equilibrium applications of agonists (0.2 μM ACh and 1 μM Deca). The increase in burst frequency by butanol and ether does not depend on the method of agonist application. Thus, the increase in burst frequency by these drugs is not related to desensitization.

If alcohols and anesthetics increase burst frequency by increasing the opening rate of the ACh receptor, then there should be a drug-induced increase in the macroscopic current response to saturating concentrations of agonist. However, ACh is a very efficacious agonist; saturating concentrations of ACh open at least 95% of the channels (20, 21). Therefore, it would be difficult to detect a drug-induced increase in the macroscopic current response to saturating concentrations of ACh. Indeed, 10 mM butanol actually decreases the macroscopic current response to 300 μM ACh by 10% (data not shown), due to the inhibitory effects of butanol. Because saturating concentrations of the partial agonist Deca (22) open very few channels, it is an ideal agonist to test for drug-induced effects on the equilibrium between open and closed channels.

Fig. 5, A-C, shows ensemble mean current responses of a single patch to 100-msec application of 100 μM Deca, with and without 10 mM butanol, at −100 mV. In Fig. 5A, only Deca is present in the perfusing solution (control). Butanol is then included in Fig. 5B along with Deca. The mean current amplitude during Deca and butanol application is increased by a factor of 2, compared with control. A second control trace (Fig. 5C) is obtained after Fig. 5B, and the current response returns to the level of the first control. For three patches, the mean current amplitude in the presence of 10 mM butanol is 191 ± 16% that of control. A similar experiment was performed with 5 mM ether instead of butanol (Fig. 5, D-F). In sharp contrast to butanol, ether does not significantly change the current response to 100 μM Deca. The results are similar when ether is transiently applied to the patch along with 100 μM Deca; the mean current amplitude in the presence of 5 mM ether is 100 ± 12% ($n = 3$) of control. With 10 mM ether, the current response to 100 μM Deca is smaller (91 ± 1%, $n = 2$) than that of control. We also performed the same sequence of experiments as shown in Fig. 5, A-C, with 200 μM hexanol. The mean current amplitude in the presence of 200 μM hexanol is 103 ± 7% ($n = 3$) of control.

The channel opening rate can be directly measured by rapid perfusion of the patch with a supersaturating concentration of ACh (18). Shown in Fig. 6 are current responses of a single patch to rapid perfusion of 10 mM ACh, in the absence and in the continuous presence of 20 mM butanol, at +50 mV. Because 20 mM butanol inhibits the current response to ACh by about 2-fold (23), the current amplitude in the presence of butanol is scaled to that of the two controls so that the onset time courses can be compared. The two control experiments were performed before and after the butanol experiment and their time courses cannot be distinguished from one another. The onset time of the controls is 40 μsec. The onset time in the presence of butanol is faster (25 μsec). On average, 20 mM butanol decreases the mean onset time from 47 ± 9 μsec ($n = 6$) to 27 ± 14 μsec ($n = 3$) ($p < 0.05$). Butanol at 10 mM decreases the onset time from 53 ± 7 μsec ($n = 7$) to 36 ± 6 μsec ($n = 4$) ($p < 0.01$). We also performed onset experiments in the presence of 3 mM pentanol and 20 mM ether. Pentanol at 3 mM decreases the mean onset time from 57 ± 14 μsec ($n = 6$) to 33 ± 12 μsec ($n = 3$) ($p < 0.05$). In contrast, ether does not change the onset time [control, 54 ± 6 μsec ($n = 7$); ether, 62 ± 12 μsec ($n = 4$) ($p > 0.1$)].

Discussion

Alcohols and volatile anesthetics appear to have both inhibitory and excitatory effects on nicotinic ACh receptor channels. The inhibitory actions of these drugs are seen as a decrease in the single-channel burst duration, a decrease in the apparent single-channel current amplitude, or an increase in the number of openings per burst. For both butanol and ether, inhibition is

[2] Y. Liu, J. F. Roper, and J. P. Dilger. Slow desensitization of the nicotinic acetylcholine receptor in BC3H-1 cells. Submitted for publication.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012

MOL PHARM

TABLE 1

Comparison of transient and equilibrium exposure to agonists in determining the increase in burst frequency produced by ether and butanol

Effects of 10 mM butanol and 15 mM ether on frequency of bursts were determined using two methods, transient and equilibrium exposure to agonists. The numbers refer to ratios of the burst frequency obtained in the presence of drug to that obtained in the absence of drug for the same patch. Values are given in the form of mean ± standard deviation. Numbers in parentheses are the numbers of patches studied. Membrane potential was −100 mV.

	Burst frequency with drug/frequency without drug			
	15 mM Ether		10 mM Butanol	
	Transient	Equilibrium	Transient	Equilibrium
ACh (0.2 μM)	2.18 ± 0.51 (2)	2.25 ± 0.44 (2)	1.98 ± 0.53 (5)	1.63 ± 0.47 (6)
Deca (1 μM)			2.53 ± 0.24 (2)	2.67 ± 0.36 (3)

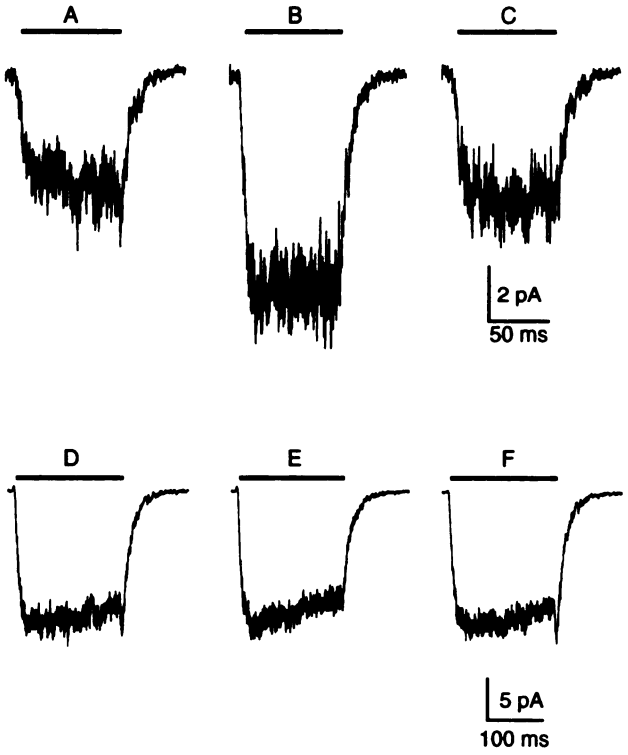


Fig. 5. A-C, Ensemble mean current responses of a single patch to rapid perfusion of 100 μM Deca with (B) and without (A and C) 10 mM butanol. The duration of Deca/butanol application was 100 msec (*solid bars*). D-F, Ensemble mean current responses of a single patch to rapid perfusion of 100 μM Deca with (E) and without (D and F) 5 mM ether. For the trace in E, ether was present before, during, and after agonist application. The duration of Deca application was 170 msec.

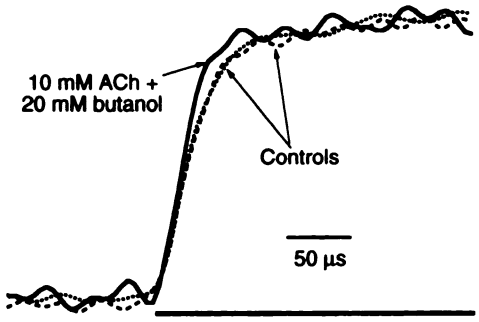
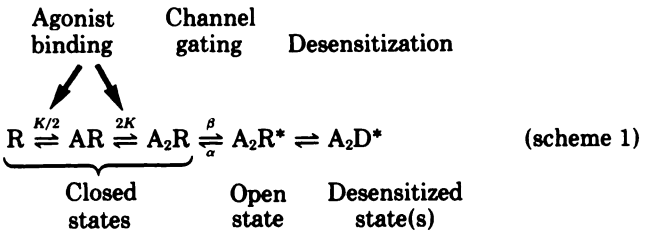


Fig. 6. Onset of current responses of a single patch to rapid application of 10 mM ACh in the absence and during the continuous presence of 20 mM butanol. Control experiments were performed before and after the butanol experiment and are indistinguishable from each other. The *butanol trace* is scaled by a factor of 2.6 so that the maximum current level matches that of the controls. *Solid bar*, time of ACh application. Membrane potential was +50 mV.



seen as a decrease in the apparent single-channel current amplitude. The inhibitory actions are well described by a model in which drug molecules bind to the channel protein and block the flow of ions through the channel (12, 13, 15).

The excitatory actions of alcohols and volatile anesthetics are not predicted by the channel-blocking model described above. They apparently arise from an independent effect of the drug on the channel. There are at least three ways in which excitatory effects of a drug on a ligand-gated ion channel can be understood, as (a) a decrease in the degree of desensitization induced by equilibrium exposure to agonist, (b) an increase in the affinity of agonist binding to the receptor, and (c) an increase in the maximum channel open probability (efficacy) for agonist caused by a change in channel gating (increased opening rate β). These mechanisms are illustrated in scheme 1.

Activation of the ACh receptor (24) is characterized by the sequential binding of two agonist molecules (A) to sites on the receptor (R), followed by the channel gating step from closed to open ($A_2R \rightarrow A_2R^*$). In this scheme, the two binding sites are assumed to have the same affinity, K, for agonist. Continuous exposure to agonist drives the receptor to a set of desensitized states (25); for simplicity, scheme 1 represents these states as a single state (A_2D^*) coming from the open state.

In this paper, we use different experimental protocols to discriminate among these alternative mechanisms. We compare the effects of the drugs under conditions of equilibrium and transient applications of agonist to test for effects on desensitization. We use a partial agonist to separate effects on agonist binding from effects on channel gating. And we use a direct method for determining the channel opening rate.

When a patch is continuously exposed to low concentrations of an agonist, a substantial fraction of the channels in the patch are desensitized (26–28).[2] If general anesthetics were to decrease the affinity of agonists for desensitized states, then channels would spend less time in desensitized states, resulting in an increase in burst frequency. To test this possibility, we performed experiments in which 0.2 μM ACh (or 1 μM Deca) was applied transiently, to avoid desensitization. The effects of

MOLECULAR PHARMACOLOGY

aspet

Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012

butanol and ether on burst frequency during transient application of agonist are similar to those observed using equilibrium application of agonist (Table 1). Thus, butanol and ether, at least at the concentrations used here, do not significantly alter the equilibrium between desensitized and nondesensitized channels. Other studies show that alcohols and some volatile anesthetics actually promote desensitization (15, 19). However, the concentrations required to produce these effects are generally higher than those used in the present study. For example, 20 mM butanol has only a small effect on steady state desensitization (19).

Under conditions of minimal desensitization and channel block by agonist, the channel open probability, p, predicted by scheme 1 is

$$p = \frac{c^2\beta/\alpha}{K^2 + 2Kc + c^2(1 + \beta/\alpha)}$$

where c is the agonist concentration, β is the channel opening rate, and α is the channel closing rate. For $\beta/\alpha \ll 1$ (partial agonism), p is approximately equal to β/α.

Deca is a partial agonist at the nicotinic ACh receptor in BC3H-1 cells (22). With 100 μM Deca, about 80% of the receptors are fully bound but only about 1% of the channels are open. If the only effect of butanol were to increase the binding affinity of the receptor for Deca, then butanol would increase the current response to 100 μM Deca by no more than 20%. We found that 10 mM butanol increases the current response by 1.9-fold. Therefore, butanol must increase β/α for Deca. Because 10 mM butanol decreases the single-channel current by ~10%, the actual increase in β/α is $1.9 \div 0.9 = 2.1$ times. If we assume that this increase in β/α is entirely due to an increase in the channel opening rate, we would expect 10 mM butanol to increase burst frequency by 2.1-fold. This is only slightly smaller than the observed increase in burst frequency of 2.5–2.7-fold (Table 1). Unfortunately, the mean open duration in the presence of butanol does not quantitatively reflect the closing rate of the channel because of unresolved brief closures. However, the observed decrease in open duration (Fig. 3) suggests that the channel closing rate increases in the presence of 10 mM butanol. If this is true, then the butanol-induced increase in β/α reflects an even larger increase in β. This could account for the entire increase in burst frequency produced by butanol.

Neither 5 nor 10 mM ether increased the current response to 100 μM Deca. It might be argued that ether, like butanol, increases the efficacy of Deca but this increase is obscured by the inhibitory effect of ether. The equilibrium constant for inhibition by ether is 43 mM.[3] If we "correct" our data for the inhibitory effects of ether, we find that, with 5 mM and 10 mM ether, the responses would both have been ~110% of control. If the increase in the channel opening rate were responsible for this 10% increase, the burst frequency would increase by only 10%, far less than the observed increase in frequency of bursts produced by ether (Fig. 4). On the other hand, if ether decreased the dissociation constant of Deca from 12.5 μM (22) to 7.5 μM, the frequency of bursts at 1 μM Deca would be 2.5 times that of control and the current response to 100 μM Deca would increase by 9%. Although we have not measured agonist binding directly, the conclusion that ether increases the burst frequency by increasing agonist affinity is entirely consistent with our data.

Hexanol at 200 μM did not significantly increase the current response to 100 μM Deca. However, this concentration of hexanol inhibits at least 30% of the channels (23). If this inhibition is "corrected," we find that 200 μM hexanol would have increased the current response to 100 μM Deca by about 50%. This is more than what can be accounted for by an increase in the Deca binding affinity alone (<20%). We conclude that the primary excitatory effect of hexanol, like butanol, is to increase the channel opening rate. In fact, if the opening rate with Deca is increased by 50% by hexanol, the burst frequency at 1 μM Deca would be increased by 44%. This is similar to the observed increase in burst frequency produced by 200 μM hexanol with 0.2 μM ACh as the agonist (Fig. 4).

Our conclusion that butanol acts by increasing the channel opening rate is strengthened by the effects of butanol on the onset of the current response to rapid perfusion of 10 mM ACh. To a first approximation, the time constant of the onset of current elicited by 10 mM ACh is equal to the reciprocal of the channel opening rate (18). Butanol and pentanol both increase the onset of current response to 10 mM ACh. We conclude that the channel opening rate is increased in the presence of butanol or pentanol. In contrast, ether does not significantly change the onset time. Therefore, it has no significant effect on the channel opening rate.

The increase in the opening rate of the ACh receptor channel observed for ethanol (9), butanol, and pentanol may be a common property of all n-alcohols. However, such effects may be obscured for the longer-chain alcohols. The alcohol concentrations required to induce enhancement and to cause inhibition may be less well separated with increasing alcohol chain length. This makes observations such as those seen in Fig. 5, A-C, increasingly difficult for longer-chain alcohols. Moreover, longer-chain alcohols inhibit the open and closed channels with different affinities (e.g., onset of current response induced by 10 mM ACh during continuous exposure to hexanol or longer-chain alcohols is accompanied by a current decay immediately following the rise phase) (23). This prevents us from interpreting the onset results in any simple way, even though the onset times are indeed faster in the presence of these alcohols.[4]

The kinetic model (12, 13, 15) proposed to interpret inhibition of ACh receptors by alcohols and volatile anesthetics cannot explain the excitatory effects of these compounds. It is therefore necessary to postulate that the excitatory effects of these drugs involve a binding site distinct from the site(s) of their inhibitory actions. This has also been suggested by others to explain the dual effects of alcohols (2, 7, 8). Alcohols and volatile anesthetics have distinct excitatory actions. Although both classes of drugs increase the frequency of single-channel bursts, ethanol, butanol, and pentanol do so by increasing the opening rate of the gate of the channel, whereas ether and isoflurane do so by increasing the affinity of the agonist binding site.

Acknowledgments

We thank Ms. Claire Mettewie for culturing cells.

[3] J. P. Dilger, 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. Submitted for publication.

[4] Unpublished observations.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012

MOL PHARM

MOLECULAR PHARMACOLOGY

References

1. Gage, P. W., R. N. McBurney, and G. T. Schneider. Effects of some aliphatic alcohols on the conductance change caused by a quantum of acetylcholine at the toad endplate. *J. Physiol. (Lond.)* **244**:409–429 (1975).
2. Pennefather, P., and D. M. J. Quastel. Actions of anesthetics on the function of nicotinic acetylcholine receptors, in *Molecular Mechanisms of Anesthesia* (B. R. Fink, ed.), Vol. 2. Plenum Press, New York, 45–58 (1980).
3. Linder, T. M., P. Pennefather, and D. M. J. Quastel. The time course of miniature endplate currents and its modification by receptor blockade and ethanol. *J. Gen. Physiol.* **83**:435–468 (1984).
4. Bradley, R. J., K. Peper, and R. Sterz. Postsynaptic effects of ethanol at the frog neuromuscular junction. *Nature (Lond.)* **284**:60–62 (1980).
5. Bradley, R. J., R. Sterz, and K. Peper. The effects of alcohols and diols at the nicotinic acetylcholine receptor of the neuromuscular junction. *Brain Res.* **295**:101–112 (1984).
6. Forman, S. A., D. L. Righi, and K. W. Miller. Ethanol increases agonist affinity for nicotinic receptors from *Torpedo*. *Biochim. Biophys. Acta* **987**:95–103 (1989).
7. Wood, S. C., S. A. Forman, and K. W. Miller. Short chain and long chain alkanols have different sites of action on nicotinic acetylcholine receptor channels from *Torpedo*. *Mol. Pharmacol.* **39**:332–338 (1991).
8. Alifimoff, J. K., B. Bugge, S. A. Forman, and K. W. Miller. Stereoselectivity of channel inhibition by secondary alkanol enantiomers at nicotinic acetylcholine receptors. *Anesthesiology* **79**:122–128 (1993).
9. Dilger, J. P., Y. Liu, J. F. Roper, and R. J. Bradley. Effects of ethanol on ACh receptor channels. *Biophys. J.* **66**:A9 (1994).
10. Gage, P. W., and O. P. Hamill. Effects of anesthetics on ion channels in synapses. *Int. Rev. Physiol.* **25**:1–45 (1981).
11. McLarnon, J. G., P. Pennefather, and 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).
12. 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. (Lond.)* **437**:431–448 (1991).
13. 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).
14. 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).
15. 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).
16. Sine, S. M., and J. H. Steinbach. Activation of a nicotinic acetylcholine receptor. *Biophys. J.* **45**:175–185 (1984).
17. 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).
18. Liu, Y., and J. P. Dilger. Opening rate of acetylcholine receptor channels. *Biophys. J.* **60**:424–432 (1991).
19. Miller, K. W., L. L. Firestone, and S. A. Forman. General anesthetic and specific effects of ethanol on acetylcholine receptors. *Ann. N. Y. Acad. Sci.* **492**:71–98 (1987).
20. 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).
21. Auerbach, A. Kinetic behavior of cloned mouse acetylcholine receptors: a semi-autonomous, stepwise model of gating. *Biophys. J.* **62**:72–73 (1992).
22. Liu, Y., and J. P. Dilger. Decamethonium is a partial agonist at the nicotinic acetylcholine receptor. *Synapse* **13**:57–62 (1993).
23. Liu, Y., H. Mody, and J. P. Dilger. Kinetics of the inhibition of ACh receptor channels by volatile anesthetics and alcohols. *Biophys. J.* **64**:A323 (1993).
24. Lingle, C. J., D. Maconochie, and J. H. Steinbach. Activation of skeletal muscle nicotinic acetylcholine receptors. *J. Membr. Biol.* **126**:195–217 (1992).
25. Dilger, J. P., and Y. Liu. Desensitization of acetylcholine receptors in BC3H-1 cells. *Pflügers Arch.* **420**:479–485 (1992).
26. Feltz, A., and A. Trautmann. Desensitization at the frog neuromuscular junction: a biphasic process. *J. Physiol. (Lond.)* **322**:257–272 (1982).
27. Franke, C., H. Hatt, H. Parnas, and J. Dudel. Recovery from the rapid desensitization of nicotinic acetylcholine receptor channels on mouse muscle. *Neurosci. Lett.* **140**:169–172 (1992).
28. Sine, S., and P. Taylor. Local anesthetics and histrionicotoxin are allosteric inhibitors of the acetylcholine receptor. *J. Biol. Chem.* **257**:8106–8114 (1982).

Send reprint requests to: James P. Dilger, Department of Anesthesiology, University at Stony Brook, Stony Brook, NY 11794–8480.

aspet

Downloaded from molpharm.aspetjournals.org at Thammasart University on December 2, 2012