Structural Comparisons of Ligand-gated Ion Channels in Open, Closed, and Desensitized States Identify a Novel Propofol-binding Site on Mammalian γ-Aminobutyric Acid Type A Receptors

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ABSTRACT

Background: Most anesthetics, particularly intravenous agents such as propofol and etomidate, enhance the actions of the neurotransmitter γ-aminobutyric acid (GABA) at the GABA type A receptor. However, there is no agreement as where anesthetics bind to the receptor. A novel approach would be to identify regions on the receptor that are state-dependent, which would account for the ability of anesthetics to affect channel opening by binding differentially to the open and closed states.

Methods: The open and closed structures of the GABA type A receptor homologues *Gloeobacter* ligand–gated ion channel and glutamate-gated chloride channel were compared, and regions in the channels that move on channel opening and closing were identified. Docking calculations were performed to investigate possible binding of propofol to the GABA type A β3 homomer in this region.

Results: A comparison between the open and closed states of the *Gloeobacter* ligand–gated ion channel and glutamate-gated chloride channel channels identified a region at the top of transmembrane domains 2 and 3 that shows maximum movement when the channels transition between the open and closed states. Docking of propofol into the GABA type A β3 homomer identified two putative binding cavities in this same region, one with a high affinity and one with a lower affinity. Both cavities were adjacent to a histidine residue that has been photolabeled by a propofol analog, and both sites would be disrupted on channel closing.

Conclusions: These calculations support the conclusion of a recent photolabeling study that propofol acts at a site at the interface between the extracellular and transmembrane domains, close to the top of transmembrane domain 2. **(ANESTHESIOLOGY 2015; 122:787-94)**

THE γ-aminobutyric acid type A ($GABA_A$) receptor is an important anesthetic target,[1–5] particularly for the intravenous agents such as propofol, etomidate, and the barbiturates.[6–8] It is not known, however, how the binding of the anesthetic to the receptor affects changes at the molecular level. Where do the anesthetics bind and how does this binding translate into increased channel opening?

To answer this question, many researchers have investigated the effects of mutating specific amino acids on the receptor, and many residues have been identified as key anesthetic determinants.[7,8] However, mutations in all four transmembrane domains (TMs), as well as in the extracellular domain, affect anesthetic responses even for the same drug, so many of these mutated sites must represent parts of the receptor important for gating as well as, potentially, binding. An alternative approach,[9] modifying chosen amino acids to cysteine and then investigating the impact of cysteine-modifying chemical reagents on anesthetic action, suffers from the same problem—how does one disentangle changes in binding from changes in gating?[10] Homology modeling of $GABA_A$ receptors has also been employed to identify plausible binding sites.[11]

What We Already Know about This Topic

- Ion channels including γ-aminobutyric acid type A receptors are sensitive to general anesthetics, but the molecular mechanisms of anesthetic effects on channel gating are unclear
- Structural studies of ion channels provide a basis for molecular modeling of anesthetic binding

What This Article Tells Us That Is New

- Molecular docking calculations of propofol binding to a γ-aminobutyric acid type A receptor identified putative binding sites in a region with maximal predicted movement during channel opening
- These calculations support a model of state-dependent binding of propofol resulting in enhanced γ-aminobutyric acid type A receptor opening

A different, potentially more direct, approach uses photolabeling. This method has revealed various putative binding sites on the $GABA_A$ receptor for etomidate,[12] neurosteroids,[13] and propofol.[14,15] Recently, we have identified a possible binding site for propofol using a propofol analog—*ortho*-propofol diazirine.[14] This reagent labeled a histidine residue that, on the basis of homology modeling,

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lies at the side of a cavity that we proposed to be the propofol-binding site.[14]

One uncertainty with photolabeling is to what extent the inevitable modification of the parent drug causes it to bind at a different site. In this article, I am taking a different tack and one that is independent of the experimental approaches mentioned above. It is based on the simple idea[16] that anesthetics bind to a particular channel conformation rather than another because an anesthetic-binding site is created by the conformational changes that occur during channel opening. Thus, in the presence of anesthetic, one channel conformation would be statistically favored over others. Consequently, possible anesthetic-binding sites might be identified by a detailed comparison of channels in different states.

Until recently, there were no crystal structures of any ligand-gated ion channel of the cys-loop receptor family in both the open and closed states. However, a structure of the proton-gated channel *Gloeobacter* ligand–gated ion channel (GLIC) from *Gloeobacter violaceus* in a closed state has now been published,[17] which can be compared with the known open state structure.[18] Also, the closed state of the glutamate-gated chloride channel (GluCl) from *Caenorhabditis elegans* has just been determined,[19] which can be compared to the previously determined structure of the channel in its open state.[20] Finally, very recently, the crystal structure of a human $GABA_A$ receptor, consisting of a pentamer of β3 subunits, has been solved in a likely desensitized state.[21] I show in this article how a comparison between these structures can give an insight into the location of anesthetic-binding sites on mammalian $GABA_A$ receptors.

Materials and Methods

Sequence Alignments

Sequence alignments were performed using Clustal Omega[22] via the European Bioinformatics Institute Web site.*

Docking Calculations

Docking of propofol into putative anesthetic-binding sites was done using AutoDock Vina[23] on a Hewlett Packard PC (Palo Alto, CA) with an Intel i7-3770 quad core processor. The initial conformation for propofol was taken from the ZINC† database,[24] and the bonds from the aromatic ring to the hydroxyl group and the two isopropyl groups were allowed to rotate freely during the docking calculations. The target structure consisted of chains A and B of the pentameric $GABA_A$ β3 receptor (Protein Data Bank ID code [PDB ID]: 4COF). Before docking, all hydrogen atoms were included, partial charges were added, and nonpolar hydrogens were merged. The grid spacing was 0.375 Å, and the initial search volume was the entire TM (125,280 $Å^3$). Once the location of the lowest-energy mode was established, which was adjacent to H267, the residue labeled by the propofol analog, *ortho*-propofol diazirine,[14] the subsequent searches were centered on this residue and the volume reduced to a 40 Å cube. All of the residues in the putative propofol-binding sites were allowed to be flexible during docking. The binding energies, ΔG, in kcal/mol calculated by AutoDock Vina were converted to binding constants, K, in molar units using $\Delta G = RT \ln(K)$, where R is the gas constant (1.99×10^{-3} kcal K^{-1} mol^{-1}) and T is room temperature (300 K).

Molecular Graphics

Images of the structures were made using the PyMOL Molecular Graphics System (Version 1.5.0.4 Schrödinger, LLC, Cambridge, MA), and the views showing movements of Cα carbon atoms on channel opening–closing were calculated using a script (colorbyrmsd.py) available via the PyMOLWiki Web page.‡

Results

Comparison between the Open and Closed GLIC Channel Structures

The structures of the proposed open (PDB ID: 3EAM)[18] and closed (PDB ID: 4LMK)[17] states of the GLIC channel from *G. violaceus* were superposed using all five subunits of the pentameric channel. The superpositions of the transmembrane and extracellular domains were done independently. For the superpositions of the extracellular domain, the first 12 residues were excluded because they were either missing or very variable between the two structures. The distances between the Cα carbon atoms in the two structures were then calculated and color-coded in the views shown in figure 1. Figure 1A provides an orientation for the reader and shows the receptor viewed from the extracellular surface with two chains (A and B) colored in yellow and green, respectively. The standard view adopted for subsequent illustrations is provided in figure 1B, which shows the channel viewed from inside the channel pore (in the direction indicated in fig. 1A). Figure 1, C and D illustrates two chains of the open and closed states of the GLIC channel, respectively, colored to show the movement of the Cα carbon atoms during channel opening–closing, with red for maximum movement and blue for little movement. A hot spot where the Cα carbon atoms move most can be seen at the top of TM2 where the maximum movement between the two structures is 5.5 Å.

Comparison between the Open and Closed GluCl Channel Structures

The structures of the proposed open[20] (PDB ID: 3RHW) and closed[19] (PDB ID: 4TNV) states of the GluCl channel from *C. elegans* were superposed using all five subunits of the pentameric channel. The superposition of the transmembrane and extracellular domains was done independently. For the superposition of the extracellular domain, 102 to 105 were excluded because they were either missing or very variable between the

* Available at: http://www.ebi.ac.uk/Tools/msa/clustalo/. Accessed December 1, 2014.

† Available at: http://zinc.docking.org/. Accessed December 1, 2014.

‡ Available at: http://pymolwiki.org/index.php/ColorByRMSD. Accessed December 1, 2014.

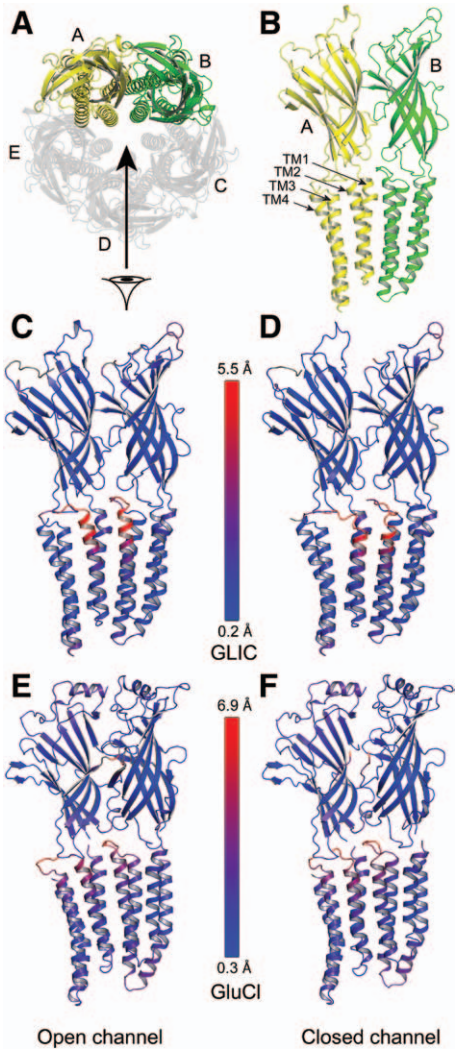

Fig. 1. The transition between the open and closed states of the pentameric ligand–gated ion channels *Gloeobacter* ligand–gated ion channel (GLIC) and glutamate-gated chloride channel (GluCl) identifies a hotspot of channel movement in the transmembrane domain (TM) at the top of TM2 and TM3. (*A*) Extracellular view of the GLIC receptor with two chains highlighted. Chain A is in *yellow* and chain B is in *green*. (*B*) A side view of the two chains A and B, viewed from the pore, in the direction indicated in the illustration in (*A*). This view is adopted in subsequent representations. (*C*) The open state of the GLIC channel (3EAM)[18] with the cartoon color-coded to represent the movement of the Cα carbon atoms on channel closing. (*D*) The closed state of the GLIC channel (4LMK)[17] with the cartoon color-coded to represent the movement of the Cα carbon atoms on channel opening. (*E*) The open state of the GluCl channel (3RHW)[20] with the cartoon color-coded to represent the movement of the Cα carbon atoms on channel closing. (*F*) The closed state of the GluCl channel (4TNV)[19] with the cartoon color-coded to represent the movement of the Cα carbon atoms on channel opening.

two structures. The distances between the Cα carbon atoms in the two structures were then calculated and color-coded in the views shown in figure 1, E and F, which illustrate the open and closed states of the GluCl channel, respectively. As with GLIC, a hot spot where the Cα carbon atoms move most can be seen at the top of TM2, but in GluCl, this movement extends along the TM2/TM3 loop to the top of TM3; the maximum movement between the two structures is 6.9 Å.

Molecular Docking of Propofol into the Structure of the $GABA_A$ β3 Homomer

A structure of the human $GABA_A$ receptor consisting of a pentamer of β3 subunits has been recently determined[21] at 3Å resolution (PDB ID: 4COF). The receptor was crystallized with an agonist (benzamidine) bound in the agonist-binding site, but with the channel closed at the base (intracellular side) of the pore. This is consistent with a desensitized state. However, despite the pore being closed at the base of the pore, the channel structure at the top of the pore much more closely resembles an open channel than a closed channel. This is illustrated by table 1, which shows a comparison between the desensitized β3 homomer and the open and closed states of GLIC and GluCl at the top of TM2/TM3—the hotspot of channel movement on opening or closing (see also the study by Miller and Aricescu[21]). Because this desensitized structure resembles an open channel structure in the region identified to bind *ortho*-propofol diazirine by photolabeling,[14] and because kinetic models show that the actions of propofol are best mimicked by binding sites on both the desensitized and open states,[7,25] I used the desensitized structure, 4COF, as a target for docking propofol.

Molecular docking identified the lowest energy putative propofol-binding site immediately adjacent to H267, the residue that had been photolabeled using *ortho*-propofol diazirine.[14] The site (site 1) showing the lowest binding energy (–8.3 kcal/mol, corresponding to a dissociation constant of 0.7 μM at 22°C) is illustrated in figure 2A. (The top four binding modes in this site had binding energies ranging from –8.3 to –7.2 kcal/mol.) The propofol molecule lies in a largely hydrophobic cleft between TM2 from chain A and TM2 from the adjacent subunit, chain B, with additional interactions with residues from TM1 of chain A. The polar oxygen of the propofol molecule lies 2.8Å from the main-chain carbonyl oxygen of Q224, and this could result in a

Table 1. A Comparison between the Desensitized $GABA_A$ β3 Homomer and the Open and Closed States of GLIC and GluCl in the TM2/TM3 Region at the Top of the Channel Pore

	GLIC Open State	GLIC Closed State	GluCl Open State	GluCl Closed State
β3 $GABA_A$ homomer	1.67 Å	4.66 Å	1.60 Å	4.65 Å

Values shown are root mean square differences in Å. The Cα carbon atoms of residues 263 to 281 in all five chains of the β3 homomer were superposed with either residues 237 to 255 of GLIC or residues 258 to 276 of GluCl in their open and closed states.

$GABA_A$ = γ-aminobutyric acid type A receptor; GLIC = *Gloeobacter* ligand–gated ion channel; GluCl = glutamate-gated chloride channel; TM2 = transmembrane domain 2; TM3 = transmembrane domain 3.

strong hydrogen bond. This is similar to the tighter of the two binding sites in human serum albumin where the propofol oxygen is 3.1 Å from a main-chain carbonyl oxygen.[26] A second putative propofol-binding site, site 2, is essentially the same as that previously identified using homology modeling[14] and is illustrated in figure 2B. This site lies mainly between TM2 and TM1 in chain A, with some interactions with residues in TM2 from the adjacent subunit, chain B. The polar hydrogen of this propofol molecule could hydrogen bond with the oxygen atom of T225. However, propofol is predicted from the docking algorithm to bind significantly weaker than in site 1, with a predicted binding energy of −6.0 kcal/mol, which corresponds to a dissociation constant of about 40 μM. (The top four binding modes in site 2 had binding energies ranging from −6.0 to −5.4 kcal/mol.)

Both sites lie within the region of maximum movement during channel opening and closing. This is shown in figure 3. Figure 3A shows the alignment of the amino acid sequences of GLIC (Q7NDN8) and GluCl (G5EBR3) with the three β subunit sequences (β1, β2, and β3) from the human $GABA_A$ receptor (P18505, P47870, and P28472, respectively) over the regions of the sequence containing the amino acid residues in both of the predicted propofol-binding sites. Comparison between which amino acid residues move on channel opening and those amino acids that line the binding cavities are shown in figure 3, B and C. These graphs show the movement (in Å) of the Cα carbon atoms, averaged over all five subunits for GLIC (fig. 3B) and GluCl (fig. 3C). Superimposed are red circles that indicate all the residues that line the propofol-binding site 1 and red crosses that indicate all the residues that line the propofol-binding site 2. There is a strong correspondence between those residues that move most on channel opening and those that line the cavities.

I also investigated a site at the lipid–protein interface, which has been proposed as a propofol-binding site based on photolabeling of α1β3 $GABA_A$ receptors by the propofol analog AziP*m*.[15] Photolabeling identified two residues in the β3 subunit (M286 in TM3 and M227 in TM1) and two residues in the α1 subunit (M236 in TM1, equivalent to M227 in the β3 subunit and I239 in TM1, equivalent to I234 in the β3 subunit). Docking centered on this region (20-Å cube centered on F289) confirmed a cavity exists in the crystal structure of the β3 homomer that is lined with seven residues (M227, L231, I234, in TM1 and M286, F289, V290, and F293 in TM3), which included the four residues, or their equivalents in α1, that were photolabeled by AziPm.[15] Docking into this site, with these residues allowed to be flexible, gave a predicted binding energy of −6.4 kcal/mol, which corresponds to a dissociation constant of 22 μM. (The top four binding modes in this site had binding energies ranging from −6.4 to −6.2 kcal/mol.) This is comparable to the weaker of the two binding sites identified above. However, this site is not state-dependent, with none of these residues moving much on channel opening or closing, based on the structural changes that occur in GLIC and GluCl (see black circles in fig. 3, B and C). It should be stressed, however, that these calculations were done on a homomeric, not a heteromeric, structure.

Changes in the Propofol-binding Sites on Channel Closure

Figure 4, A and B shows 12-Å sections through the $GABA_A$ β3 structure perpendicular to the transmembrane helices at the level of the two propofol-binding sites adjacent to H267. Both sites lie between TM1 and TM2 in the same subunit and the two TM2 domains of neighboring subunits. Figure 4, C and D shows the movements of these transmembrane

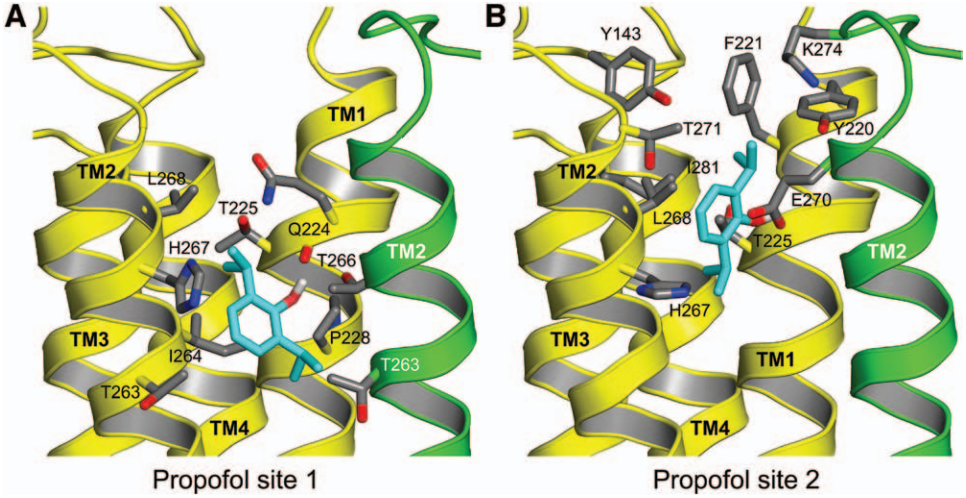

Fig. 2. Molecular docking identifies two propofol-binding sites in the γ-aminobutyric acid type A β3 homomer adjacent to H267. (*A*) Propofol is predicted to bind in site 1 with a dissociation constant of 0.7 μM. The propofol molecule (carbon atoms colored *cyan*) lies in a predominantly hydrophobic cavity, mainly between the TM2 transmembrane domains of adjacent subunits. (*B*) Propofol is predicted to bind in site 2 with a dissociation constant of about 40 μM. The propofol molecule (carbon atoms colored *cyan*) lies in a predominantly hydrophobic cavity mainly between the TM1 and TM2 domains of a single subunit. All of the residues shown were allowed to be flexible during docking and are shown in their final configurations. Subunit A is colored *yellow* and subunit B is colored *green*. TM1-4 = transmembrane domains 1-4.

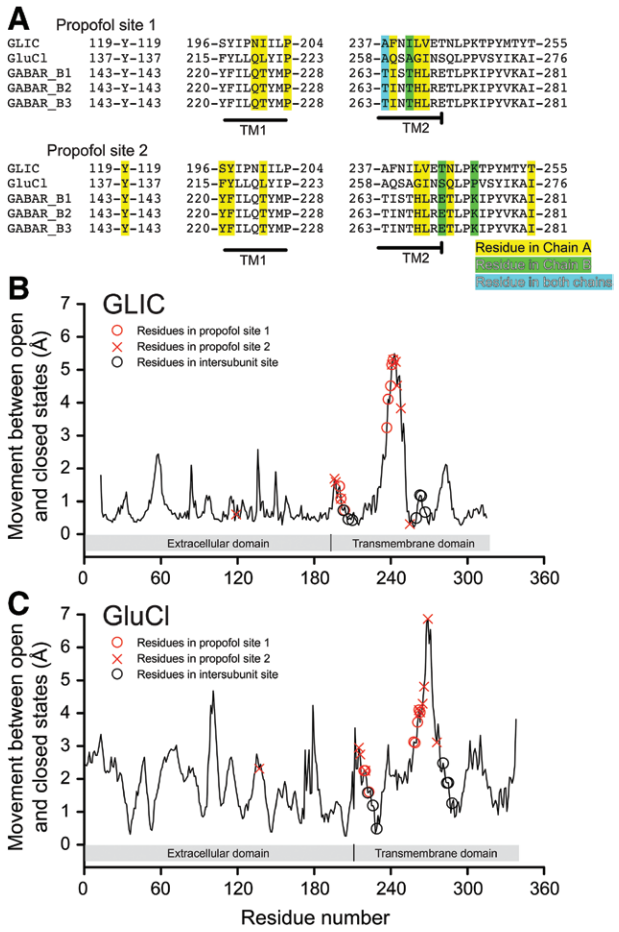

Fig. 3. There is a correspondence between which amino acid residues move most on channel opening or closing and those that line the two putative propofol-binding sites adjacent to H267. (*A*) Segments of a sequence alignment between *Gloeobacter* ligand–gated ion channel (GLIC), glutamate-gated chloride channel (GluCl), and the β subunits of the human γ-aminobutyric acid type A receptor (GABAR). Only three segments containing the amino acid residues that surround the putative anesthetic binding cavities are shown. Highlighted in *yellow* are residues in chain A, highlighted in *green* are residues in chain B, and highlighted in *blue* is one residue that is in both chain A and chain B. (*B*) The movements of the amino acid residues of GLIC, averaged over the five subunits, following the opening (or closing) of the channel. (*C*) The movements of the amino acid residues of GluCl, averaged over the five subunits, following the opening (or closing) of the channel. In *B* and *C*, the *red circles* highlight the residues that were identified as lining propofol-binding site 1 and the *red crosses* indicate the residues that were identified as lining propofol-binding site 2; both coincide with local hot spots of channel movement. The *black circles* indicate residues that line a site[15] at the interface between subunits at the protein–lipid interface. TM1-2 = transmembrane domains 1 to 2.

helices on channel closing for both GLIC (fig. 4C) and GluCl (fig. 4D). For both channels, the relative movements of TM1 and TM2 are very similar. (For GluCl, but not for GLIC, there is also a movement of TM3, but this is not relevant to the environment of the propofol-binding sites.)

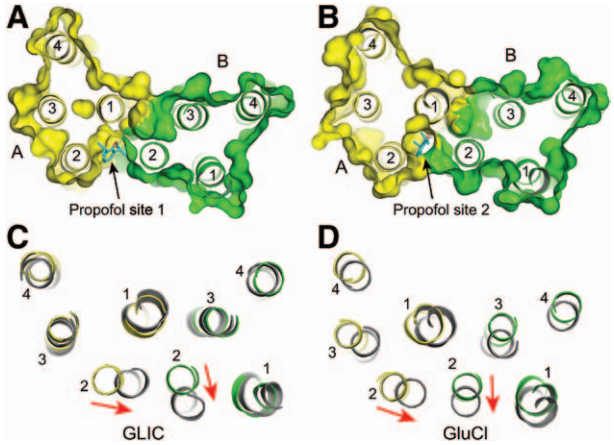

Fig. 4. The propofol-binding sites adjacent to H267 would be disrupted on channel closure. (*A*) A 12-Å slab through the γ-aminobutyric acid type A β3 homomer structure perpendicular to the transmembrane segments, centered at the level of propofol-binding site 1. This site is mainly sandwiched between the transmembrane domain 2 of adjacent subunits. (*B*) A 12-Å slab through the γ-aminobutyric acid type A β3 homomer structure perpendicular to the transmembrane segments, centered at the level of propofol-binding site 2. This site is mainly sandwiched between the transmembrane domains 1 and 2 of the same subunit. (*C*) The movements in the transmembrane domains that occur in *Gloeobacter* ligand–gated ion channel (GLIC) on channel closure, illustrated at the level of the propofol-binding sites. (*D*) The movements in the transmembrane domains that occur in glutamate-gated chloride channel (GluCl) on channel closure, illustrated at the level of the propofol-binding sites. Subunit A is colored *yellow* and subunit B is colored *green*. In (*C*) and (*D*), both subunits are colored *gray* in the closed channel.

Table 2 shows the distances between TM1 and TM2 in the same subunit and the two TM2 helices in adjacent subunits for both the open and closed states of GLIC and GluCl. These distances were calculated for the Cα carbon atoms at the level of the propofol-binding sites (residues 200 to 204 for GLIC TM1, residues 237 to 241 for GLIC TM2, 219 to 223 for GluCl TM1, and residues 258 to 262 for GluCl TM2). It is clear from these calculations that the major effect on channel closure is the coming together (by about 2.5 Å) of the TM2 helices between neighboring subunits, whereas the distance between the TM1 and TM2 helices in the same subunit is essentially unchanged. An inspection of figure 2 and figure 4, A and B shows that both propofol sites would be affected by the relative movement of the TM2 domains, with the impact probably being greater for propofol site 1.

Discussion

The idea that general anesthetics act by binding to cavities on proteins, and that some conformational states are favored if anesthetic-binding sites fortuitously exist on those conformations, but not on others, is an old one.[16,27] It is an extension of conventional thinking about the natural allosteric transitions that occur between different functional states

Table 2. Movements of the Transmembrane Domains on Channel Closing

	Channel and State			
	GLIC Open State	GLIC Closed State	GluCl Open State	GluCl Closed State
Distance between TM1 and TM2 in same subunit	11.0 Å	11.1 Å	12.7 Å	13.4 Å
Distance between TM2 domains in adjacent subunits	11.7 Å	9.0 Å	11.7 Å	9.2 Å

These movements are calculated for the average coordinate of the Cα carbon atoms in TM1 and TM2 at the level of the propofol-binding sites.
GLIC = *Gloeobacter* ligand–gated ion channel; GluCl = glutamate-gated chloride channel; TM1 = transmembrane domain 1; TM2 = transmembrane domain 2.

of a protein, which are governed by the concentration of an endogenous ligand and the relative affinities of the ligand to these different conformational states.[28] In the context of general anesthetics acting on $GABA_A$ receptors, this model has been refined and extended and accounts well for the molecular pharmacology of anesthetics binding to $GABA_A$ receptors.[7,25,29] Anesthetics binding to a cavity or cleft on a protein and displacing water, with little change in the structure of the protein, is also consistent with both thermodynamic[30] and structural observations.[26,31–33]

If this view is correct, then in addition to trying to identify anesthetic-binding sites experimentally, by mutagenesis or photolabeling, an inspection of likely anesthetic targets in different conformation states, could provide an insight as to where the anesthetics might bind. There are now two ligand-gated ion channels whose structures are known at high resolution in both the open and closed states—GLIC[17,18] and GluCl.[19,20] The first caveat that comes to mind immediately, however, is that ligand-gated ion channels exist in a complex free-energy landscape where changes in conformation can occur with minimal changes in energy. Hence, relating the state of an ion channel that has been crystallized, usually with detergents, to a known functional state is difficult. Nonetheless, the case that the two GLIC structures (3EAM and 4LMK) and the two GluCl structures (3RHW and 4TNV) are in the open and closed states is quite convincing. In any event, they certainly represent structures that exist in local energy minima and are thus likely to be functionally significant.

When comparing the two GLIC structures, what is remarkable is how the conformational changes that occur on channel opening (or closing) are restricted to a small region of the channel at the top of TM2. The changes in the extracellular domain are much smaller, perhaps not surprising because the ligand is a proton so only minor structural changes may affect gating. With the two GluCl structures, the changes in the extracellular domain are considerably larger; however, there is a remarkable correspondence with GLIC in the transmembrane domain, with a very similar hotspot of movement centered at the top of TM2. This same region has recently been identified[34] as a key transduction site for anesthetic action (which does not preclude, of course, that it is also part of, or close to, a binding site).

This region of the receptor at the top of TM2 is also the site identified by photolabeling using the propofol analog *ortho*-propofol diazirine. The recent publication of the structure of a $GABA_A$ receptor consisting of a homomer of β3 subunits,[21] one of the receptors that was used in the photolabeling studies,[14] allowed propofol binding to this site to be explored using molecular docking. The structure has an agonist bound but a closed channel pore, the definition of a desensitized state. However, this structure closely resembles an open channel at the extracellular end of the transmembrane domain.[21] Moreover, kinetic models have predicted[7,25] that propofol most likely binds to both the open and desensitized states, so docking to the β3 homomer seems reasonable. The two putative binding sites identified were both immediately adjacent to the amino acid H267 that was photolabeled.[14] One site was essentially the same at that previously proposed from homology modeling,[14] but a new site was identified, with a predicted dissociation constant of 0.7 μM, which is close to the estimated clinically relevant concentration of about 0.4 μM.[35] This higher affinity site is particularly attractive because it certainly would be disrupted, if not eliminated, on channel closure; hence, it would account for the ability of propofol to favor channel opening.

Docking has been used previously to investigate anesthetic binding to model proteins.[36] One finding of particular relevance to this study is that docking of propofol into GLIC successfully located a binding site that had been identified crystallographically.[33] This site, which is quite distinct from those that I am predicting for mammalian receptors, lies largely between TM1 and TM3. Interestingly, the amino acid residues that coordinate the propofol molecule in GLIC do not change significantly on channel opening or closing (I calculate a root mean square deviation of only 0.97 Å), suggesting this is not a state-dependent site; it is hard to see, therefore, how such a site could be pharmacologically relevant because it would be expected to bind equally to the open and closed states of the channel.

It is important to note that the structural comparisons I have made are for β homomers, and the $GABA_A$ receptor exists as a heteropentamer, and an intersubunit propofol-binding site has been proposed based on photolabeling studies on α1β3 heteromers using a propofol analog AziP*m*.[15] There is experimental evidence to support the idea that other anesthetics may also bind in this region of the receptor.[10,12,15,37,38] My docking calculations support the existence of such a site, albeit a site to which propofol binds with relatively low affinity. However, docking to this site really needs to be done using a heteromeric receptor as a template, and equally importantly, with lipids present. The lipids might improve affinity by

providing additional binding interactions, but might greatly weaken apparent affinity by competing for binding. What is clear is that the intersubunit site identified by the docking calculations I have presented is not state-dependent based on the movements that occur in GLIC and GluCl.

For the two binding sites that lie adjacent to H267, the majority of the interactions between propofol and the receptor lie within one β subunit. Moreover, the interacting residues in the neighboring subunit (two in site 1 and two in site 2), which could conceptually be an α or a γ subunit, are well conserved across α, β, and γ subunits, two of them (equivalent to T263 and K274 in the β subunits) being identical. Hence, the binding sites, although based on a β homomer, may be similar in heteromeric receptors. A combination of X-ray crystallography and site-directed mutagenesis will be required to confirm this.

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Competing Interests

The author declares no competing interests.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Sailor Advertising Card for "Compound Oxygen"

In the 1890s, Drs. Palen and Starkey of Philadelphia used the image of a sailor on the front of one of their advertising cards for the panacea "Compound Oxygen." (Sadly, on the card depicted above, both the banner inset from the back and the sailor image from the front have been damaged by a careless collector who had glued the card into a scrapbook.) Compound Oxygen was assailed by Dr. Samuel S. Wallian, who noted that, "the trash they [Palen and Starkey] send to their mail correspondents … is a barefaced swindle and utterly worthless, being nothing more than a weak solution of nitrates of lead and ammonium, or of ammonium muriate and St. Croix rum." (Copyright © the American Society of Anesthesiologists, Inc.)

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