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Unresponsive Correlated Motion in $\alpha 7$ nAChR to Halothane Binding Explains Its Functional Insensitivity to Volatile Anesthetics

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Abstract

Neuronal nicotinic acetylcholine receptors (nAChRs) have been implicated as targets for general anesthetics, but the functional responses to anesthetic modulation vary considerably among different subtypes of nAChRs. Inhaled general anesthetics, such as halothane, could effectively inhibit the channel activity of the $\alpha 4\beta 2$ nAChR, but not the homologous $\alpha 7$ nAChR. To understand why $\alpha 7$ is insensitive to inhaled general anesthetics, we performed multiple sets of 20-ns molecular dynamics (MD) simulations on the closed- and open-channel $\alpha 7$ in the absence and presence of halothane, and critically compared the results with those from our studies on the $\alpha 4\beta 2$ nAChR (Liu et al., *J. Phys. Chem. B* **2009**, 113, 12581; *J. Phys. Chem. B* **2010**, 114, 626). Several halothane binding sites with fairly high binding affinities were identified in both closed- and open-channel $\alpha 7$, suggesting that lack of sensitive functional responses of the $\alpha 7$ nAChR to halothane in the previous experiments was unlikely due to lack of halothane interaction with $\alpha 7$. The binding affinities of halothane in $\alpha 7$ seemed to be protein conformation dependent. Overall halothane affinity was higher in the closed-channel $\alpha 7$. Halothane binding to $\alpha 7$ did not induce profound changes in $\alpha 7$ structure and dynamics that could be related to the channel function. In contrast, correlated motion of the open-channel $\alpha 4\beta 2$ was reduced substantially in the presence of halothane, primarily due to the more susceptible nature of $\beta 2$ to anesthetic modulation. The amphiphilic EC/TM interface of the $\beta 2$ subunit is attractive to halothane and is susceptible to halothane perturbation, which may be why $\alpha 4\beta 2$ is functionally more sensitive to halothane than $\alpha 7$.

Keywords

anesthesia mechanism; halothane; nicotinic acetylcholine receptor; $\alpha 7$; $\alpha 4\beta 2$; membrane protein; molecular dynamics simulation

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Supporting Information Available Eight supporting figures and three additional tables are available free of charge via the Internet at <http://pubs.acs.org>.

INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) are comprised of α ($\alpha 2$ – $\alpha 10$) and β ($\beta 2$ – $\beta 4$) subunits. Different combinations of these subunits result in homo- ($\alpha 7$ – $\alpha 10$) or heteropentameric ligand-gated ion channels with diverse functional characteristics. Some of the neuronal nAChRs have been identified as possible targets of general anesthetics.^{1, 2} Their channel activity could be inhibited by anesthetics at clinically relevant concentrations. Two nAChR subtypes, $\alpha 7$ and $\alpha 4\beta 2$, are found in high abundance in the brain.^{3, 4} Despite high sequence homology ($\alpha 7$ has 62% and 57% sequence homology with $\alpha 4$ and $\beta 2$, respectively), they exhibit substantial differences in their response to inhaled anesthetics. The $\alpha 4\beta 2$ nAChR is sensitive to inhaled general anesthetics, while $\alpha 7$ is considerably less sensitive.^{5, 6}

The underlying cause for the different functional responses of $\alpha 7$ and $\alpha 4\beta 2$ to general anesthetics remains unclear. Discovering the cause may advance the current understanding of anesthetic action on the $\alpha 7$ and $\alpha 4\beta 2$ nAChR as well as homologous proteins. There are at least three possibilities that can contribute to the differences observed on $\alpha 7$ and $\alpha 4\beta 2$. First, inhaled anesthetics interact with $\alpha 4\beta 2$, but not with $\alpha 7$. Second, anesthetics interact with both proteins, but in distinctive regions of the proteins and with different binding affinities. Anesthetics may bind to $\alpha 4\beta 2$ in the regions that are important to the protein function, while to $\alpha 7$ in the regions that have no strong impact to the function of $\alpha 7$. Third, anesthetics may act similarly in both proteins, but $\alpha 7$ and $\alpha 4\beta 2$ react differently to the perturbation of anesthetics due to their intrinsic structural and dynamical difference. Ideally, these possibilities can be tested by experiments that provide high-resolution structural and dynamical information. Some recent experimental successes on homologous proteins certainly encourage studies probing anesthetic interaction with $\alpha 7$ and $\alpha 4\beta 2$ in high resolution.^{7–9} However, technical issues present a formidable obstacle in the progress of experimental structural study on $\alpha 7$ and $\alpha 4\beta 2$.

Complementary to the experimental effort, the computational approach provides meaningful predictions that can be substantiated by experimental evidence. Modeling, MD simulations, and normal mode analysis have illustrated structural and dynamic features for nAChRs and predicted plausible channel gating mechanisms.^{10–14} MD simulations and various experiments have revealed the importance of the interface of the extracellular (EC) domain and the transmembrane (TM) domain in the channel function, especially the interplay of the TM2–TM3 linker with the Cys and $\beta 1$ – $\beta 2$ loops.^{15–17} Computations have also provided insights for anesthetic binding to proteins and anesthetic effects on protein structures and dynamics.^{18–23} These insights were often used to guide experimental design or add more details to the experimental findings.^{18, 23–28}

In this study, we investigated how the inhaled general anesthetic halothane interacted with the $\alpha 7$ nAChR over 20-ns MD simulations on the closed- and open-channel $\alpha 7$ in the absence and presence of halothane molecules. To discover the reason why $\alpha 7$ is much less sensitive than $\alpha 4\beta 2$ to anesthetic inhibition, the simulation results of $\alpha 7$ were compared with the data from the previous MD simulations on $\alpha 4\beta 2$.^{21, 22} Halothane was found to interact with $\alpha 7$ directly. The halothane binding sites in $\alpha 7$ were distributed in EC and TM domains as well as the EC/TM interface, which was also the case in $\alpha 4\beta 2$. The correlated motion between EC and TM domains, particularly at the EC/TM interface, was found resilient to halothane perturbation in $\alpha 7$ and $\alpha 4$, but not in $\beta 2$. Because of its unique EC/TM interface, the $\beta 2$ subunits may bring the $\alpha 4\beta 2$ nAChR to react more sensitively to halothane disturbance. Dynamic susceptibility to halothane perturbation in the $\beta 2$ subunit has been perceived as a reason why $\alpha 4\beta 2$ is functionally more sensitive to halothane than $\alpha 7$.

METHODS

The open- and closed-channel $\alpha 7$ systems were prepared using the same method reported previously.²⁹ Briefly, the sequence of human $\alpha 7$ nAChR (P36544) was obtained from the ExPASy Molecular Biology Server (<http://us.expasy.org>).³⁰ The closed-channel $\alpha 7$ structure was generated by homology modeling using the structure of *T. marmorata* nAChR³¹ as a template (pdb code: 2bg9). The resulting closed-channel $\alpha 7$ model was energy minimized for 10,000 steps with a 500 kcal/mol/Å² harmonic restraint on its backbone atoms using NAMD 2.6³² and evaluated subsequently using the PROCHECK program.³³ The open-channel $\alpha 7$ was generated in the same way as we did previously for generating open-channel $\alpha 4\beta 2$ nAChR.²⁹ Elastic-network normal mode analysis (NMA)³⁴ was performed on the closed-channel $\alpha 7$ nAChR using the online *elNémo* server.³⁵ The lowest frequency eigenvectors, corresponding to a twist-to-open motion, were applied to the closed-channel $\alpha 7$ model through multiple cycles. In each cycle, heavy atoms of $\alpha 7$ experienced only small displacements, the model was energy minimized, and the pore radius was evaluated using the Hole program.³⁶ The final minimum pore radius at the hydrophobic girdle was 4.0 Å, which was close to the experimentally obtained value of 3.7 Å for the open-channel.³⁷ Two nicotine molecules were docked at the agonist-binding sites for open-channel $\alpha 7$. The choice of nicotine rather than other agonists was based on our comparative studies on $\alpha 4\beta 2$,^{21, 22, 29} in which nicotine was used as an agonist for the open-channel simulations because of its high affinity to $\alpha 4\beta 2$ and the availability of the nicotine-bound AChBP crystal structure.³⁸ The open- and closed-channel $\alpha 7$ were separately immersed into a previously prepared water and lipids box^{29, 39} and energy-minimized for 50,000 steps with a harmonic restraint of 500 kcal/mol/Å² on protein backbones.

All MD simulations were performed using NAMD 2.6³² and CHARMM27 force-field parameters⁴⁰ on BigBen at the Pittsburgh Supercomputer Center. In order to be comparable with the simulations for $\alpha 4\beta 2$,^{21, 22} the constant pressure of 1 atm and the constant temperature of 303 K (NPT ensemble) were also applied to the $\alpha 7$ simulations. Each open- and closed-channel system first went to a NPT equilibration with an initial harmonic restraint of 250 kcal/mol/Å² applied to the protein Ca atoms. The restraint was removed gradually over a ~2 ns time period. The systems then went to unrestrained simulations for multiple ns before taking snapshots of the open- and closed-channel $\alpha 7$ for halothane docking. The docking to the $\alpha 7$ snapshots at four different time points (3.5, 4.5 5.5 and 6.5 ns) showed similarities (Figure S1 and S2). Halothanes present in the snapshot at 5.5 ns were carried on for additional 20-ns simulations. The subsequent 20-ns MD simulations were performed on four individual systems: a closed channel without halothane, a closed channel in the presence of halothane, an open channel without halothane, and an open channel in the presence of halothane. Each halothane system underwent two additional replica simulations with different random seeds, yielding three statistically independent trajectories for each halothane system. The same protocols reported previously were used for the MD simulations.^{21, 29} Halothane parameters for docking and MD simulations were taken from our previous publication.⁴¹

The initial halothane sites in $\alpha 7$ were identified using Autodock4,⁴² through a Lamarckian genetic algorithm with a grid spacing of 0.375 Å. Structural snapshots of both closed- and open-channel $\alpha 7$ at aforementioned time points of simulations were collected for halothane docking. We performed 500 independent halothane dockings on each of these structures. Based on the binding energies and occupancies from docking and similarity to the sites observed in *T. marmorata* nAChR,⁴³ five and four high halothane occupancy sites were determined for the open- and closed-channel $\alpha 7$, respectively. One halothane was manually placed at the interface of the extracellular (EC) and transmembrane (TM) domains in the closed channel for the purpose of comparison with the open channel. Thus, five halothane molecules were present in both open- and closed-channel systems for MD simulations. Detailed descriptions of the

selection of halothane binding sites, including the related figures and tables (Figures S1 and S2, Tables S1 and S2) have been provided in the Supporting Information.

Halothane binding energies ($\Delta\Delta G_{\text{binding}}$) were calculated for each halothane site in both open- and closed-channel $\alpha 7$ using the Free Energy Perturbation (FEP)^{44, 45} implementation in NAMD-2.6.³² The same calculation protocol used previously for the $\alpha 4\beta 2$ systems²¹ was adopted in the calculations for $\alpha 7$ systems except that the time step was changed from 2 fs to 1 fs. The $\Delta\Delta G_{\text{binding}}$ is defined as the subtraction result of the free energy of ligand water interactions ($\Delta G_{\text{L, W}}$) from that of ligand protein interactions ($\Delta G_{\text{P, L}}$).

The Gaussian network model (GNM)^{46, 47} was used to analyze if halothane had any effect on the global dynamics of the $\alpha 7$ nAChR. C α atoms represented residues and the interactions were cutoff at 10 Å. The five slowest modes of the GNM calculations on the structures after 20-ns MD simulations were included in the data analysis.

The pore radius profiles were obtained using the HOLE program³⁶ with a step size of 0.25 Å along the pore axis. Average profiles for both open- and closed- channel $\alpha 7$ were calculated using 100 frames from the last 1-ns simulation. The water profiles within the pore at each simulation time point were calculated by counting the number of water molecules within 2.5 Å windows along the pore axis. Each reported water profile resulted from the average of profiles at 100 time points over the last 1 ns of simulation.

The VMD program³² was used for visualizing MD trajectories, generating figures, and calculating Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF). RMSF was calculated on the data sampling the last 7-ns simulations of the $\alpha 7$ systems. For comparison, the same data analysis was also performed on the $\alpha 4\beta 2$ data acquired previously.^{21, 22}

RESULTS AND DISCUSSION

Closed and Open-channel $\alpha 7$ Models

The closed- and open-channel $\alpha 7$ models (Figure 1) were generated through the same strategy as used for previously constructing $\alpha 4\beta 2$ nAChRs.²⁹ Subunit packing and channel integrity were maintained over the course of 20-ns of simulation. RMSD of backbone atoms reached plateaus gradually in both conformations and stabilized in the last 7 ns of the simulations. At the end of the simulations, the pore radius at the hydrophobic girdle was ~4.0 Å and ~2.2 Å for the open channel and closed channel, respectively (Figure S3). The constricting residues for the closed channel were L256 and to a lesser extent V252, in agreement with Law's $\alpha 7$ model.¹³ Interestingly, the narrowest pore region (~3.0 Å radius) in our open-channel model occurred at residue T245, which is close to the intracellular (IC) end of the channel. In the narrow pore region, the hydroxyl group of T245 could interact effectively with an ion to facilitate partial desolvation of the ion, and thus assist ion permeation through the pore. The pore profile with a wide-open EC entrance and a much narrower IC exit (presumably for Na⁺) for the open-channel of $\alpha 7$ is quite similar to that found in the X-ray structure of GLIC.⁸

Halothane in $\alpha 7$

Although previous experiments suggested that halothane had no functional impact on the $\alpha 7$ nAChR,^{5, 6} we found multiple halothane binding sites in both the closed- and open-channel $\alpha 7$ nAChR (Figure 1). Most halothane molecules did not move significantly away from their initial positions during the simulations. Final halothane sites in the three independent simulations mostly overlapped (Figure S4). The binding energies of halothane molecules at

their final sites of the first 20-ns simulations were calculated using the FEP method and summarized in Table 1.

Several features are noteworthy. First, halothane-binding sites sampled all representative regions of the $\alpha 7$ nAChR, including the EC and TM domains and the EC/TM interface. Second, most binding sites were at comparable locations in the closed- and open-channel $\alpha 7$ nAChR. Third, halothane binding energies varied significantly among individual sites. Halo-5, surrounded primarily by non-polar residues at the intracellular end of the TM domains, showed the lowest binding energy in both channel conformations. Halo-3 and halo-4 at the EC/TM interface exhibited lower binding energies in the closed channel than in the open channel. However, halo-1 and halo-2 in the EC domain had lower binding energies in the open channel. Collectively, the variation in binding energies reflected differences in local binding environments. Some details on halothane binding environment are provided in Figures S5 and S6.

Despite the existence of multiple halothane sites in $\alpha 7$, the pattern of halothane-binding sites in $\alpha 7$ did not show the five-fold symmetry that one might expect from a homopentamer, such as the $\alpha 7$ nAChR. This is understandable if one considers asymmetric motion in $\alpha 7$.^{10, 11, 48} Asymmetric anesthetic bindings in a highly symmetric protein assembly were also observed in high-resolution X-ray structures of apoferritin complexed with anesthetics,^{23, 49} in which motion-induced subtle changes at potential binding sites might differentiate one equivalent site from another.

Differences of halothane behavior in $\alpha 7$ and $\alpha 4\beta 2$

Multiple halothane sites in the $\alpha 4\beta 2$ nAChR were identified by our previous computational studies.^{21, 22} The existence of halothane binding sites in $\alpha 4\beta 2$ was in good agreement with the finding that the channel function of the $\alpha 4\beta 2$ nAChR could be inhibited by halothane.^{5, 6} In the case of the $\alpha 7$ nAChR, on which halothane showed no obvious impact in electrophysiology measurements,^{5, 6} one might expect no significant halothane binding. However, our data suggested multiple binding sites in $\alpha 7$. Moreover, halothane binding energies in $\alpha 7$ were comparable with those in $\alpha 4\beta 2$.

How can halothane molecules act on $\alpha 7$ differently from $\alpha 4\beta 2$ to induce different functional impacts? First, the halothane sites exclusive to $\alpha 4\beta 2$ may hold primary responsibility for producing functional inhibition. $\alpha 4\beta 2$ had halothane binding sites in the TM domains toward the extracellular end, either between the $\alpha 4$ and $\beta 2$ subunits or within a $\beta 2$ subunit, with K_d values less than 0.2 mM,^{21, 22} while equivalent sites did not exist in $\alpha 7$. Second, it is possible that different functional responses may result from intrinsic differences in the structural and dynamic properties of $\alpha 7$ and $\alpha 4\beta 2$ to similar halothane bindings. Our previous studies demonstrated that a halothane-binding site similar to halo-3 or halo-4 in Figure 1 could elicit considerable structural and dynamic changes in $\beta 2$, but not in $\alpha 4$.^{21, 22} If $\alpha 7$ is similar to $\alpha 4$ in its structural and dynamic responses to halothane binding, insensitive functional response to halothane binding in $\alpha 7$ should be anticipated. Indeed, it seems to be the case.

Distinct response of the EC/TM interfacial structure and dynamics to halothane binding between $\alpha 7$ and $\beta 2$

Interactions of the TM2-TM3 linker with the Cys loop and the $\beta 1$ - $\beta 2$ linker at the EC/TM interface are critical for propagating signals of agonist binding in the EC domain to channel gating in the TM domains.^{29, 50-54} We found previously that such interactions in $\beta 2$ were much more susceptible to halothane perturbation than those in $\alpha 4$.^{21, 22} In this study, we noticed that $\alpha 7$ more closely resembled $\alpha 4$, bearing similar resilience to halothane binding at the EC/TM interface.

Figure 2 compares representative EC/TM interfacial structures of the $\alpha 7$, $\alpha 4$, and $\beta 2$ subunits in the open-channel conformations in the absence and presence of halothane at the end of 20-ns simulations. The interactions between the EC and TM domains are dominated by hydrophobic contacts. Although some halothane molecules were initially docked at the EC/TM interface in $\alpha 7$ and $\alpha 4$, they moved away during the simulations (Figure S7) and made no strong impact to the interfacial interaction. In contrast, the hydrophobic interaction in the same region of the $\beta 2$ subunit was disrupted by polar contacts (Figure 2C) and salt bridges (R48 and D268 or D140 and K274),²⁹ creating a more amphiphilic environment. A halothane molecule moved between the Cys loop and TM2-TM3 linker of the $\beta 2$ subunit, weakened the interfacial interactions, and consequently generated a wide gap between the EC and TM domains (Figure 2F). Our early experiments proved that anesthetics prefer an amphiphilic environment to a hydrophilic region.^{28, 55, 56} Thus, it is understandable why we did not observe the same halothane binding and subsequent effect in either $\alpha 7$ or $\alpha 4$ as that in $\beta 2$.

The cross-correlation maps in Figure 3 integrated all GNM modes that demonstrated motional dependency between $\alpha 7$ residues in the absence and presence of halothane. For comparison, the corresponding maps of $\beta 2$ were included in Figure 3. In the control systems, the Cys loop and the $\beta 1$ - $\beta 2$ linker were strongly correlated with the TM2-TM3 linker in both $\alpha 7$ and $\beta 2$ subunits, indicating the capacity of these contact points within the EC/TM interface to act as the actuation points for propagating signal from the agonist binding site to the channel gate. In the presence halothane, the correlations between these loops and linkers remained almost the same in $\alpha 7$, but diminished in $\beta 2$. Insensitivity of correlated motion of $\alpha 7$ to halothane was substantiated by the results from the other trajectories as well (Figure S8). Such a sensitive response of $\beta 2$ and an insensitive response of $\alpha 7$ to halothane appeared consistent with halothane induced functional responses of $\alpha 7$ and $\alpha 4\beta 2$.^{5, 6}

The data presented in Figures 2 and 3 were from the open-channel systems. One may wonder what happened to the closed-channel $\alpha 7$. Interestingly, overall higher binding affinity of halothane in the closed-channel conformation did not produce a profound effect on the structure and correlated motions of the $\alpha 7$ subunit. A similar phenomenon was also observed in the closed-channel $\alpha 4\beta 2$, where halothane had higher affinity in the closed system and produced less structural and dynamical consequences on the $\alpha 4\beta 2$ than that in the open system.^{21, 22} Taken together, these results suggest that high binding affinity of anesthetics may not be sufficient to assure functional changes in these receptors.

Halothane induced fewer changes in residue flexibility in $\alpha 7$ than in $\alpha 4\beta 2$

To assess whether halothane affected the local motion of $\alpha 7$, we calculated C_{α} RMSF for the closed- and open-channel $\alpha 7$ in both the control and halothane systems. For comparison, the same RMSF analysis was also performed on the previous $\alpha 4\beta 2$ simulation data. As expected, residues in large loops and linkers, particularly at the N- and C-termini, show greater RMSF values than residues in helices or β sheets. Because of this terminal effect, residues at the N- and C-termini were excluded in our examination of halothane's effect on the RMSF. For a clear illustration of RMSF changes between the halothane and control systems, the average ratios of the backbone RMSFs of each subunit type in the halothane system to the control system were calculated. As shown in Figure 4, the average RMSF ratios for the majority of residues have values close to one, indicating that the motion of these residues on the picosecond to nanosecond time scale was almost unaffected by halothane. The elevated ratios around residues 305 to 307 were due to the disconnection between TM3 and TM4. The significant changes in the RMSF ratios of the halothane system to the control system were observed in the A- and F-loops of the $\beta 2$ subunit, but the changes did not occur to $\alpha 7$ and $\alpha 4$. Interestingly, for most residues in the F-loop, their RMSF changes between the control and halothane systems are comparable to the changes between the open and closed channels (Table S3). Thus,

halothane affected the RMSF of these residues in a way as if to shift an open-channel conformation to a closed channel conformation. It is known that the F-loop is important for coupling agonist binding to channel gating.^{52, 57} Mutations in the homologous loops in the δ and ϵ subunits have been shown to cause a dramatic decrease in channel conductance.⁵⁸ The ability of halothane to change the RMSF of the F-loop in $\alpha 4\beta 2$ but not in $\alpha 7$ might be another source differentiating halothane functional impact on $\alpha 4\beta 2$ from $\alpha 7$.

CONCLUSIONS

Our study revealed several important points regarding anesthetic action on $\alpha 7$ nAChR. First, lack of sensitive functional responses of the $\alpha 7$ nAChR to halothane in previous experiments was unlikely due to lack of halothane interaction with $\alpha 7$. Multiple halothane binding sites exist in both closed- and open-channel $\alpha 7$. Some of the sites have fairly high binding affinities. Second, the binding sites and affinities of halothane in $\alpha 7$ are dependent on the protein conformation. Overall halothane affinity was higher in the closed-channel $\alpha 7$, similar to previous observations of $\alpha 4\beta 2$.^{21, 22} Third, halothane binding to $\alpha 7$ did not induce profound changes in the structure and dynamics of $\alpha 7$ that could be related to the channel function. This is probably the most distinctive difference between $\alpha 7$ and $\alpha 4\beta 2$. The favorable interaction between halothane and the amphiphilic EC/TM interface of the $\beta 2$ subunit brings about changes in dynamics at this interface. These changes may be the reason why $\alpha 4\beta 2$ is functionally more sensitive to halothane than $\alpha 7$.

It is worth noting that the previous experiments of anesthetic sensitivity on $\alpha 7$ were performed on the recombinant homomeric $\alpha 7$ nAChR,^{6, 59} as reflected in our homopentameric $\alpha 7$ structural models. But in nature, $\alpha 7$ could also form heteromeric functional channels with other types of subunits. The $\alpha 7$ and $\beta 2$ subunits of nAChR were found co-expressed within individual interneurons, signifying a possibility of the $\alpha 7$ and $\beta 2$ co-assembled nAChR.⁶⁰ Co-expression of the rat $\alpha 7$ and $\beta 2$ in *Xenopus* oocytes resulted in functional nAChR channels that had lower sensitivity to agonists and a slower rate of desensitization than a recombinant homomeric $\alpha 7$ nAChR.⁶¹ Moreover, the $\alpha 7\beta 2$ nAChR, but not the homomeric $\alpha 7$ nAChR, was found to be highly sensitive to functional inhibition by pathologically relevant concentrations of oligomeric forms of amyloid β_{1-42} .⁶² Based on what has been found in our computational study, we predict that halothane will inhibit the function of the $\alpha 7\beta 2$ nAChR, as it does to the $\alpha 4\beta 2$ nAChR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The closed- (A) and open-channel (B) structures of $\alpha 7$ at the end of 20-ns simulations. For clarity, the lipids and water molecules in the simulation systems are not shown. Five halothane-binding sites, labeled 1-5 for each system, at the beginning and end of the simulations are highlighted with transparent and solid halothane molecules in vdW format, respectively. Extracellular (EC) and transmembrane (TM) domains are labeled and the EC-TM interface is marked with a dash line.

Figure 2.

Residue packing of the $\beta 1$ - $\beta 2$ loop, Cys-loop, and TM2-TM3 linker at the EC-TM interface of the open-channel conformations of $\alpha 7$ (A and D), $\alpha 4$ (B and E) and $\beta 2$ (C and F) in the absence (A, B, and C) and presence (D, E, and F) of halothane (black, vdW). The residues are colored according to their types, white: nonpolar; green: polar; red: acidic; blue: basic. The residues in the TM2-TM3 linker are highlighted in grey shadow, the residues in the $\beta 1\beta 2$ loop and the Cys-loop are covered with transparent yellow surface. Nonpolar contacts dominate the interaction at the EC-TM interfaces of all three subunits. Unlike $\alpha 7$ and $\alpha 4$, $\beta 2$ could form salt bridges between K274 and D140 or R48 and D268. Halothane at the $\beta 2$ EC/TM interface introduced a sizeable gap that interrupted the correlated motion between the EC and TM domains.

Figure 3.

The cross correlation maps from 5 lowest frequency modes of GNM analysis for the open-channel $\alpha 7$ (upper right panels) and $\beta 2$ (lower left panels) in the halothane (A) and control (B) systems. The difference (C) between the halothane and control systems (A minus B) shows that $\alpha 7$ displays limited changes in the correlated motion, but $\beta 2$ has a substantial decrease (blue) in its correlated motion between the Cys loop and the TM domains.

Figure 4.

(A) The ratio of the RMSFs in the presence and absence of halothane for the open-channel $\alpha 7$ (black) and $\alpha 4\beta 2$ ($\alpha 4$ -blue and $\beta 2$ -red). The RMSF was calculated using the data from the last 7-ns simulation of each system and averaged for the same type of subunits. Ratios close to 1 indicate that the flexibility of residues in both control and halothane systems are nearly identical. Two significant changes in the presence of halothane are observed in A-loop and F-loop of $\beta 2$. The large variations around residues 305 to 307 were due to the ending residues of TM3 and TM4. (B) The residues around the A-loop (magenta) and F-loop (also referred to as $\beta 8$ -9 loop) in the $\beta 2$ subunit showed a large RMSF increase and decrease in the halothane systems, respectively.

Table 1

Binding energies and disassociation constants of halothane in closed- and open-channel $\alpha 7$ calculated using FEP based on the first simulation.^{44, 45}

Site ID	Binding Energy (kcal/mol)		K_d (mM)	
	open	closed	open	closed
halo-1	-6.3	-3.1	2.9×10^{-2}	5.8
halo-2	-3.7	-2.7	2.1	11.3
halo-3	-5.3	-7.0	1.5×10^{-1}	8.9×10^{-3}
halo-4	-2.9	-6.8	8.1	1.2×10^{-2}
halo-5	-7.3	-8.7	5.4×10^{-3}	5.3×10^{-4}