Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition

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ABSTRACT **Molecular cloning of cDNAs coding for ligand-gated ion channel subunits makes it possible to study the pharmacology of recombinant receptors with defined subunit compositions. Many laboratories have used these techniques recently to study actions of agents that produce general anesthesia. We review the effects of volatile and intravenous anesthetics on recombinant $GABA_A$, glycine, AMPA, kainate, NMDA, and $5HT_3$ receptors. Evidence for and against specific ligand-gated ion channel subunits as targets responsible for anesthesia or the side effects of anesthetic agents is discussed for each type of receptor. Subunit specific actions of some of the agents suggest that construction and testing of certain chimeric receptor subunits may be useful for defining the amino acid sequences responsible for anesthetic actions.—Harris, R. A., Mihic, S. J., Dildy-Mayfield, J. E., Machu, T. K. Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. *FASEB J.* 9, 1454–1462 (1995)**

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DESPITE THEIR UBIQUITOUS APPLICATION in medicine, we still do not understand the mechanisms of action of agents that produce general anesthesia. There has, however, been a paradigm shift in the study of anesthetic mechanisms, and there are encouraging signs that the 1990s will be as important as the 1890s for understanding anesthetic drugs. In the 1890s, Meyer and Overton (1) proposed the relationship that dominated thinking in this area throughout this century. They noted that the potency of an anesthetic is proportional to its lipid solubility. This relationship was extended to implicate the lipid regions of the nerve cell, particularly the lipid bilayer of the plasma membrane, as the site of action of anesthetics. However, despite intensive study, the action of anesthetics on lipid structure has failed to provide a mechanism for changes in neuronal function that could account for anesthesia. More recently, emphasis has shifted to the study of anesthetic actions on specific proteins that determine neuronal excitability. The search for proteins that are affected by a wide range of anesthetic agents and are important for neuronal function has focused on the ligand-gated ion channels (2, 3). This superfamily of ion channels represents the site of action of the major inhibitory γ-aminobutyric acid (GABA)[3] and glycine) and excitatory (glutamate and aspartate) neurotransmitters of the central nervous system (CNS). Anesthetic-induced changes in the functioning of these receptor systems would change the balance between inhibitory and excitatory influences in the CNS, making the ligand-gated ion channels logical candidates for study. Initial studies of anesthetic action on these receptors used brain preparations containing many different types of receptors, as well as pre- and postsynaptic elements (4–6). These pioneering studies, although demonstrating that both inhibitory and excitatory synaptic transmission are sensitive to a variety of anesthetics, did not define the site of anesthetic action.

A major advance in the reductionist approach to studying synaptic mechanisms occurred in the late 1980s and early 1990s when the cDNAs that code for the protein subunits of these ligand-gated ion channels were cloned and sequenced. This work provided two remarkable insights: first, many different neurotransmitter receptors are structurally related even though their associated channels have very different ion selectivities; and second, some of the receptors could be formed from many different subunits. The latter has tremendous implications for pharmacology because it provides many different receptor subtypes that all respond to the same neurotransmitter but display distinct modulation by drugs. Such studies are possible because specific receptor subunits can be expressed in cells (e.g., *Xenopus* oocytes or embryonic kidney cells) that do not contain these receptors endogenously. In this way, different receptors can be compared in the same cellular context and membrane environment. This approach has been particularly successful for the $GABA_A$ receptor where subunit requirements for drugs such as benzodiazepines have been defined (7, 8).

Recently, these receptor expression techniques have been applied to study anesthetic agents, and sufficient data have accumulated from multiple laboratories to answer, or at least

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[3]Abbreviations: GABA, γ-aminobutyric acid; F3, 1-chloro-1,2,2-trifluorocyclobutane; F6, 1,2-dichlorohexafluorocyclobutane; F8, 2,3-chlorooctafluorobutane; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid; GluR, glutamate receptor; NMDA, N-methyl-D-aspartate; DHP, 3α-hydroxy-5α-pregnan-20-one; BuOH, butanol; EtOH, ethanol; pento, pentobarbital; CNS, central nervous system.

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address, several key questions. These include: *1)* Which ligand-gated ion channels are sufficiently sensitive to selected anesthetic agents to be considered as likely sites of action? *2)* Is a specific ligand-gated ion channel sensitive to a wide variety of anesthetics or is sensitivity restricted to certain classes of chemicals? *3)* How important is subunit composition for the anesthetic sensitivity of each class of ligand-gated ion channels? *4)* Does the anesthetic sensitivity of a specific channel depend on the expression system or only on the subunit composition?

The goal of this review is to summarize existing data on the effects of volatile and nonvolatile anesthetics on ligand-gated ion channels of defined subunit composition and to use these data to attempt to answer the four questions posed above. We will not present data from neuronal preparations, but will refer the reader to articles that review this rather large body of literature. Effects of each anesthetic on specific subunits are summarized with a rough estimate of the change in receptor function produced by a given concentration of the anesthetic. Concentrations are given as approximate multiples of the anesthetic concentration for each drug. As noted by Franks and Lieb (3), the anesthetic dose-response curve is extremely steep in vivo, and a concentration of 1.2-fold the EC_{50} is sufficient to anesthetize most animals whereas a concentration of 2- to 4-fold the EC_{50} will produce untoward actions, which may include death. Thus, actions achieved with more than twice the anesthetic level may not be relevant to anesthetic actions in vivo. However, it should also be appreciated that anesthetic concentrations of some of the drugs are not precisely known. For example, anesthetic concentrations of octanol and butanol were determined in amphibians, but not in mammals. Most of the results reported here were obtained at room temperature, and anesthetic concentrations are determined at physiological temperatures, introducing another potential error for the volatile anesthetics (3). Finally, the extensive protein binding of propofol leads to very different estimates of its anesthetic concentration (3).

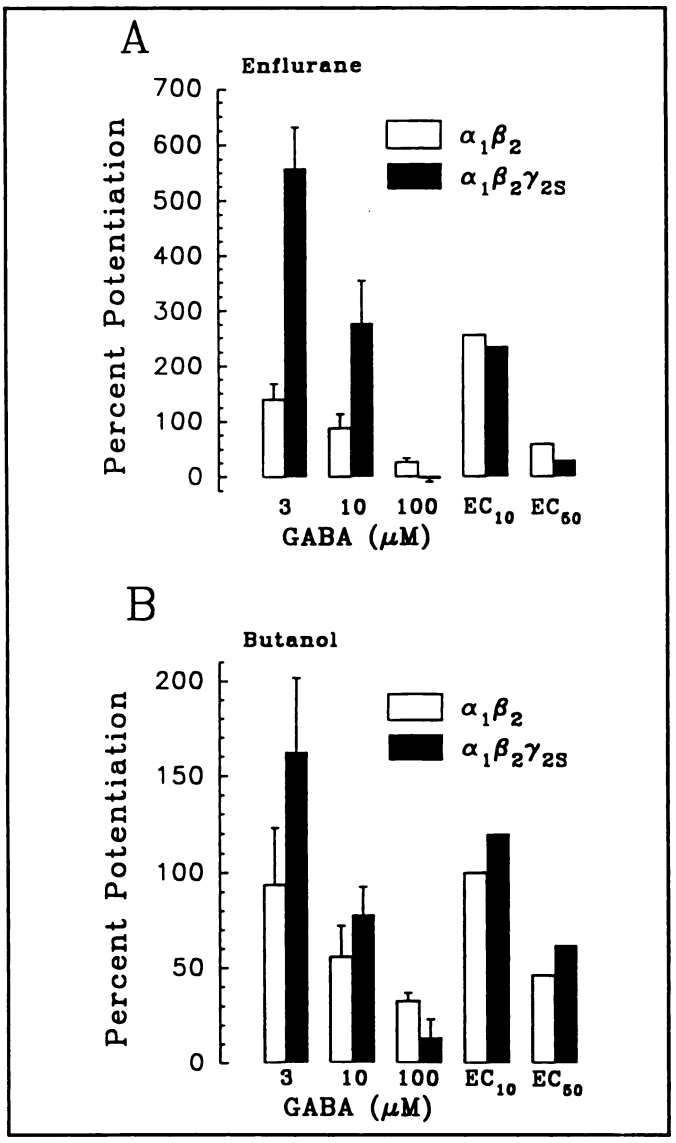

Figure 1. Potentiation of GABA action by enflurane or butanol depends on the concentration of GABA as well as the subunit composition of the receptor. Percent potentiation produced by enflurane (1.5 mM, panel *A*) or butanol (20 mM, panel *B*) is shown as a function of GABA concentration both as absolute concentration (μM) and as the effective concentration (EC) for receptors with subunit composition of $\alpha_1\beta_2$ (open bars) and $\alpha_1\beta_2\gamma_2S$ (filled bars). Subunits were human α_1 and γ_2S and rat β_2, and were expressed in *Xenopus* oocytes. Data from Mihic et al. (10, 11).

$GABA_A$ RECEPTORS

Because the $GABA_A$ receptor mediates inhibitory neurotransmission, one would expect anesthetics to enhance this inhibition by potentiating GABAergic currents. Indeed, studies of neuronal preparations showed that these receptors are sensitive to a wide variety of anesthetic agents, and potentiation of GABA action can usually be detected with anesthetic or subanesthetic concentrations of these drugs (2, 3). The $GABA_A$ receptor has an extremely complex array of subunits resulting in many possible receptor subtypes (7, 8), making it popular for studies of effects of anesthetics on expressed receptors.

Estimation of the amount of potentiation of $GABA_A$ receptor function by anesthetics, as well as the comparison of anesthetic sensitivity of different subunit combinations, is complicated considerably by the strong dependence of anesthetic action on extent of occupation of the GABA site. This is shown in **Fig. 1** where the potentiation of GABA actions by enflurane and butanol is given for two different subunit combinations at different concentrations of GABA. Both anesthetics produce a greater potentiation with the $\alpha_1\beta_2\gamma_2$ than with the $\alpha_1\beta_2$ receptor if the concentration of GABA is low (3–10 μM), but the subunit dependence is reversed at high concentrations (e.g., 100 μM) of GABA. However, these two receptors differ in their sensitivities to GABA, with the EC_{50} being about 6 μM for $\alpha_1\beta_2$ and 17 μM for $\alpha_1\beta_2\gamma_2$, and comparison of anesthetic effects at *equieffective* concentrations of GABA gives a different subunit dependence. For example, at EC_{10} or EC_{50} concentrations of GABA, the two receptors are about equally sensitive to anesthetics (Fig. 1). Thus, quantitative comparisons of anesthetic sensitivity of

GABA are not possible unless one determines the anesthetic effects over a range of GABA concentrations. This issue is discussed in more detail elsewhere (9–11).

Examination of anesthetic actions on defined $GABA_A$ receptors (**Table 1** and **Table 2**) reveals several consistent findings.

First, a wide variety of chemically distinct anesthetics potentiate the actions of GABA, and these effects are generally detectable at anesthetic or subanesthetic concentrations. There is no systematic variation among chemical classes; in fact, more variation exists among some agents in the same class than among classes. For example, ethanol, butanol, octanol, and propofol are all alkanols, but at the concentrations taken as anesthetic, butanol and octanol produce a pronounced potentiation whereas propofol and ethanol produce weak actions. However, these discrepancies may be because this group of compounds has some of the least reliable values for anesthetic concentrations in vivo (as discussed above).

Second, no single subunit is required for action of any anesthetic agent. It is remarkable that so many different subunit combinations display similar sensitivity to such a range of anesthetic agents. Notable exceptions are the ρ subunits (12–14). These are the only GABA receptors tested so far that are not affected by pentobarbital, alphaxalone, or propofol but are inhibited by alcohols and volatile anesthetics (S. J. Mihic and R. A. Harris, unpublished results). The inhibitory action of anesthetics on ρ receptors is seen with submaximal but not maximal concentrations of GABA, and this may explain why no effect of isoflurane was observed by Harrison et al. (13). The anesthetic sensitivity of the ρ receptor is similar to that of α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) receptors (see below) in that short-chain alcohols are effective inhibitors, but long-chain alcohols, volatile anesthetics, pentobarbital, and propofol produce little or no effect. However, these receptors are quite different in many respects from other $GABA_A$ receptors. For example, they share only 30–38% homology with most other subunits, and homomeric ρ receptors are resistant to bicuculline and are found primarily in the retina. In fact, it has been suggested that they should be termed $GABA_C$ receptors (12). The δ subunit also has a low homology with other $GABA_A$ receptor subunits. Although it forms functional homomeric receptors, it is not known whether these channels are affected by anesthetics other than pentobarbital. Incorporation of the δ subunit into heteromeric receptors (containing α, β, and γ subunits) does not alter the sensitivity of these receptors to pentobarbital (15).

Third, the expression system does not appear to determine the actions of the compounds tested to date, although direct comparisons are limited. *Xenopus* oocytes, human embryonic kidney cells (HEK293), and mouse fibroblasts (Ltk^- cells) have been transfected with several subunit combinations for anesthetic studies. Pentobarbital has been tested in all three systems and the enhancement of GABA action is similar for these three cell types as well as for neuronal $GABA_A$ responses. Similar octanol sensitivity is observed on receptors expressed in oocytes and HEK cells.

In regard to the $GABA_A$ receptor, two other issues arise. One is the direct activation of the channel by anesthetics. It is clear that barbiturates, 3α-hydroxy-5α-pregnan-20-one (DHP), and propofol can activate the channel in the absence of GABA (16, 17), but volatile anesthetics generally do not activate the channel directly (10, 13). The direct actions of pentobarbital and propofol appear to require a β subunit; thus, they display much more subunit specificity than the enhancement of GABA action and likely represent a distinct site on the receptor complex (17). However, the concentrations required to produce the direct actions are generally greater than those required to potentiate GABA actions. This observation, together with the finding that volatile anesthetics do not activate the channel, suggests that this phenomenon may not be relevant to anesthesia. A second issue is the action of low, nonanesthetic concentrations of ethanol on $GABA_A$ receptors. There are numerous reports that ethanol at concentrations of 5–30 mM (e.g., 10- to 60-fold less than the anesthetic concentration) enhances GABA action (18, 19). The effects of anesthetic concentrations of ethanol display no subunit specificity, but the low-dose actions require a γ subunit (20). In addition, there is evidence that phosphorylation of the γ_2L splice variant is required for sensitivity to low concentrations of ethanol (21). (In Table 1, the short and long splice variants of the γ_2 subunit are not distinguished because there is no indication that they differ in sensitivity to anesthetic concentrations of any of these drugs.) However, the effects of ethanol on recombinant $GABA_A$ receptors are controversial, and several investigators have not found enhancement of GABA action by subanesthetic concentrations of ethanol (e.g., 10–100 mM) (22–24). This issue is discussed in detail elsewhere (18, 25).

Another important consideration for any candidate site of anesthetic action is the effect (or lack thereof) of nonanesthetic drugs. Recently, several fluorinated hydrocarbons were found to be nonanesthetic even though they reach the brain in concentrations that would be predicted (from the Meyer-Overton relationship) to be anesthetic (26, 27). In addition, some other membrane-active agents are not anesthetic (28). Although solubility in olive oil is sufficient to predict the potency of most anesthetics, it is not capable of classifying these compounds as nonanesthetics. Accordingly, it was of interest to determine whether recombinant $GABA_A$ receptors are superior to olive oil as predictors of anesthetic potency. In contrast to anesthetics, structurally similar nonanesthetics do not enhance GABA action, even at concentrations predicted by Meyer-Overton to be anesthetic (10, 28, 29) (see Table 5).

In summary, recombinant $GABA_A$ receptors are sensitive to all anesthetics tested so far but are not affected by structurally related nonanesthetics. The enhancement is extremely dependent on the concentration of GABA, and appreciable (e.g., 200–600%) enhancement can be obtained at or below the anesthetic EC_{50} if the GABA concentration is low (e.g., EC_{5-10}). With high concentrations of GABA, there is little or no enhancement by most anesthetics, and in the case of enflurane there is a slight inhibition of GABA action (Fig. 1) (9–11, 30). Enhancement of GABA action by anesthetics

TABLE 1. *Potentiation of $GABA_A$ receptor function by nonvolatile anesthetics*[a]

	Drugs[b]					
Subunits	Pento	Propofol	DHP	Octanol	BuOH	EtOH
Oocytes						
$h\beta_1$	+++/2/1	+++/6/1				
$h\rho_1$	0/0.2-20/2	0/4/3		0/1/3	- -/1/3	- - -/1/3
$r\delta$	++/1/4					
drGR	+/0.6/5		+/10/5			
$b\alpha_1\beta_1$	+++/0.5/6		+++/1/5			
$b\alpha_2\beta_1$			+/1/7			
$b\alpha_3\beta_1$			+++/1/7			
$h\alpha_1\beta_1$		+/2/1		++/0.5/8	+++/1/9	+/0.7/9
$h\alpha_2\beta_1$		+++/6/10				
$h\alpha_5\beta_1$		+++/6/10			+++/1/8	+/0.7/9
$h\alpha_1r\beta_2$		+++/6/10			+++/1/9	+/0.7/9
$h\alpha_1\gamma_2$		+++/6/1				
$h\alpha_1r\beta2h\gamma_2$	+++/6/10				+++/1/9	+/0.7/9
$h\alpha_1\beta_1\gamma_2$	++/0.5/11	+++/2/1	+++/3/12		+++/1/9	+/0.7/9
$h\alpha_1\beta_2\gamma_2$			+++/3/12			
$h\alpha_1\beta_3\gamma_2$			+++/3/12			
$b\alpha_1\beta_1\gamma_2$			+++/1/7	++/0.5/8		
$b\alpha_2\beta_1\gamma_2$			+/1/7			
$b\alpha_3\beta_2\gamma_2$			+/1/7			
$b\alpha_1\beta_1\gamma_1$	+++/0.5/6					
$r\alpha_1\beta_1\gamma_2$						0/0.3/13
$r\alpha_1\beta_2\gamma_2$						0/0.3/13
Transfected cells						
$h\beta_1$			+++/1/14			
$h\alpha_1\beta_1$			+++/1/14			
$b\alpha_1\beta_1$	+++/0.6/15					
$r\alpha_1\beta_2$					++/2/16	
$r\alpha_5\beta_2$	+++/2/17					
$h\alpha_1\beta_1\gamma_2$			+++/1/14			
$b\alpha_1\beta_1\gamma_2$	+++/0.6/15					
$r\alpha_5\beta_2\gamma_3$	+/2/17					
$r\alpha_1\beta_2\gamma_2$				++/2/18		0/0.3/18
$r\alpha_6\beta_2\gamma_2$				++/2/18		0/0.3/18

[a]Species from which clones were derived are noted with subunits (b, bovine; dr, drosophila [drGR is *Drosophila* GABA receptor]; h, human; r, rat). Drugs are pentobarbital (pento), 3α-hydroxy-5α-pregnan-20-one (DHP), butanol (BuOH), and ethanol (EtOH). [b]The first symbol for each entry denotes the amount of potentiation of receptor function as + for 20–50%, ++ for 50–100%, and +++ for > 100%. Amount of inhibition of receptor function is given as - for 20–50%, - - for 50–75%, and - - - for 75–100%. The second symbol denotes the drug concentration used as a multiple of the anesthetic concentration. Whenever possible, data obtained with concentrations near anesthetic were selected for presentation. The third symbol is the literature reference, given below. Anesthetic concentrations were taken as: pentobarbital, 50 μM; propofol, 0.8 μM; DHP, 0.3 μM; octanol, 60 μM; butanol (BuOH), 20 mM; ethanol (EtOH), 300 mM. These values were estimated from several different sources including Franks and Lieb (3), Roth and Miller (49), and E. I. Eger (personal communication for ethanol and butanol concentrations). Values are for mammals except for octanol where an EC_{50} of 60 μM was estimated in tadpoles; for butanol we used 20 mM based on EC_{50} of 12 mM in tadpoles, 17 mM in rats, and 76 mM in newts; and for ethanol we used 300 mM based on EC_{50} of 190 mM in tadpoles and 350 mM in rats. All data for tadpoles and newts are from Roth and Miller (49). References: 1. (17); 2. (12); 3. S. J. Mihic and R. A. Harris, unpublished results; 4. (50); 5. (51); 6. (52); 7. (53); 8. Mihic et al., unpublished results; 9. (11); 10. (54); 11. (55); 12. (56); 13. (22); 14. (16); 15. (57); 16. (24); 17. (58); 18. (23).

TABLE 2. *Potentiation of $GABA_A$* receptor function by volatile anesthetics[a]

	Drugs[b]		
Subunits	Enflurane	Isoflurane	F3
Oocytes			
$h\rho_1$	-/2/1	-/2/1	
$h\alpha_1\beta_1$	+++/3/2		
$h\alpha_{1r}\beta_2$	+++/2/3		++/1/3
$h\alpha_1\beta_1\gamma_2$	+++/3/2		
$h\alpha_{1r}\beta_2h\gamma_2$	+++/2/3		+++/1/3
Transfected cells			
$h\rho_1$		0/2-7/4	
$h\alpha_1\beta_1$		++/4-8/4	
$h\alpha_2\beta_1$		+,+++/5-7/4	
$h\alpha_2\gamma_2$		+,+++/3/4	
$h\alpha_1\beta_1\gamma_2$		+,+++/2-4/4	
$h\alpha_2\beta_1\gamma_2$		+,+++/0.6-6/4	

[a]Species from which clones were derived are noted with subunits (b, bovine; h, human; r, rat). F3 is 1-chloro-1,2,2-trifluorocyclobutane. [b]Amount of inhibition of receptor function is given as - for 20–50%, - - for 50–75%, and - - - for 75–100%; amount of potentiation of receptor function is denoted as + for 20–50%, ++ for 50–100%, and +++ for > 100%. The concentration used is given as a multiple of the anesthetic concentration (see legend to Table 1). Whenever possible, data obtained with concentrations near anesthetic were selected for presentation. Anesthetic concentrations were taken as: enflurane, 0.6 mM; isoflurane, 0.3 mM; halothane, 0.25 mM; and F3, 0.9 mM. Sources: (3, 10). References: 1. S. J. Mihic and R. A. Harris, unpublished results; 2. (9); 3. (10); 4. (13).

does not require any specific subunit and is similar for a wide variety of different subunit combinations, although it is not found with homomeric ρ subunits. It is possible that the anesthetics act directly on one or more sites that are conserved among the α, β, and γ subunits but not found in the ρ subunits (13).

GLYCINE RECEPTORS

There is only limited information on the effects of anesthetics on recombinant glycine receptors. Harrison et al. (13) transiently expressed glycine α_2 subunits in HEK 293 cells and found that isoflurane enhanced glycine responses (**Table 3**). Using *Xenopus* oocytes, we found that anesthetic (EC_{50}) concentrations of halothane, butanol, and ethanol enhanced glycine responses of homomeric receptors formed from α_1 or α_2 subunits. This anesthetic sensitivity is consistent with studies of effects of volatile anesthetics and propofol on neuronal preparations (29, 31, 32). In view of recent interest in the spinal cord as a site of anesthetic action (33–35), the glycine receptor emerges as a promising candidate target for these drugs. Clearly, additional studies of recombinant receptors are warranted. In particular, the physiological receptor likely contains β subunits in addition to α_1 or α_2 subunits (36), and it will be of interest to determine whether the β subunit alters the anesthetic sensitivity or selectivity of the α subunits.

AMPA/KAINATE RECEPTORS

Because glutamate receptors produce neuronal excitation, one would expect anesthetics to reduce the function of these receptors. Molecular cloning and expression studies defined several populations of glutamate receptors that are distinct from the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. Four glutamate receptor subunits (GluR1-4) are termed AMPA receptors because α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) acts as a high-affinity agonist at these receptors, and three subunits (GluR5-7) are designated as kainate receptors based on sensitivity to this agonist and insensitivity to AMPA (37). There are only a few studies of anesthetic action on these receptors in neuronal systems (3) or expression systems (**Table 4**). The AMPA receptors are weakly inhibited by several volatile and nonvolatile anesthetics. However, two alcohols, propofol and ethanol, are exceptions. Propofol produces no effect on AMPA receptors, even at concentrations far above those required for anesthesia. In contrast, ethanol strongly inhibits AMPA receptor function at subanesthetic to anesthetic concentrations (Table 4) (38).

Only a few drugs distinguish between AMPA and kainate receptors, and it is remarkable that several volatile anesthetics produce opposite actions on these two types of receptors. Enflurane, isoflurane, halothane, and trifluorocyclobutane (F3) all inhibit (weakly) AMPA receptor function yet enhance kainate (GluR6) responses (Table 4). In contrast, butanol and ethanol inhibit both AMPA and kainate receptors, but propofol has little effect on either subtype of receptor (Table 4). The nonanesthetic halocarbons do not alter the function of AMPA or kainate receptors (**Table 5**).

In summary, inhibition of AMPA and kainate receptors may be important for the anesthetic and intoxicating effects of ethanol and other short-chain alcohols, but studies of recombinant receptors suggest that the drug sensitivity of AMPA responses is not sufficient for them to play a primary role in anesthetic actions of volatile or intravenous agents. However, the sensitivity of AMPA receptors to ethanol can be increased by elevation of intracellular calcium (38). If this is also true for

TABLE 3. *Potentiation of glycine receptor function by anesthetics*[a]

	Isoflurane	Halothane	Butanol	Ethanol
Oocytes				
α_1		++/1/1	+++/1/1	+/0.7/1
α_2		++/1/1	+++/1/1	+/0.7/1
HEK293 cells				
α_2	+ to +++/2-3/2			

[a]See Tables 1 and 2 for anesthetic concentrations and other details. Amount of potentiation of receptor function is denoted as: + for 20–50%, ++ for 50–100%, and +++ for > 100%. References: 1. T. Machu, unpublished results; 2. (13).

TABLE 4. *Effects of anesthetics on recombinant AMPA/kainate receptors*

	Enflurane	Isoflurane	Halothane	F3	Pento	Propofol	Octanol	Butanol	Ethanol
Oocytes									
GluR 1	-/2/1	-/10/1		0/2/1		0/30/1			-/0.3/2
	-/8/1								
GluR 3	-/2/1	-/10/1	-/2/1	-/2/1	0/2/1	0/30/1	-/1.5/1	-/.3/1	-/0.3/2
	-/8/1		-/4/1		-/6/1			-/1/1	- - -/1/2
GluR 2 + 3	-/2/1	-/10/1		0/2/1					
	-/8/1								-/0.3/2
GluR 6	+/1/1	+/2/1	+/1/1	+/0.5/1	-/2/1	+/30/1	-/1.5/1	-/.3/1	-/0.3/2
	++/2/1	++/4/1	+++/4/1	+++/2/1	-/6/1			-/1/1	-/1/2
GluR 1 + 3									-/0.3/2
GluR 1 + 2 + 3									-/0.3/2
Transfected cells									
GluR1									-/0.3/3
GluR 4									-/1/3
GluR 1 + 4									-/1/3
GluR 2 + 4									-/1/3

[a]Amount of inhibition of receptor function is given as - for 20–50%, - - for 50–75%, and - - - for 75–100%; potentiation of receptor function is denoted as + for 20–50%, ++ for 50–100%, and +++ for > 100%. The concentration used is given as a multiple of the anesthetic concentration (see legend to Table 1). Whenever possible, data obtained with concentrations near anesthetic were selected for presentation. Anesthetic concentrations are given in legends to Tables 1 and 2. For the oocyte studies, the "flop" splice variants were used, for the transfected cells, "flip" splice variants were used. F3 is 1-chloro-1,2,2-trifluorocyclobutane. References: 1. J. E. Dildy-Mayfield, unpublished results; 2. (38); 3. (59).

other anesthetic agents, then AMPA receptors of some neurons (i.e., those activated by neurotransmitters that increase calcium levels) might be sufficiently sensitive to anesthetics to be candidate targets. The enhancement of kainate action on homomeric GluR6 receptors by volatile anesthetics is quite unexpected and would be expected to increase brain excitability. This could contribute to the stimulatory effects of subanesthetic concentrations or even the seizure-like activity sometimes seen during anesthesia with enflurane. Alternatively, activation of kainate receptors in appropriate pathways could enhance inhibitory neurotransmission and thereby participate in anesthesia.

NMDA RECEPTORS

This is the most widely studied of the glutamate receptor subtypes, but there have been relatively few reports of anesthetic action on these receptors in either neuronal or expression systems. Neuronal NMDA receptors are resistant to pentobarbital but sensitive to ketamine and alkanols (3). Ethanol inhibits the function of homomeric recombinant receptors from two splice variants of the rat NMDA R1 subunit (R1-LL and R1-SS) (39) as well as heteromeric receptors composed of R1 + R2A, R1 + R2B, and R1 + R2C (40) at a concentration of 100 mM. The mouse subunit homologous to R1 is $\zeta 1$, R2A is $\varepsilon 1$, R2B is $\varepsilon 2$, and R2C is $\varepsilon 3$. For these subunits, the $\zeta 1$ subunit in combination with $\varepsilon 1$ or $\varepsilon 2$ is sensitive to ethanol (25–44% inhibition with 100 mM) (41). However, homomeric ζ_1 is not affected by ethanol, and $\zeta 1$ with $\varepsilon 3$ shows only 19% inhibition by 100 mM ethanol (41). It is not clear why homomeric rat R1 receptors were reported to be sensitive to ethanol, whereas the equivalent mouse receptors were not, although these homomeric receptors express poorly and it is difficult to estimate drug effects on the small currents observed.

In summary, as for AMPA receptors, inhibition of NMDA receptors may be important for anesthetic and intoxicating effects of ketamine, ethanol, and other short-chain alcohols, but there are insufficient studies to define their role in anesthetic actions of volatile agents. Studies of ethanol on

TABLE 5. *Effects of nonanesthetic halocarbons on GABA*$_A$ and AMPA/kainate receptor function[a]

	F6	F8
$h\alpha_1r\beta_2$		0/5/1
$h\alpha_1\beta_1\gamma_2$	0/1.5-4/1	0/0.5-4/1
$h\alpha_1r\beta_2h\gamma_2$		0/5/1
GluR 3	0/1.5-4/2	0/0.5-4/2
GluR 6	0/1.5-4/2	0/0.5-4/2

[a]None of these compounds produced significant (> 20%) inhibition or enhancement of GABA or glutamate responses on these types of receptors. None of these compounds is anesthetic in vivo, but the concentration used is denoted as a multiple of the predicted "anesthetic" EC_{50} based on the Meyer-Overton relationship (10, 26). F6 is 1,2-dichlorohexafluorocyclobutane; F8 is 2,3-chlorooctafluorobutane. References: 1. (10); 2. J. E. Dildy-Mayfield, unpublished results.

recombinant NMDA receptors suggest that not all subunits are equally sensitive. It will be of interest to see if this result can be extended to volatile anesthetics.

5HT$_3$ RECEPTORS

The 5HT$_3$ receptor is a cation channel activated by serotonin that has a discrete localization in brain (e.g., area postrema). Endogenous 5HT$_3$ receptor responses are enhanced by anesthetic concentrations of ethanol and diethyl ether but inhibited (weakly) by pentobarbital (42). Thus far, only one 5HT$_3$ subunit has been cloned, and initial studies demonstrate that most anesthetic agents enhance the function of this homomeric receptor expressed in *Xenopus* oocytes (43). In this study, ethanol, butanol, isoflurane, halothane, and F3 all enhanced the action of serotonin on 5HT$_3$ receptors at levels corresponding to one- to twofold the anesthetic concentration. However, propofol was ineffective even at 30-fold the anesthetic concentration.

Enhancement of 5HT$_3$ receptor function is unlikely to be responsible for anesthesia but may cause the nausea and vomiting produced by many anesthetic agents (43). This suggestion is strengthened by the observation that propofol produces less nausea than most anesthetics (44) and does not enhance 5HT$_3$ receptor function.

OTHER RECEPTORS

The only member of the superfamily of ligand-gated ion channels not discussed in this review is the nicotinic acetylcholine receptor (nAChR). Although there is considerable evidence for effects of anesthetics on the nAChR (3, 45), we did not find any report of effects of these drugs on recombinant nAChR. An ATP-activated cation channel has recently been cloned but is structurally unrelated to the ligand-gated ion channels discussed here (46, 47). Anesthetic sensitivity of the recombinant receptor has not been reported, but the neuronal receptor is inhibited by ethanol and propanol but not by longer chain alcohols or the anesthetic trichloroethanol (48). These results suggest that this receptor is not a site of anesthetic action, but studies with volatile anesthetics on the cloned receptors are warranted.

FUTURE DIRECTIONS

The results summarized in this article provide several possibilities for defining receptor regions, or perhaps even specific amino acids, that are required for anesthetic action. For example, demonstration of distinct drug actions on two homologous proteins allows use of chimeric proteins to define regions critical for drug action. Thus, it might be possible to show the site of propofol action by making chimeric receptors based on the propofol-sensitive β_1 subunit of the GABA$_A$ receptor in which regions have been exchanged with the propofol-insensitive 5-HT$_3$ receptor. For the purpose of discussion, let us assume that exchange of a specific transmembrane region (e.g., TM3) between these receptors prevents propofol action on the β_1 subunit but confers sensitivity to the 5-HT$_3$ receptor. This could be followed by mutation of specific amino acids in this region with the hope of identifying the amino acids responsible for propofol action. One of the many pitfalls in this approach is that the chimeric cDNAs may not express functional proteins. This is more likely to be a problem if the receptors have low homology, as is the case with the β_1 GABA$_A$ receptor and the 5-HT$_3$ receptor. A better choice would probably be the ρ and β_1 subunits, which have about 37% sequence identity at the amino acid level. In addition, receptors formed from the ρ subunits are resistant to propofol and pentobarbital, but those formed from β_1 subunits are sensitive to both these drugs. Another promising possibility is the study of chimeras of GluR3 and GluR6 subunits. Because volatile anesthetics inhibit the function of receptors formed from GluR3 subunits, but enhance function of GluR6 receptors, chimeras of these two subunits should be able to define the subunit regions or even the amino acids responsible for these distinct actions of anesthetics.

An important issue is how to interpret the results of chimera/mutagenesis studies. Taking our hypothetical result of a single amino acid conferring or abolishing propofol sensitivity, can we conclude that this amino acid is a critical part of the propofol binding site on the channel? Can this approach define a "receptor" for this anesthetic? For many drugs, the next step is straightforward: measuring the binding of radioactive drug to cells transfected with the mutated receptor will determine whether the mutation alters the drug-receptor interaction or another step in the transduction mechanism. However, the anesthetic interactions are of such low affinity (μM to mM) that ligand binding is not an option, and even the most successful mutagenesis study may not be able to define an "anesthetic receptor." The question of whether a mutation directly alters the binding of the anesthetic to the channel or indirectly disrupts its effect on channel function is of particular importance to the differences between the GluR3 and GluR6 receptors. Here the anesthetics act on both receptors, but the actions are opposite, and chimeric receptors will likely identify protein sequences that are responsible for transducing the action of the anesthetic rather than the binding of the anesthetic. Despite concerns about the interpretation of these studies, they bring a truly molecular approach to the study of anesthetic action. It is possible that this strategy will succeed in identifying sequences that are common to "anesthetic-sensitive" proteins. These may be found in other ion channels or proteins unrelated to known ion channels, making it possible to infer from sequence homology that a protein may be important for anesthesia.

CONCLUSIONS

At first glance, it appears that almost all subunit combinations of ligand-gated ion channels are sensitive to most anesthetics tested. This presents a looming shadow of nonspecificity at

least as dark as that raised by Meyer and Overton (1) at the end of the last century. However, closer analysis suggests that there may be light at the end of the tunnel. For example, the finding that ρ subunits differ from all other $GABA_A$ receptors in their anesthetic sensitivity might allow construction of chimeras and mutants that will define the site of action of anesthetics. In addition, the opposite effects of volatile anesthetics on AMPA and kainate receptors should be a valuable clue to the molecular mechanisms responsible for these actions. Testing a range of chemically distinct anesthetics on the same receptor in the same cellular environment has led to clear distinctions between the actions of agents such as propofol and ethanol. From the number of blank areas in the tables, it is clear that such multianesthetic comparisons are relatively rare, but it is hoped that they will be forthcoming as these techniques are used in more laboratories. These studies force us to revisit another issue of 100 years ago. Implicit in the ideas that have developed from the observations of Meyer and Overton (1) is a unitary site and mechanism of action for all anesthetic agents. Is this valid, or are there multiple mechanisms of anesthesia and does each agent have a unique spectrum of activities on multiple receptors? Recombinant receptors provide the potential to identify molecular sites of action for each anesthetic agent and should finally provide the answer to this question.

In summary, our first real insights into understanding anesthetic actions came with the pioneering work of Meyer and Overton (1) at the turn of the century. During the latter half of this century, our initial notions that volatile anesthetics act on lipids have been supplanted by the belief that their primary sites of action are instead proteins, most likely ligand-gated ion channels. By the turn of the 21st century, it is conceivable that our understanding will reach the molecular level, with the identification of specific amino acids mediating the effects of volatile anesthetics. FJ

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