Molecular Discrimination of Barbital Enantiomer at the Propofol Binding Site of the Human β3 Homomeric GABA_A Receptor

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Abstract

Intravenous anesthetic barbitals act on the GABA_A receptor, and this receptor is the principal molecular target of loss of consciousness. Their action and side effects differ according to the enantiomer. Elucidation of their enantiomeric action is essential for a safe anesthetic agent. This study investigates molecular mechanisms of discrimination of enantiomeric barbital in the binding site of the GABA_A receptor. Amobarbital, (R)-, (S)-pentobarbital, and (R)-, (S)-isobarbital bonded to the TM2-TM2’ transmembrane domain (TMD), i.e., the propofol binding site. There was a 2.9 kcal mol\(^{-1}\) difference in enantiomeric pentobarbital bindings. Pentobarbital discrimination in the TM2-TM2’ TMD site was caused not only by the barbital ring’s hydrogen-bond, but also by steric fittings of the methyl-group adjacent to the chiral carbon atom.

Key Words: barbital, enantiomer, propofol, GABA_A receptor, molecular recognition

Area of Interest: Molecular recognition and molecular modeling
1. Introduction

Enantiomers are a pair of two stereoisomers that have different absolute configurations around asymmetric carbon atoms, even those with identical chemical structures in a single symmetric environment. Generally, the enantiomers of an anesthetic have different actions from one another. We can develop safe anesthetics having a small side-action/action ratio by using one distinctively. In clinical practice, barbital anesthetics are provided as a racemi mixture without specifying the enantiomeric ratio. For further safety in anesthetic development, enantiomeric ratio control is indispensable together with clarifying the action and side actions of individual enantiomers [1].

Isoflurane, barbitals, etomidate, and neuro steroids have been reported to have different anesthetic potencies, the minimum alveolar concentration (MAC), between enantiomers [2–5]. Resolution of the action difference and its mechanisms of enantiomer difference, leads to the development of anesthetics with fewer side effects. It remains an unsolved problem to understand how enantiomeric anesthetics recognize and act at anesthetic binding sites. By focusing on the binding mode at the molecular target site of enantiomeric barbital, this study clarifies the mechanisms responsible for molecular discrimination of an enantiomer at its binding site.

The GABA_A receptor (GABA_A,R) has an important role in the loss of consciousness by anesthesia [6, 7], and is a ligand-gated Cl^- channel consisting of pentameric subunits, which exists the post-synaptic membrane of inhibitory neurons of the central nerve system [8]. Potentiation, Cl-current enhancement of channel conduction, is observed at clinically relevant concentrations of anesthetic [9, 10], which plays an important role in the loss of consciousness of anesthesia due to the argument of central nervous system inhibition. Anesthetic binding sites of barbital, propofol, benzodiazepine, etomidate, and ethanol of GABA_A,R have been identified with amino acid residues [11]. In 2014, the three-dimensional structure of GABA_A,R was resolved [8], and a precise discussion based on steric structural information with atomic resolution has become possible.

Molecular docking is a computer simulation that predicts the binding mode, interactions and its energy of small molecules to known structural molecules [12, 13]. Combined with the precise GABA_A,R structure and docking, we undertook to examine the binding site of the enantiomeric anesthetic in GABA_A,R, its binding mode and energy with atomic coordinate accuracy, and to resolve the enantiomeric anesthetic binding mode in GABA_A,R.

We address such questions as to how enantiomeric anesthetics bind to the anesthetic target site, and why there are differences between the enantiomers in their mode of binding. To clarify the mechanisms of enantiomeric molecular recognition and binding to GABA_A,R, we investigated the binding mode and energy of amobarbital, pentobarbital, and isobarbital by using docking simulation.

2. Materials and Methods

2.1 GABA_A,R structure preparations

The three-dimensional structure of the GABA_A,R (4COF) [8] (3Å resolution) was obtained from the Protein Data Bank (PDB). Benzamidine molecules included in the 4COF agonist binding sites were removed for docking. Missing hydrogen atoms of the 4COF structure were amended. To avoid overlapping added hydrogens with the heavy atoms and others, these hydrogen positions were optimized by energy minimization. Protonation of the 4COF at pH 7 was assigned with the MOE.
Protonate 3D program [14]. The complete GABA\textsubscript{A}R structure at pH 7, including hydrogen atoms, was used for examining the flexible docking of barbitals.

2.2 Docking simulation

The Molecular Operating Environment (MOE) 2016.0802 (Chemical Computing Group, Inc., Quebec, Canada) [15] was used to perform the three-dimensional presentation, structural preparation, docking program execution, and interaction analysis. The ASEDock (2016.01.07) program on the MOE platform [16, 17] (MOLSIS Inc., Tokyo) was used for docking with default parameters. The ASEDock treats the ligands as flexible molecules, but maintains the ligand’s chirality. 4COF main chain atoms were fixed, but the side chain was kept flexible for docking. We adopted the Amber10:EHT force field [18, 19] to set partial charge and force-field parameters. Total binding energy was calculated by molecular mechanics using the MOE, within which the Generalized Born/Volume Integral Implicit Solvent Model [20, 21] was used for the solvation energy estimation. For ASEDock validation, benzamidine was docked to GABA\textsubscript{A}R and examined to reproduce benzamidine binding and mode of the 4COF structure.

First, amobarbital was flexibly docked to 4COF to determine the binding site of exhaustive GABA\textsubscript{A}R. Then, (R)-pentobarbital, (S)-pentobarbital, (R)-isobarbital, and (S)-isobarbital were docked to the whole 4COF structure.

2.3 Binding interactions analysis

Molecular interactions between barbital and its binding site were analyzed and represented by using the Ligand Interaction Diagrams in the MOE [22].

3. Results

3.1 Benzamidine binding reproducibility of ASEDock

Benzamidine docked to the 4COF GABA\textsubscript{A}R structure, and reproduced the binding site and mode of the benzamidine-GABA\textsubscript{A}R complex of 4COF.

3.2 Amobarbital binding sites and interactions

Figure 1 shows the four most stable amobarbital bindings of GABA\textsubscript{A}R, which are the agonist binding site (A), TM2-TM2’ inter-subunit transmembrane domain (TMD) site (B), TM2-TM3-TM1’ inter-subunit TMD site (C), and inter-subunit Extracellular domain (ECD) site (D) of GABA\textsubscript{A}R, respectively.

Binding energies of amobarbital calculated by ASEDock are shown in Table 1. Each binding energy of the site was -31.9, -36.5, -31.6 and -31.9 kcal mol\textsuperscript{-1}, respectively, and (B) the TM2-TM2’ site turned out to be the most stable binding site. The binding energy difference between the TM2-TM2’ and TM2-TM3-TM1’ sites turned out to be 4.9 kcal mol\textsuperscript{-1} (Table 1).

From MOE-Interaction analysis of the TM2-TM2’ site binding, NH in the amobarbital ring became a proton donor and hydrogen-bonded to the Gln224 backbone of the TM2-TM2’ site (Figure 2).
Figure 1. Secondary structure presentation of the four most stable amobarbital binding sites (#1–4) of the GABA<sub>A</sub> receptor. Side view (left) and bottom view from cytoplasm (right).

#1: TM2-TM2’ inter-subunit transmembrane domain (TMD) site, #2: agonist binding site, #3: inter-subunit extracellular domain (ECD) site, #4: TM2-TM3-TM1’ TMD site. Each binding energy was -36.5, -31.9, -31.9, and -31.6 kcal mol<sup>-1</sup>, respectively.

Table 1. The two most stable binding energies of barbitals (kcal mol<sup>-1</sup>) at the TM2-TM2’ and TM2-TM3-TM1’ sites

| barbital       | TM2-TM2’ | TM2-TM3-TM1’ |
|----------------|----------|--------------|
| amobarbital    | -36.5    | -31.6        |
| (R)-pentobarbital | -34.4    | -28.3        |
| (S)-pentobarbital | -31.5    | -28.7        |
| (R)-isobarbital | -31.1    | -28.9        |
| (S)-isobarbital | -32.0    | -29.1        |
| propofol       | -26.3    | -23.5        |
Figure 2. Amobarbital (brown) binding and propofol (blue) high affinity site binding. Both anesthetics hydrogen-bonded commonly to the backbone carbonyl of Gln224 at the TM2-TM2’ site.

3.3 Enantiomeric pentobarbital binding of site, mode, and interactions

(R)-pentobarbital and (S)-pentobarbital bindings are shown in Figure 3. (R)- and (S)-pentobarbital bound to the TM2-TM2’ TMD site of GABAAR. In comparison, each docking result of (R)- and (S)-pentobarbital is shown in the superimposed form. While the methyl groups adjacent to the asymmetric carbon atom of (R)- and (S)-pentobarbital are directed oppositely, binding of the barbital rings and ethyl and propyl groups almost overlapped in the TM2-TM2’ sites.
Figure 3. Independent dockings of (R)- and (S)-pentobarbital to the TM2-TM2’ inter-subunit of the transmembrane domain (TMD) of GABA_\text{A} receptor

A, B: These bindings are represented as a superposition of the enantiomers. C: (R)- and (S)-pentobarbital bindings to the TM2-TM2’ inter-subunit of TMD of the GABA_\text{A} receptor. The background GABA_\text{A} receptor structure is suppressed for clarity.

Binding energies of (R)- and (S)-pentobarbital calculated by ASEDock are shown in Table 1. Each binding energy in the TM2-TM2’ site was -34.4 and -31.5 kcal mol\(^{-1}\), respectively. (R)-pentobarbital bound to the TM2-TM2’ site more firmly, by 2.9 kcal mol\(^{-1}\), than (S)-pentobarbital did. Molecular discrimination of the pentobarbital enantiomers turned out to have a 2.9 kcal mol\(^{-1}\) difference due to enantiomeric fitting to the TM2-TM2’ site.

From the MOE-Interaction analysis at the TM2-TM2’ site binding, NH in the barbital ring of pentobarbital became a proton donor and hydrogen-bonded to Glu270 of the TM2-TM2’ site (Figure 4).

(R)- and (S)-pentobarbital’s NH of the barbital rings hydrogen-bonded to the Glu270 side-chain with 176 and 179 pm distances, respectively. Ethyl and propyl groups interact with TM2 His267 and TM2’ Thr263, respectively. Methyl group orientations adjacent to the chiral carbon are directed in opposite ways (Figure 5).
Figure 4. Binding interactions of (R)-pentobarbital with the TM2-TM2’ site and those of (S)-pentobarbital (MOE-interactions) NH’s of the barbital-ring of the enantiomers hydrogen-bonded to Glu270 of the TM2-TM2’ site.

Figure 5. Molecular recognition of the pentobarbital enantiomer in the TM2-TM2’ site NH of barbital rings hydrogen-bonded to the Glu270 side-chain with 176 and 179 pm distances, respectively. Ethyl and propyl groups interact with TM2 His267 and TM2’ Thr263, respectively. Methyl group orientations adjacent to the chiral carbon are directed in opposite ways.
3.4 Enantiomeric isobarbital binding site and energy

Likewise, pentobarbital, (R)- and (S)-isobarbital docked to the TM2-TM2’ TMD site of GABA_A R. Each binding energy in the TM2-TM2’ site was -31.1 and -32.0 kcal mol^{-1}, respectively (Table 1). Also, NH in the barbital ring of isobarbital became a proton donor and hydrogen-bonded to Glu270 of the TM2-TM2’ site.

4. Discussion

Due to a photolabel experiment of GABA_A R with 5-allyl-1-methyl-5- (m-trifluoromethyl)diazirinylphenyl) barbituric acid (m-TFD-MPAB), m-TFD-MPAB bound to the inter-subunit TM2-TM2’ TMD [23]. As that methodology differs from that used in this study, a simple comparison is difficult, but the amobarbital binding site of this study, TM2-TM2’ inter-subunit TMD, is consistent with the photolabeling result of m-TFD-MPAB.

Regarding molecular recognition of enantiomers, it has been understood that the binding cavity exclusively discriminates it from steric fitting like a lock and key. In the results of this study, enantiomers of (R)- and (S)-pentobarbital bound to the same binding cavity (TM2-TM2’ inter-subunit TMD domain) (Figure 3), and they commonly hydrogen-bonded to Glu270 (Figures 4, 5). The R-body bonded more strongly than the S-body by 2.9 kcal mol^{-1}. This corresponds to a 100:1 binding preference from the Boltzmann distribution. The enantiomers turned out to bind to the common binding cavity, but the binding affinity of the enantiomers may differentiate their anesthetic actions.

This study clarified the enantiomeric selectivity from the binding mode analysis of the pentobarbital enantiomer. First, barbital rings of amobarbital and pentobarbital hydrogen-bonded to Gln224, Glu270 of the binding cavity, respectively, which became the principal binding element of barbitals. (Figures 2, 4) Then, a propyl group attached to an asymmetric carbon atom. This became the second binding element in the binding cavity with hydrophobic interactions. Lastly, a methyl group adjacent to the asymmetric carbon atom limited its orientation (Figure 5) and produced a 2.9 kcal mol^{-1} difference in the binding energy by the enantiomer. We have reported that isobarbital binds to the agonist site of the nicotinic acetylcholine receptor, and also in GABA_A R have speculated that the enantiomer binds in a one-point contact manner in-line with the characteristics of the barbital structure [24]. This study also clarifies that the cause of the enantiomeric binding selectivity in the GABA_A R binding site is not simply from binding in a one-point contact manner of the barbital ring, but from steric fitting of methyl group adjacent to an asymmetric carbon atom (steric fitting from a chiral carbon atom).

Amobarbital, pentobarbital, and isobarbital turn out to have a common binding site with propofol. Propofol binding sites of the GABA_A receptor have been investigated precisely and there exist both high and low affinity sites. The TM2-TM2’ inter-subunit TMD site of amobarbital binding is identical with the propofol high affinity site. (Figure 2A) Also, the TM2-TM3-TM1’ TMD site of amobarbital corresponds to the low affinity site of propofol. The binding energy difference of the amobarbital sites is 4.9 kcal mol^{-1}, which corresponds to a 10000:4 ratio of binding distribution. (Table 1) Thus, amobarbital is considered to have selectivity to the high affinity site of propofol rather than the agonist binding site, inter-subunit extracellular ECD, or propofol low affinity site. As for pentobarbital and isobarbital, you will find that they also bind to the propofol high affinity site. Propofol and amobarbital interact to hydrogen-bond to Gln 224 of the TM2 helix, and pentobarbital and isobarbital interact to hydrogen-bond to Glu270 of the helix. Even with their different chemical structures propofol and barbitals hydrogen-bonded to TM2-TM2’ TMD site and
produce loss of awareness. Amobarbital, pentobarbital, and isobarbital are considered to bind to the common binding cavity with hydrogen-bond and to produce a pharmacological action.

So far, potentiation of the GABA$_\text{A}$ receptor, one of the reasons that even anesthetics with their diverse chemical structures produce loss of consciousness, shows that a common binding cavity exists in the GABA$_\text{A}$ receptor and the anesthetics may potentiate that receptor via common interactions. Further study to elucidate the mechanism of the potentiation is necessary [9, 10].

Potentiation of the GABA$_\text{A}$ receptor, i.e., channel conduction from the agonist binding, has been observed due to augmentation of the conductance or prolongation of the channel open-time from barbital binding [9,10]. The open-close transition of the channel has been recognized as a distance change, tilt and rotation of TM2 helixes [14]. The structure used this study, 4COF, corresponds to an open structure, that is to say, the barbital molecule in this study bound between TM2 helixes which would modify the distance, rotational and tilting fluctuations for channel open-close dynamics. Barbital binding to TM2 helixes of the open structure may modulate distances, tilting, and rotation of the helix to prolong the life time of the open structure. GABA$_\text{A}$ potentiation has been reported to be (S)- > (R)-pentobarbital [5], where their binding strengths are inversely correlated to the potentiation. The mechanisms by which barbitals prolong channel open-time or augment channel conductance still remain for future investigation.

5. Conclusion

Amobarbital, pentobarbital, and isobarbital bonded to the TM2-TM2’ inter-subunit TMD site, i.e., the propofol high affinity site of the GABA$_\text{A}$ receptor. Molecular discrimination of enantiomeric pentobarbital was 2.9 kcal mol$^{-1}$ at the TM2-TM2’ site. The hydrogen-bond of the barbital ring was the dominant binding element. Pentobarbital discrimination in the TM2-TM2’ TMD site was caused not only by the barbital ring’s hydrogen-bond, but also by steric fitting of a methyl-group adjacent to the chiral carbon atom. Molecular design modification adjacent to the chiral carbon atom will lead to optimization of the enantiomeric actions, and to the development of anesthetics with a further small side effect/action ratio.

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