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ACCELERATED COMMUNICATION

A Discrete Site for General Anesthetics on a Postsynaptic Receptor

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SUMMARY

General anesthetics depress central nervous system excitability via a mechanism that probably involves effects on synaptic ion channels, but the fundamental molecular nature of the site where they act is unknown. Although the importance of hydrophobicity for general anesthetic drug potency has long been established, it remains uncertain whether these "nonspecific" drugs act on membrane proteins directly or by modification of the physical properties of the lipid membrane or the lipid-protein interface. We find that specific mutations in the acetylcholine receptor pore-forming M2 domains enhance the sensitivity of the receptor to the general anesthetics isoflurane, hexanol, and octanol, suggesting that these agents act by binding directly to a discrete protein site at or near these residues. The sensitivity of the receptor to block by general anesthetics increases with increased hydrophobicity of these residues, demonstrating that hydrophobic forces dominate the interaction of drugs with their protein site. Furthermore, octanol inhibits both wild-type and mutant nicotinic acetylcholine receptors preferentially after channel opening, which is consistent with a mechanism where drugs bind within the receptor's pore. Similar sites on postsynaptic ion channels in brain may represent general anesthetic targets for modulating consciousness.

General anesthetic drugs have been used clinically for nearly 150 years, yet their mechanism of action remains unknown. Neuronal ion channels are considered likely targets for anesthetics because they underlie excitability in the central nervous system. Postsynaptic ligand-gated ion channels are particularly sensitive at concentrations that anesthetize experimental animals and humans (1).

A fundamental question regarding the molecular mechanism of general anesthesia is whether drug effects on ion channels are mediated indirectly via lipid-protein interactions or via direct drug binding to protein sites. General anesthetics are considered "nonspecific" drugs because they lack the strong molecular structure-function dependence characteristic of most drugs that act by binding to proteins (2). The major determinant of anesthetic potency in animal or human behavioral tests is a simple physical property of the drug: its hydrophobicity as determined by partitioning into a nonpolar bulk phase (3). This fact has engendered a variety of proposed mechanisms whereby anesthetic perturbations of lipid membranes cause depression of neuronal function. On the other hand, some water-soluble proteins unassociated with lipid exhibit similar nonspecific hydrophobic interactions with anesthetics (4, 5), implying that drug binding to sites on ion channel proteins might underlie anesthetic actions.

General anesthetic interactions with peripheral nAChRs have been characterized in far more detail than for other postsynaptic ion channels, but the molecular mechanism in this model system is also unclear (6). At clinically relevant concentrations, anesthetics inhibit agonist-gated cation translocation in nAChRs. The presence of some discrete inhibitory site for anesthetics is suggested by rapid flux studies in nAChR-rich *Torpedo* vesicles showing apparent competition between long-chain alcohols (7). This site has been proposed to be either within the transmembrane pore or at the lipid-protein interface. In support of an anesthetic site within

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine.

the pore are single-channel studies (8) showing that alcohols and inhaled anesthetics produce brief closures within open channel bursts, similar to those produced by charged open channel blockers (9), although these anesthetics do not inhibit the open state exclusively (10). In support of anesthetic action on lipids or at the lipid-protein interface is the finding that changes in membrane sterol content alter general anesthetic inhibition of nAChRs (11).

We tested the hypothesis that general anesthetics act on the nAChRs by binding directly to protein sites within the pore by assessing the ability of mutations in the pore-forming region to change the sensitivity of the receptor to general anesthetics. The pore-forming region of the nAChR protein, and even specific amino acid residues that line the pore, have been identified previously (12–15). The five subunits ($\alpha_2\beta\gamma\delta$) are disposed pseudosymmetrically around the central pore, and each contributes the second of four putative membrane-spanning regions (M2) to the pore lining. The sequences of the various M2 regions are well conserved and in general rather hydrophobic (Table 1), making the pore a potential binding site for general anesthetics. A particular set of nonhydrophobic M2 residues, at the 10′ positions, appears to interact with the hydrophobic end of a charged channel blocker, QX-222 (15). Because hydrophobicity is the major determinant of general anesthetic potency for inhibiting nAChRs (6), we reasoned that if general anesthetics bind in the pore, then replacing the nonhydrophobic M2 residues at the 10′ position with hydrophobic residues might enhance general anesthetic binding.

Sensitivity to the general anesthetics octanol, hexanol, and isoflurane was assessed with voltage-clamped membrane patches from *Xenopus* oocytes that expressed the four subunits of either wild-type or mutated mouse muscle nAChRs. A perfusion system capable of producing rapid (submillisecond) changes in the concentration(s) of agonist and/or anesthetic was used to elicit currents from patches expressing receptors. Both the rate of onset of anesthetic inhibition and the concentration dependence of steady-state channel inhibition were assessed in rapidly perfused patches. To determine whether the open state was preferentially inhibited and whether mutant channels were blocked by the same mechanism, we also tested the state dependence of octanol inhibition in both wild-type and mutant channels.

The results show that changing the hydrophobicity of a specific region within the nAChR pore causes a dramatic change in sensitivity to general anesthetics. Both wild-type and mutated nAChR channels are blocked by octanol preferentially in the open state. These results strongly support the hypothesis that these drugs affect nAChRs directly by acting at a protein site within the pore.

Materials and Methods

Site-directed mutagenesis. cDNAs for α, β, γ, and δ subunits of the mouse muscle nAChR were provided by Dr. Jim Boulter (Salk Institute, La Jolla, CA) in pSP64 plasmids. Oligonucleotide-directed mutations were made in subunit cDNAs in pBluescript (Stratagene, La Jolla, CA) (16) and confirmed by dideoxynucleotide sequencing. For α subunits, 800-base pair *Bsu*36I/*Bgl*II cDNA fragments containing mutated M2 coding sequences were subcloned into a pSP64 plasmid containing *Xenopus* globin noncoding sequences (pSP64T, Ref. 17) to enhance translation. The βT10′I mutant cDNA was provided by Dr. Cesar Labarca (California Institute of Technology, Pasadena, CA) in pGEM2-SP6.

Xenopus oocyte expression. Detailed methods for oocyte expression were described previously (16). Messenger RNAs were transcribed *in vitro* with SP6 RNA polymerase (Promega, Madison, WI) from linearized cDNAs. Subunit mRNAs were isolated with affinity beads (RNAid, BIO-101, Vista, CA), mixed stoichiometrically at 2 α:β:γ:δ, and microinjected into oocytes (25–50 nl). After incubation for 48–96 hr, oocytes were stripped of their vitelline membranes and used for electrophysiology.

Patch-clamp electrophysiology. Patch pipettes were polished to give open tip resistance of 2–5 MΩ. Oocyte membrane patches were pulled in the outside-out configuration and held at −50 mV. Inside and outside buffers were symmetrical K-100 (97 mM KCl, 1 mM $MgCl_2$, 0.2 mM EGTA, 5 mM K-HEPES, pH 7.5). Currents through the patch-clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, CA) were filtered (eight-pole bessel, 5 kHz) and digitized at 5–10 kHz with a 486-class personal computer, a 12-bit analog/digital converter (National Instruments, Austin, TX), and custom software.

Rapid perfusion. Rapid perfusion was achieved according to the method of Liu and Dilger (18). Patches were continuously perfused with K-100 buffer (with or without anesthetic) until a computer-controlled solenoid pinch valve switched the perfusate to K-100 containing ACh with or without anesthetic. High concentrations of ACh (100 μM) were used to avoid interference from changes in apparent ACh agonist site affinity (K_{ACh}) induced by anesthetics (19) or by pore mutations (12, 20). ACh exposure periods were 50–350 msec, and patches were "recovered" in control perfusate for 10 sec between ACh exposures. Solution exchange times (0.2–0.9 msec) were measured by recording junction currents at open-patch pipette tips while switching the ionic strength of the perfusion solutions.

Data Analysis. Current traces displayed in figures are averages of 8 or 16 traces from a single patch aligned at the midpoints of the rapid current rise (channel opening). Control currents (ACh alone) were checked before and after experiments where patches were exposed to anesthetics. Data were not analyzed if peak control currents differed by >10%. Nonlinear least-squares analysis was performed with Origin (Microcal, Northampton, MA) software on a 486-class personal computer with Microsoft Windows.

Anesthetics. Octanol and hexanol (puriss. grade; Fluka Chemical) were weighed directly into K-100 buffer to make stock solutions that were diluted to the final experimental concentrations. Isoflu-

TABLE 1
Amino acid sequences of mouse muscle M2 domains

		1′	2′	3′	4′	5′	6′	7′	8′	9′	10′	11′	12′	13′	14′	15′	16′	17′	18′	19′	20′
α	K242	M	T	L	S	I	S	V	L	L	S	L	T	V	F	L	L	V	I	V	E
β	K253	M	G	L	S	I	F	A	L	L	T	L	T	V	F	L	L	L	L	A	D
γ	K251	C	T	V	A	T	N	V	L	L	A	Q	T	V	F	L	F	L	V	A	K
δ	K256	T	S	V	A	I	S	V	L	L	A	Q	S	V	F	L	L	L	I	S	K

Sequence alignment and terminology follows that of Ref. 28. The first residue is numbered from the amino terminus, which is supposed to be at the cytoplasmic end of the pore. Single-letter amino acid code: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

rane was delivered in air via a calibrated vaporizer and was equilibrated by bubbling through the K-100 perfusate for a minimum of 20 min. This solution was perfused continuously to minimize evaporative and absorptive losses.

Results

Kinetics of octanol inhibition in wild-type receptors. Fig. 1 demonstrates the kinetics of octanol inhibition in wild-type nAChRs. When perfused with ACh alone, patches containing wild-type nAChRs (Fig. 1A, *control*) show an inward current that peaks rapidly due to channel opening and then declines with the time course of desensitization, which on this time scale appears as a single exponential with a time constant of 60 ± 8 msec. During simultaneous rapid perfusion with ACh and octanol, the current decays in two phases (Fig. 1A, 5–80 μM). The slower decay phase corresponds to the rate of receptor desensitization seen in the control experiment with ACh alone, whereas the new faster phase represents the onset of octanol inhibition. Both the rate and extent of this faster phase increase with octanol concentration. The rate of fast current decay increases linearly with octanol concentration, giving an apparent association rate of nearly 10^7 M^{-1}·sec^{-1} (Fig. 1B). The extent of inhibition increases as a single Langmuir isotherm with a $K_{0.5}$ of 23 ± 3 μM (Fig. 1C). These results are consistent with a simple bimolecular reaction mechanism where octanol binding is the rate-limiting step in inhibition.

Mutations in the M2 domains alter the sensitivity of receptors to anesthetics. Mutating the α subunit 10′ serine to the hydrophobic amino acid isoleucine (αS10′I) dramatically increases the nAChR sensitivity to both octanol and a clinically used inhaled anesthetic, isoflurane (Fig. 2). Fig. 2 (*top*) shows the response of the wild-type and the αS10′I mutant channels to 20 μM octanol, a concentration near the $K_{0.5}$ for the wild-type. Although 20 μM octanol produces a 48% steady-state inhibition of wild-type channels, this same octanol concentration inhibits 82% of current through αS10′I mutant channels. The αS10′I mutation also

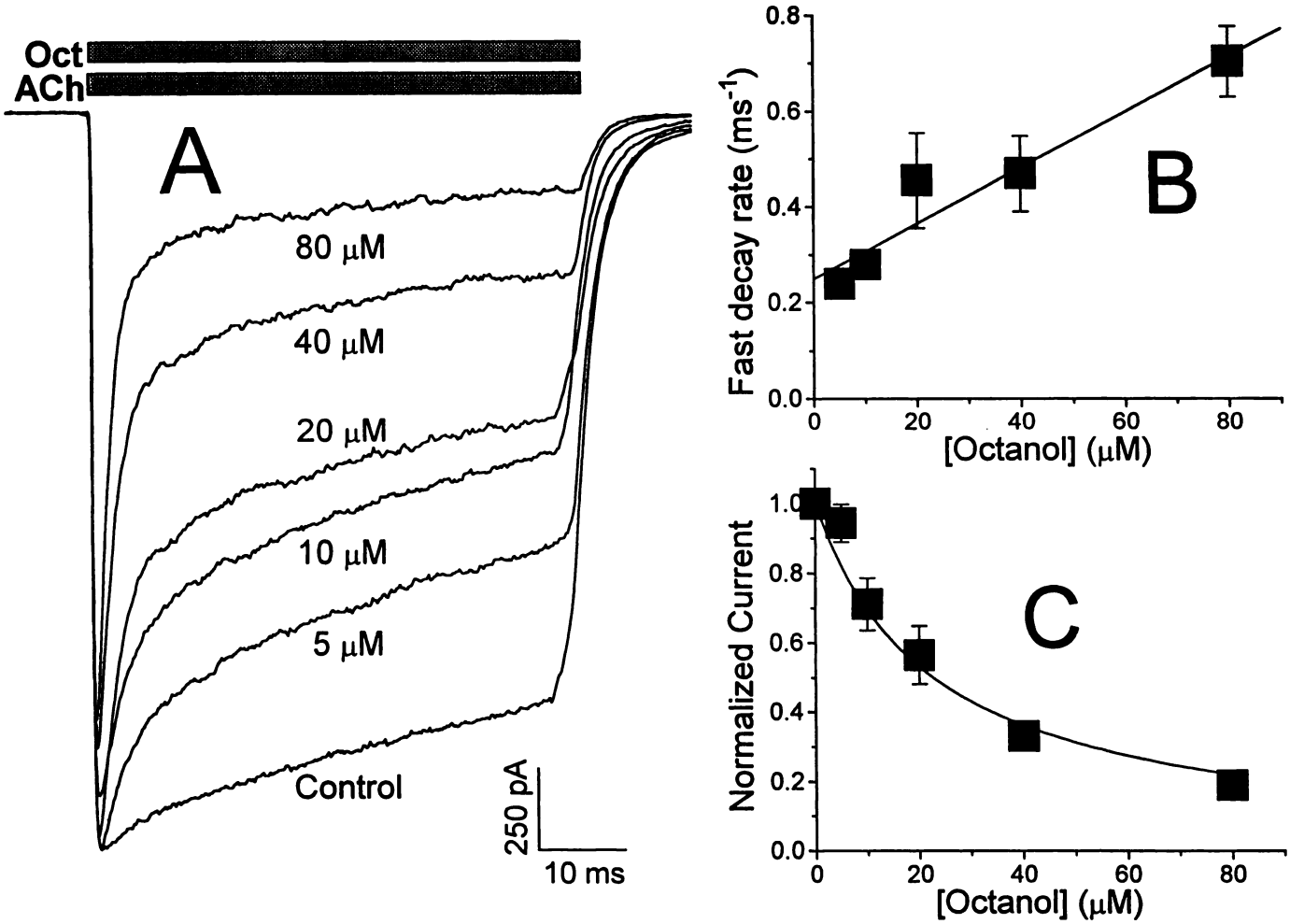

Fig. 1. Kinetics and concentration dependence of octanol inhibition. A, Current traces from a single oocyte patch expressing wild-type nAChRs exposed to 100 μM ACh alone (control) or 100 μM ACh mixed with octanol at the concentrations indicated. B, (rate of onset) Single or double exponential decay functions [$I = I_{final} + \Sigma I_i \times (\exp -(t/\tau_i))$] were fitted to averaged current decay traces from A by nonlinear least-squares analysis. Results of fitted fast decay rates ($1/\tau_1$) were combined with data from other patches (at least three patches per point) and plotted (mean ± standard error) against [octanol]. Linear least-squares analysis gives intercept of 243 ± 40/sec and slope of (5.7 ± 0.1) × 10^6 M^{-1}·sec^{-1}. C (steady state inhibition), Fraction of current remaining after octanol inhibition was estimated by subtracting out the rapid decay and normalizing to peak control currents [$(I_{oct}-I_{1,\,oct})/I_{ctrl}$]. Data from at least three patches per point were combined (mean ± standard error) and plotted against [octanol]. A logistic function, $F = 1.0 - ([octanol]^N/([octanol]^N + K_{oct}^N))$ was fitted to the data by nonlinear least-squares analysis. Fitted parameters are K_{oct} = 24.5 ± 1.7 μM and n = 1.3 ± 0.2. A fit with the N constrained at 1.0 (Langmuir isotherm) gives K_{oct} = 23 ± 3.

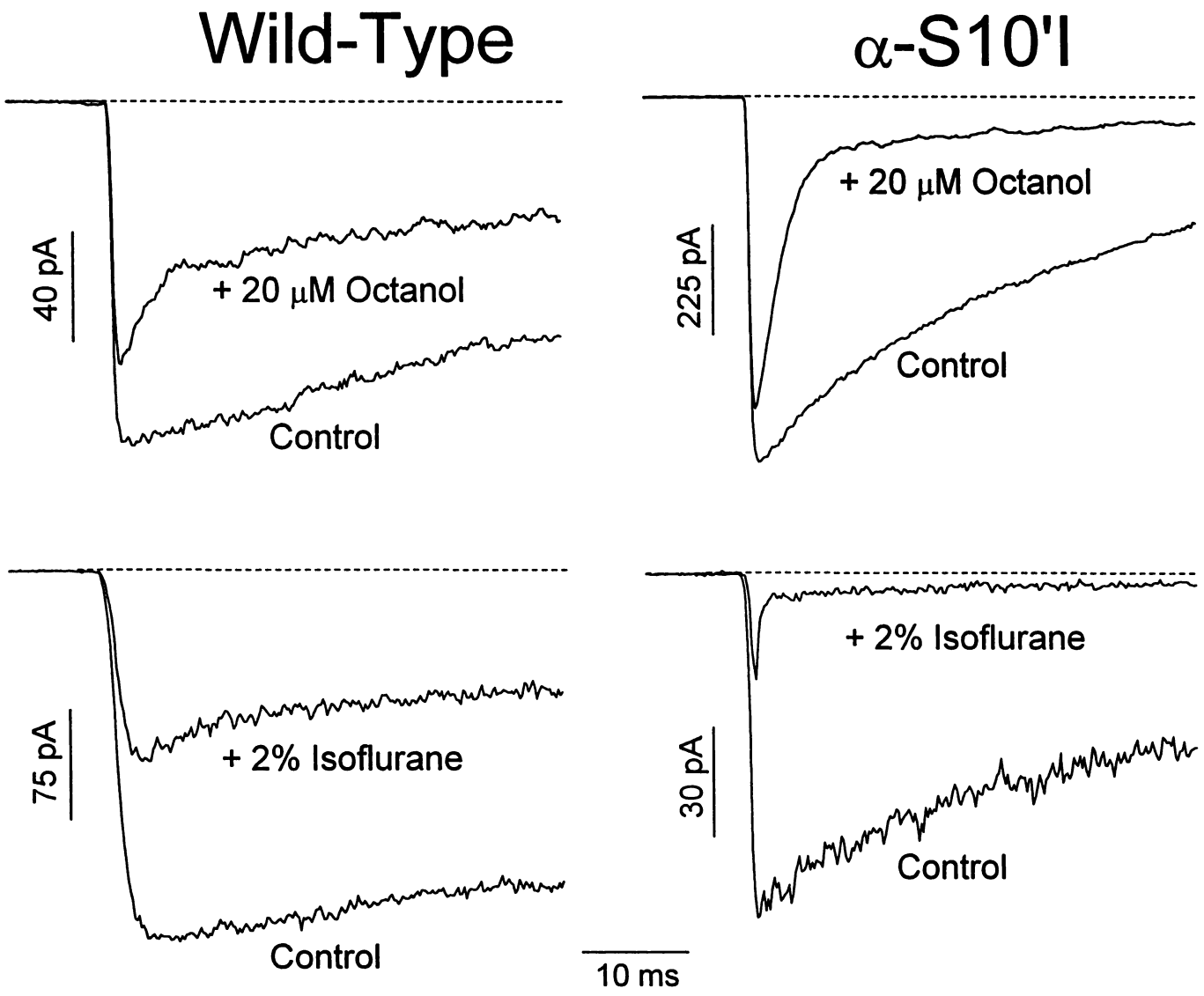

Fig. 2. Mutating a serine to isoleucine in the α subunit M2 domain enhances channel sensitivity to block by general anesthetics. Each panel displays both a control (100 μM ACh) average current trace (eight experiments) and an anesthetic-inhibited average current trace elicited by simultaneous perfusion with 100 μM ACh and anesthetic. *Left*, in wild-type patches, 20 μM octanol blocks 48%, and at 2% vapor pressure (1 mM aqueous based on a water/gas partition coefficient of 1.08 at 25°) isoflurane blocks 60% of control current. *Right*, in αS10′I mutant patches, 20 μM octanol blocks 82% and 1 mM isoflurane blocks 93% of control current. All washout control traces (not shown) had peak currents within 5% of initial controls.

enhances receptor sensitivity to isoflurane, a clinically used inhaled anesthetic (Fig. 2, *bottom*). A concentration that blocks 58% of the wild-type channels inhibits 93% of the mutant channels. Thus, the αS10′I mutation in the nAChR pore produces a ~5-fold increase in sensitivity to octanol and a 9-fold increase in sensitivity to isoflurane.

Octanol preferentially inhibits the open channel of wild-type and mutant receptors. Octanol blockade of the αS10′I mutant has a mechanism essentially like that of the wild-type channel: in both, octanol inhibits preferentially the open state. Fig. 3 represents a comparison of the effect of simultaneous presentation of octanol and ACh with the effect of octanol preincubation. When octanol and ACh are presented simultaneously (Fig. 3, *middle*), the decline in current represents the onset of inhibition for channels that are mostly open. This is apparent as the fast phase of decline in the current; note the small reduction in peak currents attributable to the rapid onset of inhibition. When octanol is presented well before ACh (1–6 minutes' exposure), there is some octanol inhibition of closed channels (8). This inhibition of closed channels causes further reduction in the peak currents (Fig. 3, *bottom*), but there is still substantial rapid current decay after ACh is presented. Thus, for octanol, the equilibrium inhibition of open channels must be greater than that for closed channels. For wild-type channels equilibrated with 20 μM octanol, there is 12% inhibition at the peak and 40% inhibition at steady state: this corresponds to a 5-fold increase in affinity on opening. For the mutant channels, the difference is larger and better resolved (because of the slower onset of inhibition): inhibition increases from 29% at the current peak to 85% at steady state, corresponding to a 14-fold increase in affinity. The preferential binding to the open state (although not absolute) suggests that the anesthetic may act within the gated pore of the channel; this is

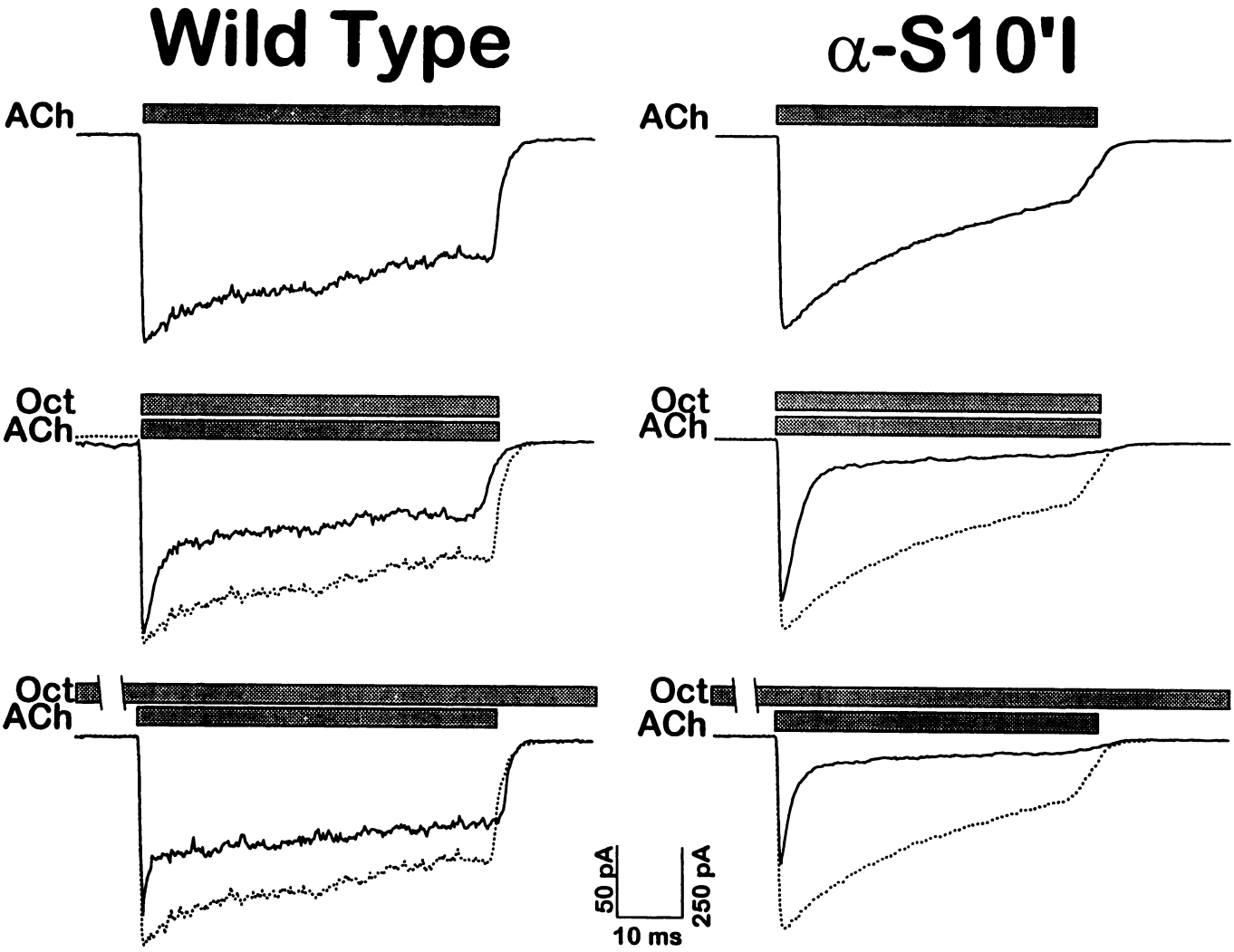

Fig. 3. Octanol preferentially inhibits both wild-type and mutant nAChRs after ACh binds. A single oocyte patch expressing wild-type receptors (*left*) and another expressing αS10′I mutant receptors (*right*) were exposed to different octanol and ACh rapid perfusion protocols. *Shaded bars*, perfusion periods for ACh and octanol. Single or double exponential decay functions [$I = I_{final} + \Sigma I_i \times (\exp -(t/\tau_i))$] were fitted to averaged current decay traces by nonlinear least-squares analysis. Parameters are reported as mean ± standard error. *Top*, control responses to rapid perfusion with 100 μM ACh alone demonstrate rapid current rise and monoexponential decay (desensitization); *wild-type*: $I = -98 \pm 8$ pA, $\tau = 60 \pm 8$ msec, $I_{final} = -44 \pm 8$ pA. *αS10′I*: $I = -574 \pm 17$ pA, $\tau = 25 \pm 2$ msec, $I_{final} = -144 \pm 10$ pA. *Middle*, Responses to simultaneous perfusion with 20 μM octanol + 100 μM ACh show two phases of current decay, octanol block followed by desensitization; *wild-type*: $I_1 = -61 \pm 2$ pA, $\tau_1 = 2.4 \pm 0.7$ msec, $I_2 = -57 \pm 12$ pA, $\tau_2 = 70 \pm 18$ msec, $I_{final} = -22 \pm 5$ pA. *αS10′I*: $I_1 = -566 \pm 14$ pA, $\tau_1 = 2.8 \pm 0.3$ msec, $I_2 = -80 \pm 11$ pA, $\tau_2 = 50 \pm 16$ msec, $I_{final} = -10 \pm 5$ pA. *Bottom*, responses to simultaneous perfusion with 20 μM octanol + 100 μM ACh during continuous perfusion with 20 μM octanol (initiated from 1–6 min before ACh application) demonstrate that octanol preferentially blocks after ACh perfusion begins; *wild-type*: $I_1 = -35 \pm 3$ pA, $\tau_1 = 0.7 \pm 0.1$ msec, $I_2 = -61 \pm 18$ pA, $\tau_2 = 75 \pm 23$ msec, $I_{final} = -24 \pm 5$ pA. *αS10′I*: $I_1 = -400 \pm 5$ pA, $\tau_1 = 1.7 \pm 0.1$ msec, $I_2 = -95 \pm 4$ pA, $\tau_2 = 39 \pm 4$ msec, $I_{final} = -18 \pm 5$ pA.

the main evidence that other, charged blockers of the channel act in the pore (9, 10, 21).

Sensitivity to anesthetics correlates with the hydrophobicity of M2 10′ residues. We further tested the involvement of the α10′ position by varying the hydrophobicity of the substituted amino acid. Steady state inhibition by hexanol, which blocks faster than octanol, was measured in wild-type receptors and mutants. As amino acids of increasing hydrophobicity are substituted for serine at α10′, the sensitivity to hexanol increases. For valine, isoleucine, and phenylalanine substitutions, the half-blocking hexanol concentration, K_{hex}, was reduced by factors of approximately 2, 4, and 8, respectively, compared with the wild-type (Figs. 4 and 5). This clear correlation of side-chain hydrophobicity with hexanol sensitivity is a further argument that the mutations have directly affected the binding site for general anesthetics.

To test whether subunits other than the α subunit contribute to the general anesthetic site, a mutation homologous to αS10′I was made on the β subunit. Coexpression of βT10′I with αS10′I results in a further 2-fold increase in sensitivity to hexanol compared with αS10′I alone (10-fold total change from wild-type), demonstrating that at least three of the five M2 domains contribute to general anesthetic binding (Figs. 4 and 5). The additive involvement of multiple subunits is similar to the results for the local anesthetic QX-222 (15).

Charged channel blockers are affected by mutations at the 6′ position of M2 (15), but we find that general anesthetics interact far more weakly here than with the 10′ position. Substituting phenylalanine for serine at the 6′ position (αS6′F) produces

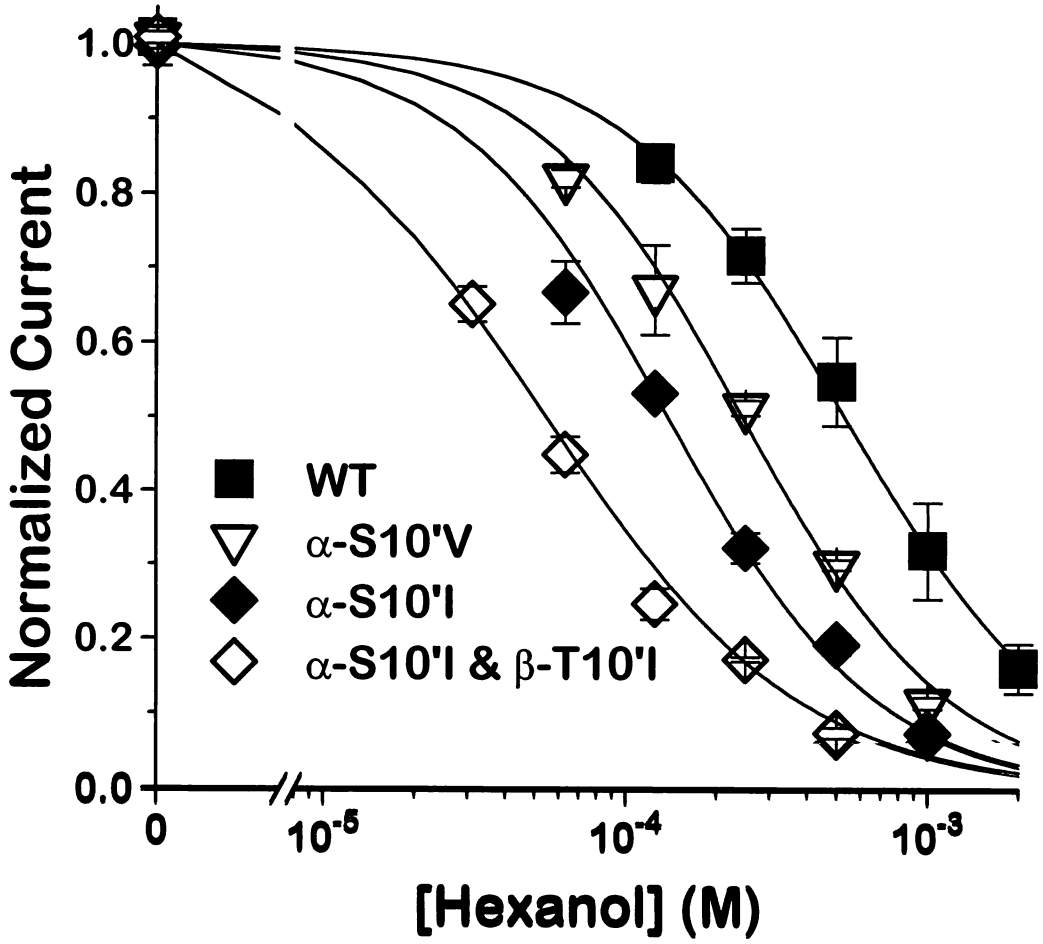

Fig. 4. Channel sensitivity to general anesthetics increases as the hydrophobicity of 10′ residues increases. Hexanol concentration-responses for wild-type (*WT*) and three mutant subunit mixtures are shown. Equilibrium currents in the presence of 100 μM ACh and hexanol were normalized to current elicited by ACh alone, as described in Fig. 1. Data from at least three patches at each hexanol concentration were averaged (mean ± standard error) and plotted against [hexanol]. A logistic function (Fig. 1 legend) was fitted to data points for each receptor type. Fitted K_{hex} values are as follows: *WT*, 530 ± 30 μM; *αS10′V*, 240 ± 20 μM; *αS10′I*, 140 ± 10 μM; *αS10′F*, 66 ± 10 μM (data not shown); *αS10′I/βT10′I*, 55 ± 6 μM. Fitted Hill coefficients were in the range of 1.0–1.3, with errors of 0.1–0.2.

only a 1.6-fold decrease in K_{hex} (300 ± 30 μM), compared with an 8-fold decrease for the same substitution at the 10′ position (αS10′F; K_{hex} = 66 ± 10 μM). This small effect is seen even though the αS6′F mutation has a larger effect on the apparent affinity of the receptor for ACh activation than the αS10′F mutation (10-fold versus 3-fold decrease in K_{ACh}).

Discussion

By combining site-directed mutagenesis with rapid-perfusion patch-clamp electrophysiology, we tested the hypothesis that general anesthetics inhibit nAChRs by binding to a site within the receptor's pore. We find that mutations altering the hydrophobicity of a site in the pore-forming region dramatically affect the sensitivity of channels to block by anesthetics. Furthermore, both wild-type and mutant channels are preferentially blocked in the open state by octanol.

The most important finding of the present study was that the sensitivity of nAChRs to general anesthetic inhibition is determined by specific amino acid side chains, supporting a mechanism where these nonpolar drugs bind directly to a protein site. Direct binding to a protein site therefore appears to be compatible with the relative absence of structural specificity of general anesthetic action on nAChRs (22). Similar "nonspecific" pharmacology is characteristic of general anesthetic action in experimental animals and humans (2, 3, 23). This observation has been used to argue for either a membrane lipid site or a global perturbation of protein structure, but our findings suggest that it is also consistent with a discrete hydrophobic protein binding site, as shown previously for firefly luciferase (4). Our conclusion is also consistent with studies showing modest stereoselectivity of anesthetic effects on some animals and synaptic receptors (1, 24).

Directed mutagenesis by itself cannot conclusively demonstrate that general anesthetics act by binding within the pore. The anesthetic molecule could bind elsewhere, and its actions could be affected by our mutations through a remote allosteric change in the protein structure. The simplest class of allosteric models, in which the change in anesthetic sensitivity is secondary to a change in the gating of the receptor channel, is effectively ruled out by data showing that for 10′ mutations, changes in gating (K_{ACh} and τ_{desens}) caused by our mutations are only weakly correlated with and much smaller than the changes in anesthetic sensitivity (Fig. 5). Also, mutations at the 10′ position in the pore alter anesthetic sensitivity in parallel with the hydrophobicity of the side chain (Fig. 5). This correlation is predicted for a direct inter-

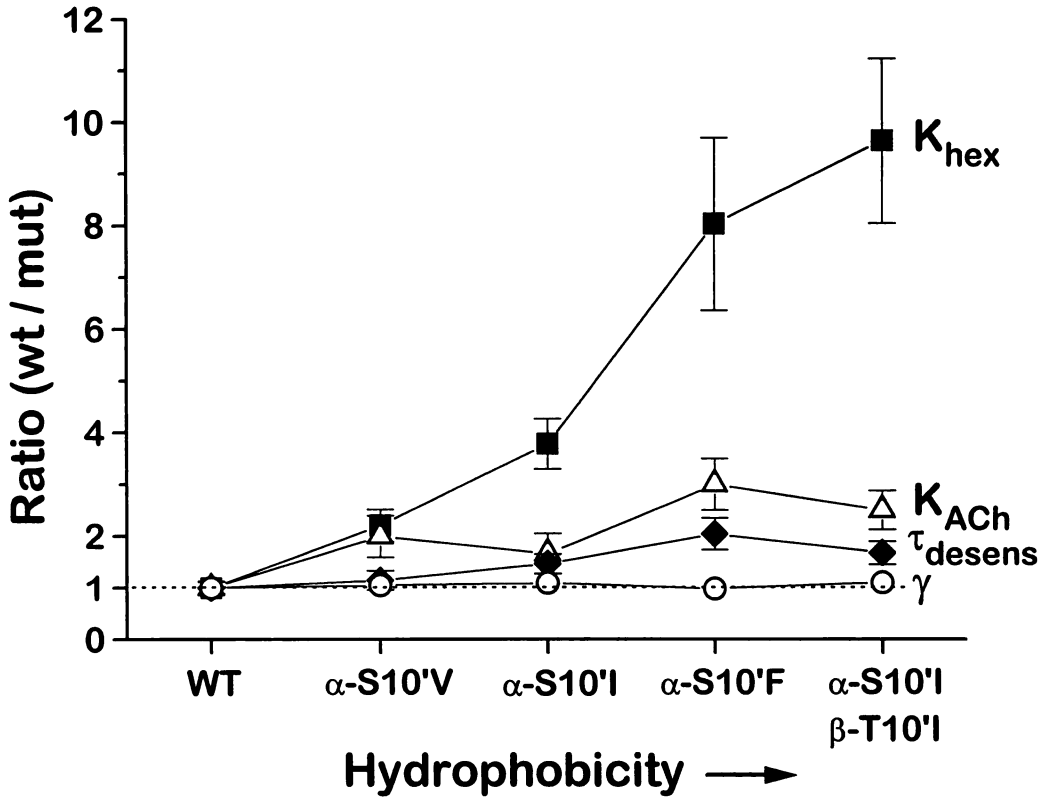

Fig. 5. Gating and desensitization parameters are relatively unaffected by hydrophobic mutations at 10′ residues. The wild-type/mutant ratios of parameters reflecting hexanol sensitivity (K_{hex} ± standard deviation), apparent ACh sensitivity (K_{ACh} ± standard deviation), desensitization kinetics (τ_{desens} ± standard deviation), and single-channel conductance (γ ± standard deviation) are plotted on the ordinate axis against an abscissa that reflects overall side-chain hydrophobicity at the 10′ positions of M2 domains. Apparent hexanol dissociation constants (K_{hex}) are given in Fig. 4 legend. K_{ACh} values for each channel type (wild-type value, 30 ± 2 μM) were determined by measuring average peak current responses (eight events) in outside-out patches (V_m = −50 mV) during rapid perfusion with varying ACh concentrations (range, 1 μM to 1 mM). Responses were normalized to average control currents measured with saturating ACh concentrations (0.1–1 mM) in the same patch. A logistic function, $I/I_{cont} = [ACh]^N/([ACh]^N + K_{ACh}^N)$ was fitted to data points from at least three patches at each concentration. Single-channel conductances for each channel type (γ; wild-type value, 48.6 ± 0.5 pS) were measured in excised inside-out patches held at −50 mV. The concentration of ACh in the patch pipette was 200 nM to 1 μM. Fifty single-channel conductances from at least three patches were averaged. Desensitization time constants for each channel type (τ_{desens}; wild-type value, 47 ± 3 msec) were determined by recording averaged (eight events) currents from outside-out membrane patches during rapid perfusion with 100 μM ACh. Perfusion periods were lengthened for slowly desensitizing receptors (e.g., wild-type). Single exponential functions were fitted to the current decay phases. Between 5 and 15 fitted decay constants were averaged.

action between protein side chains and general anesthetics but not for a remote interaction. Finally, the kinetics of octanol inhibition argue for a direct bimolecular reaction resulting in inhibition, with a strong preference for the open state. Thus, the simplest hypothesis consistent with our data is that anesthetics bind directly to the pore.

The similarity of inhibitory mechanisms for the wild-type and mutant channels makes it very unlikely that our mutations have produced a new highly sensitive site for octanol; it is more likely that the mutations increase the affinity of the existing site for anesthetics. The exact location and extent of the general anesthetic site in the wild-type nAChR channel are not specified by our results. The additive effects of mutations on α and β subunits suggest that multiple subunits contribute to the general anesthetic site. A similar model, in which all five M2 domains contribute to the site, has been proposed for the charged blocker QX-222 (15). Comparison of hydrophobic mutations at different positions on the α M2 domain suggests that general anesthetics bind closer to 10′ than to 6′. Because the serines, threonines, and alanines found at the 10′ position in wild-type nAChRs are not particularly hydrophobic, it seems likely that in the wild-type channel, the many nearby leucine residues (see Table 1) would also interact favorably with general anesthetics to form the wild-type binding site. This gives a picture of an extended hydrophobic interaction surface for general anesthetics; we suppose that placing hydrophobic residues at the 10′ site extends the favorable interaction surface still further. This picture is consonant with the suggestion that sites within the pore for local and general anesthetics are quite close, if not overlapping (25). It is also consistent with the broad pattern of reaction in the pore by hydrophobic and amphipathic photolabels (26).

Our results have profound implications for the understanding of general anesthetic actions in the central nervous system because they support a mechanism by which general anesthetics interact directly with synaptic proteins. Neuronal postsynaptic ion channels such as γ-aminobutyric $acid_A$ and 5-$hydroxytryptamine_3$ receptors are structurally homologous to nAChRs and are also modulated by general anesthetics (1, 27). Some or all of these receptors may contain protein sites that interact with general anesthetics to depress consciousness, pain perception, and memory formation.

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