Molecular Docking of Barbital Enantiomers to the Nicotinic Acetylcholine Receptor: Implications for the Mechanism of Anesthesia

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Abstract

In the design of anesthetics, the elucidation of molecular targets and their mechanisms of action are essential. The GABA_A receptor is known to be an important molecular target involved in producing loss of consciousness. However, the precise anesthetic target site and its characteristics are unclear. To elucidate the characteristics of the anesthetic binding site, we used the nicotinic acetylcholine (nACh) receptor as a model, as it is in the same superfamily as the GABA_A receptor, and the two receptors share a similar structure. In this study, we specifically examined the binding and molecular interactions of barbital enantiomers with the nACh receptor. We used docking simulation to study the binding mode (position, orientation, conformation) of amobarbital, and barbital enantiomers (isobarbital, pentobarbital) with the nACh receptor in its resting state. The nACh receptor structure was obtained from the Protein Data Bank. For flexible docking, the ASEDock 2005 program of the Molecular Operating Environment system was used. Amobarbital docked to the agonist binding site and channel pore of the nACh receptor. (R)- and (S)-isobarbital, and (R)- and (S)-pentobarbital docked to the agonist binding site, and R and S enantiomers docked to positions where the barbital rings were almost superimposed. In a situation where the dominant enantiomeric binding originates from its substructure without chiral point, the enantiomeric contribution to molecular discrimination turns out to be relatively small even if it contains chiral carbon. In this study, the major binding interactions between drug and the receptor were from barbital ring binding. Steric structural differences from chirality of the alkyl side chain did not produce large differences in drug binding forces. Similarly in the anesthetic action site, chiral carbon of the side chain of barbitals may not produce large differences in binding force, as these interactions are the nature of barbital structures. This implies that the barbital anesthetic binding site has no strict selectivity in discriminating between R and S enantiomers.

Key Words: acetylcholine receptor, barbital, docking simulation, enantiomer, molecular target of anesthesia

Area of Interest: Molecular Recognition
1. Introduction

Molecular targets of general anesthesia in the central nervous system (CNS) have not been specified at the neuronal level, particularly with respect to such components as the lipid bilayer, the cytoskeleton, and ion-channels [1]. However, many neuronal ion-channels that are modified functionally by anesthetics at clinically relevant concentrations have been considered as potential targets [2][3].

If stereoisomers of anesthetics produce different functional changes in a macromolecule such as a protein, this should be considered as important evidence that the anesthetic is acting on the macromolecule. Chirality has been proposed as a means for distinguishing molecular targets that are relevant to anesthetic action from those that are not [4]. Isoflurane, etomidate, barbital, certain alcohols, and neurosteroids are known as enantiomeric anesthetics. There is experimental evidence to show that the enantiomers of isoflurane differ by 17-53% [5][6] in their anesthetic immobilizing activity. The minimum alveolar anesthetic concentration (MAC) of the enantiomers of 2-butanol and 2-pentanol differ by 17 and 38% [7], respectively, which is similar to the amount reported for the isomers of isoflurane. Based on these facts, the aim of our current study was to determine how enantiomeric anesthetics bind to the site of a neuronal component, and to determine why there are differences between enantiomers in their mode of binding.

The GABA<sub>A</sub> receptor is an ionotropic receptor and ligand-gated ion channel. It is widely understood that drugs acting at the GABA<sub>A</sub> receptor can produce loss of consciousness through the depression of neuronal excitability and synaptic transmission. In this study, we chose to use the nicotinic acetylcholine (nACh) receptor as a model because this receptor has a structure of five subunits, pseudo-symmetrically arranged with a central ion-conducting pore, and is similar to that of the GABA<sub>A</sub> receptor. Both receptors belong to the same superfamily and have sequence similarity between them [8]. In addition, the 3D structure of the nACh receptor in the resting state has been reported [9][10], which has facilitated more precise research on this receptor. Isoflurane, 2-butanol, 2-pentanol, barbital, ketamine, etomidate, and steroid anesthetics are enantiomeric anesthetics which inhibit the conduction of Na<sup>+</sup> through the nACh receptor [11][12][13]. Barbital have inhibitory actions on the excitatory membrane. However, whether general inhibition of the membrane relates to mechanisms of anesthesia is still a matter of discussion. The goal of our study was to use the structure of nACh to determine how the nACh receptor recognizes barbital enantiomers at the intermolecular level.

The binding mode (distance, orientation, conformation) and binding energy of small molecules to macromolecules can be predicted by flexible docking simulations [14]. This enables the estimation of the binding mode and the anesthetic binding site of the receptor [15]. Using the ASEDock 2005 program [16], and the structure of the nACh receptor, we first searched for the binding site of amobarbital, which has been reported in a binding study [12]. Then, we investigated the binding site of barbital enantiomers in the nACh receptor, as well as their binding mode, and primary binding interactions (i.e. the mechanism of molecular recognition of barbital enantiomers by nACh receptors).

2. Materials and Methods

2.1 Structure preparations for docking
The structures of the nACh receptor in the resting state (PDB: 2BG9) [10] (4 Å resolution), and the acetylcholine-binding protein (AChBP) in complex with nicotine, (PDB: 1UW6) [17], were obtained from the Protein Data Bank (PDB). Missing hydrogen atoms were added and original structures were amended. To avoid overlapping these added atoms with the heavy atoms and other added atoms, their positions were optimized by energy minimization. The complete nACh receptor structure, including hydrogen atoms, was used for examining the flexible docking of barbital. The AChBP structure was determined by removing the coordinates of the nicotine molecule from 1UW6.

2.2 Docking simulations

The Molecular Operating Environment (MOE) 2007.0902 (Chemical Computing Group, Inc., Quebec, Canada) was used to perform the structural preparation, program execution, 3D presentation, and interaction analysis. For docking, the ASEDock 2005 program of the MOE platform [16][18] (Ryoka Systems Inc., Tokyo) was used. Briefly ASEDock is a docking program that uses shape similarity assessments between a concave portion (i.e., concavity) of a protein and its ligand, to search the structures of ligand-receptor complexes. All receptor atoms were fixed for docking. The ASEDock program treats the ligands as flexible molecules. The program allows rotatable bonds of the ligands to produce conformations that fit to the binding region of the receptor while it conserves the configuration of the chiral centers. We adopted the Merck Molecular Force Field 94s (MMFF94s) [19][20] to set partial charge and force-field parameters. Total binding energy and its components, electrostatic (U\textsubscript{ele}), van der Waals (U\textsubscript{vdw}), solvation energy (U\textsubscript{solv}), and ligand energy (U\textsubscript{lig}), were calculated by molecular mechanics using the MOE. For the U\textsubscript{solv} estimation the Generalized Born/Volume Integral Implicit Solvent Model was used [21][22].

The validity of the ASEDock 2005 simulation was confirmed by docking the nicotine to the AChBP structure, and comparing it with the structure of the experimental nicotine-AChBP complex (PDB: 1UW6) [17].

First, amobarbital was flexibly docked to 2BG9 to determine the binding site of the nACh receptor. Then, (R)-isobarbital, (S)-isobarbital, (R)-pentobarbital, and (S)-pentobarbital (Figure 1) were docked to 2BG9.

![amobarbital](image)

![Figure 1. Amobarbital and enantiomers of isobarbital and pentobarbital structures.](image)
2.3 Determination of molecular interactions

Molecular interactions between barbital and its binding site were analyzed and represented using the Ligand Interactions Diagram in the MOE program [23]. Three binding groups (R₁, R₂, R₃) exist around the chiral carbon of the isobarbital molecule. The contribution of each binding group (Rᵢ) around the chiral carbon was estimated from the energy difference between isobarbital binding and the binding of the molecular analog, one of whose groups was substituted with hydrogen (Rᵢ = H). The contribution of the Rᵢ group to binding was estimated by Δ(Rᵢ) = Uₜₐₜₒₜₜ (isobarb) - Uₜₐₜₒₜₜ (Rᵢ = H).

3. Results

To begin our studies, we confirmed the validity of the ASEDock 2005 module of the MOE program. We then used this program to predict the mode of nicotine binding to the AChBP, and compared it with the X-ray structure. We found that the nicotine molecule binds to the agonist binding site located between the α and β subunits. The nicotine binding site from the ASEDock simulation (red) and nicotine (blue)-AChBP complex (1UW6) [17] are also shown in Figure 2 for comparison. The root mean squared deviation (RMSD) between these two was 0.355 Å.

![Figure 2. Confirmation of ASEDock 2005 validity.](image)

Amobarbital binding is shown in Figure 3. Amobarbital bound to the agonist binding site and the pore region of the nACh receptor with a binding energy of -18.8 kcal/mol (rank 1) and -16.2 (rank2) kcal/mol, respectively.

The binding of (R)-isobarbital and (S)-isobarbital is shown in Figures 4A and 4B. (R)- and (S)-isobarbital, and (R)- and (S)-pentobarbital bound to the agonist binding site of the nACh receptor.
Figure 3. Amobarbital binding to agonist binding site (rank 1) and inside pore (rank 2). Binding energies are -18.8 and -16.2 kcal/mol, respectively.

Figure 4. (R)-isobarbital (red) and (S)-isobarbital (light blue) enantiomer binding from independent dockings are represented as a superimposition. A, B: The agonist site of nicotinic acetylcholine receptor. C: Background receptor structure was suppressed for clarity. Barbital rings almost overlapped even though they are enantiomers.
For comparison, each docking site of (R)- and (S)-isobarbital is represented in the superimposed form in Figure 4C. Apparently, binding of the barbital rings of (R)- and (S)-isobarbital almost overlapped with the binding site of the nACh receptor. Molecular mechanics calculations were used to determine total binding energies of (R)- and (S)-isobarbital and their components as shown in Table 1. The difference in the binding energy between these enantiomers was not significant (the accuracy of energy calculations in ASEDock 2005 is several kcal/mol). The enantiomeric binding energies of pentobarbital behaved the same way as isobarbital (Table 2). The solvation energy of the R body complex and that of the S body complex were different. Considering the accuracy the Generalized Born/Volume Integral Solvent Model, we regard this difference to be negligible.

### Table 1. Total binding energy (U\text{total}) of the isobarbital enantiomer and its components at agonist site

|       | U\text{total} | U\text{ele} | U\text{vdw} | U\text{solv} | U\text{lig} |
|-------|----------------|-------------|-------------|-------------|-------------|
| (R)   | -152.65        | -55.52      | -12.95      | 50.48       | -134.66     |
| (S)   | -150.60        | -55.39      | -14.30      | 51.87       | -132.78     |

\[ \text{U\text{total}} = \text{U\text{ele}} + \text{U\text{vdw}} + \text{U\text{solv}} + \text{U\text{lig}} \text{ (kcal/mol)} \]

### Table 2. Total binding energy (U\text{total}) of pentobarbital enantiomer and its components at agonist site

|       | U\text{total} | U\text{ele} | U\text{vdw} | U\text{solv} | U\text{lig} |
|-------|----------------|-------------|-------------|-------------|-------------|
| (R)   | -149.60        | -47.93      | -15.58      | 48.09       | -134.19     |
| (S)   | -150.91        | -48.41      | -14.95      | 45.48       | -133.62     |

\[ \text{U\text{total}} = \text{U\text{ele}} + \text{U\text{vdw}} + \text{U\text{solv}} + \text{U\text{lig}} \text{ (kcal/mol)} \]

The molecular interactions between barbital and the binding cavity was analyzed using the Ligand Interactions Diagrams in the MOE [23]. The interaction of isobarbital with the amino acid residues in the binding cavity are shown in Figure 5. The NH group in the barbital ring of either the (R)- or (S)-isobarbital hydrogen, bonded to Asp152 in the binding site (Figure. 5A, 5B). In addition to this hydrogen bond, the R body of isobarbital hydrogen bonded to Tyr190 of the site (Figure 5A).

Binding groups of (R)-isobarbital around the chiral carbon atom are shown in Figure 6. Binding contributions of substitution groups around the chiral carbon \( \Delta U(R_1) \) were estimated, and are shown in Table 3. The table shows quantitatively that the contribution of \( R_1 \) (i.e., barbital ring) was a major one \( \Delta U(R_1) \gg \Delta U(R_2) = \Delta U(R_3) \). The methyl- and ethyl groups around the chiral carbon interacted via van der Waals interactions.
Figure 5. A: Binding interactions of (R)-isobarbital. B: Binding interactions of (S)-isobarbital (MOE-Interactions).

Figure 6. (R)-isobarbital: binding groups adjacent to the asymmetric carbon.

\[ R_1 = C_4H_2O_3N_2(C_2H_5)-CH_2^- \text{ (barbital ring)}, \quad R_2 = CH_3CH_2-, \quad R_3 = CH_3^- \]

Table 3. Energy contribution of \( R_i \) group binding (\( \Delta U(R_i) \))^a

|      | \( (R) \)     |         | (S)     |         |
|------|-----------|---------|---------|---------|
|      | \( R_1 \) | \( R_2 \) | \( R_3 \) | \( R_1 \) | \( R_2 \) | \( R_3 \) |
| isobarbital | -146    | +4      | +4      | -149    | +4      | +5      |
| pentobarbital | -148    | +6      | +4      | -146    | +3      | +4      |

^a\( R_i \) group contribution \( \Delta U(R_i) \) is estimated by the energy difference between an \( R_i \)-barbital and its hydrogen-substituted form.

\[ \Delta U(R_i) = U_{total}(R_i) - U_{total}(R_i=H) \text{ (kcal/mol)} \]
4. Discussion

In this study, we used the ASEDock 2005 program of the MOE platform to perform docking simulations, as the reliability of this program was previously validated. The predicted and experimentally resolved structures of 59 known complexes were compared, and 98% of the predicted ligand positions were reproduced within 2 Å of the RMSD [16]. In general, if these predictions are within 2 Å RMSD of the experimental observations, they are regarded as having good reproducibility [16]. In this study, we also confirmed the validity of this program using the nicotine-AChBP complex (1UW6) as shown in Figure 2. The predictions were within 0.355 Å RMSD, which is considered to be an exceptionally good result. We therefore consider ASEDock 2005 to be a reliable method for predicting the binding mode in macromolecular structures.

Pentobarbital enantiomers are known to bind to acetylcholine binding sites and inhibit choline-stimulated ion flux [24]. The result of our docking simulation suggests that pentobarbital binds to the acetylcholine binding site. Arias et al. reported that amobarbital binds to the pore region due to the fact that tetracaine [25] or 3-(trifluoromethyl)-3-\((\text{[125I]}\text{ iodophenyl})\) diazirine (TID) [26] (binding site of these two is pore.) competitively inhibit amobarbital binding [12]. Upon close examination of their experimental procedures, we found that they controlled the receptor state, resting and desensitized, in the absence or presence of 1 mM carbamylcholine. They regarded the receptor to be in its resting state in the absence of 1 mM carbamylcholine. However, we believe that the resting state receptor will transit to its desensitized state with their experimental procedure of amobarbital addition [27]. Thus, our results for amobarbital with resting state, which bound to the acetylcholine binding site, are difficult to compare with their experimental results.

In our simulation, amobarbital bound to the pore region of the nACh receptor in resting state (rank 2 in Figure 3), and was the only compound that could bind to the pore site. Other barbitals studied here bound to the nicotine binding site and did not bind to the pore region. Thus, our simulation suggests that the pore site is an inferior binding site compared with the nicotinic binding site for barbitals. Further experimental evidence is necessary to resolve the contribution of the secondary binding sites, i.e., the pore site.

The major finding in this study is the compatibility of \((R)\)- and \((S)\)-barbitals to the agonist binding site of the nACh receptor. First, barbital ring hydrogens bind strongly to the site and the alkyl chain has a small influence on the molecular orientation of the chiral barbital, as seen in Table 3 (in general, the hydrogen bond is considered to be a strong interaction compared with van der Waals interactions [28]). We consider that enantiomeric binding of isobarbital or pentobarbital comes mostly from the barbital ring. Second, total binding energies of \((R)\)-isobarbital and \((S)\)-isobarbital did not differ significantly, and neither did those of pentobarbital isomers (Tables 1 and 2). Third, the relation \(\Delta U(R_1)>>\Delta U(R_2) = \Delta U(R_3)\) was valid from the estimation of \(\Delta U(R_i)\). These facts suggest that the compatibility of enantiomer binding to this site, or rather, the molecular discrimination between \(R\) and \(S\) enantiomers, is relatively weak. The general idea that enantiomers behave like a key in a lock, with an all-or-nothing fit, has been commonly accepted. The concept of enantiomers has led to the belief that molecular discrimination comes from exclusive binding. This study clarifies that in the case of a single binding element around the chiral carbon (the barbital ring in this study is dominant), enantiomeric discrimination in binding would be relatively small. Pentobarbital enantiomers inhibit cholinergically stimulated cation flux, and show a stereoselectivity ratio of 1.5 [24]. Our findings explain this weak selectivity of pentobarbital inhibitions.

The enantiomeric selectivity of barbital binding comes from their structural feature of one-point contact at the site (their binding come mostly from the barbital ring). We can glean insight into the
characteristics of the anesthetic binding site from features of barbitals. The GABA\textsubscript{A} receptor is an important molecular target of anesthesia \cite{1}\cite{3}, as it contains the binding site of barbital. We speculate that barbital binds to the GABA\textsubscript{A} receptor binding site in a one-point contact manner, because one-point binding is in-line with the characteristics of barbital structure. Barbital binding at the action site is presumed to have loose fitting characteristics at the molecular interaction level. In other words, the barbital binding site may have such properties that allow binding modes to not be strictly dependent on the 3D molecular shape of barbital.

5. Conclusion

We studied enantiomeric anesthetic fitting in its binding site to understand how anesthetics recognize these sites. The major binding force of barbital enantiomers turns out to be from barbital ring binding. In the case where a single binding element around an asymmetric carbon is dominant, molecular discrimination between enantiomers turns out to be relatively indistinct, even though asymmetric carbons exist in the anesthetic molecule. The barbital anesthesia site appears to recognize enantiomers indiscriminately.

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