Comparative binding character of two general anaesthetics for sites on human serum albumin

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Propofol and halothane are clinically used general anaesthetics, which are transported primarily by HSA (human serum albumin) in the blood. Binding characteristics are therefore of interest for both the pharmacokinetics and pharmacodynamics of these drugs. We characterized anaesthetic–HSA interactions in solution using elution chromatography, ITC (isothermal titration calorimetry), hydrogen-exchange experiments and geometric analyses of high-resolution structures. Binding affinity of propofol to HSA was determined to have a K_d of 65 μ M and a stoichiometry of approx. 2, whereas the binding of halothane to HSA showed a K_d of 1.6 mM and a stoichiometry of approx. 7. Anaesthetic–HSA interactions are exothermic, with propofol having a larger negative enthalpy change relative to halothane. Hydrogen-exchange

studies in isolated recombinant domains of HSA showed that propofol-binding sites are primarily found in domain III, whereas halothane sites are more widely distributed. Both location and stoichiometry from these solution studies agree with data derived from X-ray crystal-structure studies, and further analyses of the architecture of sites from these structures suggested that greater hydrophobic contacts, van der Waals interactions and hydrogen-bond formation account for the stronger binding of propofol as compared with the less potent anaesthetic, halothane.

Key words: affinity chromatography, binding force, human serum albumin (HSA), isothermal titration calorimetry (ITC), propofol.

INTRODUCTION

Propofol (2,6-di-isopropylphenol) is a short-acting intravenous anaesthetic agent for controlled sedation, short surgical procedures, rapid induction and maintenance of anaesthesia when administered by infusion [1,2]. Halothane (1-bromo-1-chloro-2,2,2-trifluoroethane) is a potent inhaled anaesthetic, still used clinically for induction of anaesthesia in children and maintenance of anaesthesia in adults. Both anaesthetics are thought to act by enhancing the action of inhibitory ligand-gated ion channels [2], but the lack of structural detail for these receptors, and the difficulty of studying binding of low-affinity ligands, have prevented a characterization of unique features underlying the structure–activity relationship. High-resolution structures for the anaesthetic–HSA (human serum albumin) complexes are available [3], which provide the capability to relate structural features to binding energetics obtained from solution experiments, resulting in a better understanding of the structure–activity relationship.

HSA transports a significant amount of propofol and halothane in the blood, and therefore an understanding of the binding interactions of these two anaesthetics with HSA will also allow refinement of pharmacokinetic models. Furthermore, because of the plethora of drugs that bind HSA, an understanding of both energetics and binding sites will allow for prediction of competition, which is crucial for safe combination therapy. An example is the interaction between propofol and halothane, since they are commonly used together clinically.

In the present study, we have characterized anaesthetic–HSA-binding interactions using several solution approaches: elution chromatography, ITC (isothermal titration calorimetry) and hydrogen exchange. The distribution of binding sites among the three major domains of HSA was studied by applying hydrogen

exchange to the individual recombinant domains. The solution experimental data was related to the X-ray crystal-structure data in order to understand the structure–activity relationships.

EXPERIMENTAL

Materials

HSA (essentially fatty-acid-free) was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.) and used without further purification. Halothane was obtained from Halocarbon Laboratories (Hackensack, NJ, U.S.A.). The thymol preservative and any contaminating water in halothane were removed before use with an aluminium oxide column. ³H₂O was obtained from ICN (Costa Mesa, CA, U.S.A.) at 100 mCi/ml. AffiGel 10 was purchased from Bio-Rad Laboratories (Hercules, CA, U.S.A.). All other chemicals were of reagent grade or better, obtained from the Sigma–Aldrich Chemical Co. (St Louis, MO, U.S.A.).

Synthesis and purification of HSA domains I, II and III

For the synthesis and purification of HSA fragments (domain I, amino acid positions 1–197; domain II, amino acid positions 189–385; and domain III, amino acid positions 381–585), we used a modified protocol based on a technique published previously [4]. Using pHIL-D2 HSA as the template, DNA oligonucleotides were used to amplify sequences corresponding to each HSA domain by PCR. All amplified fragments were digested with the DNA restriction enzymes *Xho*I and *Eco*RI. All fragments were ligated into pPIC9 (Invitrogen, Inc., San Diego, CA, U.S.A.) in frame with the α -mating factor secretion signal sequence, which was cleaved at the expected position, liberating the translation product of the

Abbreviations used: CNS, central nervous system; HSA, human serum albumin; ITC, isothermal titration calorimetry; MPEOE, modified partial equalization of orbital electronegativity.

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cloned cDNA fragment for each of the domains. The methods used to verify the DNA sequence of each yeast clone expressing domains was identical with that used to analyse clones expressing full-length HSA. Since all three HSA domains bind to Cibacron Blue Sepharose 6B resin (Sigma Chemicals), the HSA domains were expressed and purified using methods identical with those used to purify full-length HSA [5, 6].

ITC

ITC allows measurement of the magnitude of the binding affinity, and the two contributing thermodynamic terms: enthalpy (ΔH) and entropy (ΔS) changes. Experiments with halothane and propofol titration of HSA at 20 °C were determined by using a VP-ITC MicroCalorimeter (MicroCal, Inc., Northampton, MA, U.S.A.). Sodium phosphate buffer (20 mM, pH 7.0), containing 130 mM NaCl, was used in the present study. Low-affinity interactions and the limitation of the solubility of both halothane and propofol made it difficult to obtain the optimal practical molar ratio to derive all the thermodynamic parameters of the interactions, even when using sequential titrations (see below). We designed the experiment to ensure that most of the binding sites were occupied at the end of the titrations. The sample cell contained either 30 μ M or 60 μ M HSA solution for the halothane or propofol titration respectively, whereas the reference cell contained water. Ligand (at concentrations of 0.5 mM for propofol and 10 mM for halothane) was loaded into the injector and titrated in 10–30 μ l aliquots 5 min apart into the HSA sample solution. Sequential titrations were performed to ensure full occupancy of the binding sites by loading and titrating with the same ligand without removing the samples from the cell, until the titration signal was essentially constant. The titrations were linked together for data analysis using ConCat32 software distributed from MicroCal, Inc. The signals of ligand into buffer, buffer into protein, and buffer into buffer were subtracted using simple maths after separate titrations. Origin 5.0 software was used to fit thermodynamic parameters to the heat profiles.

Chromatography

Elution chromatography was used as described previously [7]. Briefly, 3 ml of AffiGel 10 was washed in cold distilled water and transferred to a graduated cylinder. Excess water was removed, and coupling was accomplished by adding 2.5 ml of the HSA solution (50 mg/ml) and gently mixing. After coupling for 2 h at 25 $\pm$ 1 °C, the gel was allowed to settle, and the supernatant was removed. To block all remaining unreacted groups, the gel was then incubated with 0.3 M glycine, pH 7.0, for an additional 30 min. A control gel (without protein) was prepared by reacting 3.0 ml of 0.3 M glycine, pH 7.0, with 3.0 ml of gel for 2 h, as described above. Aliquots of the pre- and post-coupling solutions were saved to determine the immobilized mass. Protein assays were performed with the Bio-Rad reagent. The coupled gel was washed with degassed mobile phase (10 mM sodium phosphate, pH 7.0), and packed into Bio-Rad MT-2 columns (holding approx. 2.5 ml of gel). Columns were connected to a Shimadzu LC-600 Liquid Chromatograph pump (Columbia, MD, U.S.A.) and then flushed with mobile phase at 0.4 ml/min until a steady baseline, as detected by UV absorbance at 210 nm (Shimadzu SPD-6AV), was established.

Zonal elution chromatography

With the apparatus running at 0.4 ml/min, 50 μ l of 10 mM halothane or 0.5 mM propofol in the mobile phase was injected into the chromatography apparatus at zero time. Absorbance

traces were monitored for 60 min and stored on a computer. Retention times for the halothane or propofol peaks from the protein column were then compared with that of the control (glycine) column.

The effect of ionic strength on binding of propofol to HSA was examined by varying the mobile phase salt concentration. The column was equilibrated with 10 mM sodium phosphate buffer containing 0 mM, 130 mM or 500 mM NaCl at a flow rate of 0.4 ml/min. Aliquots (50 μ l) of 0.5 mM propofol in the same mobile phase were injected at zero time, and absorbance (210 nm) was monitored for 60 min. Retention times for the propofol peaks from the different sodium chloride concentrations, and for the different columns (HSA and glycine), were compared.

Continuous (or frontal) elution chromatography

In these experiments, propofol was dissolved directly in the mobile phase at various concentrations. Flow from a pump at 0.4 ml/min was initiated at zero time and stopped at 60 min. Data collection was carried out as for zonal elution chromatography (described above). The sigmoid elution profile was monitored and recorded. Zonal elution allowed the qualitative detection of ligand binding. A reproducible rightward shift in the peak (as compared with a glycine control column) demonstrates binding, and the magnitude of the shift reflects both affinity and stoichiometry. Continuous elution chromatography allowed separation of these binding parameters. The chromatographic relationship of interest is determined by:

$$1/(V_{50} - V_0) = K_{M/H}/M_T + [\text{propofol}]_0/M_T$$

where V_{50} is the volume of eluate at which the gel matrix is half-saturated, V_0 is the unretarded elution volume for propofol (determined by equivalent runs on the control glycine column), $K_{M/H}$ is the apparent dissociation constant of the protein–propofol complex, M_T is the total capacity for propofol binding within the column (analogous to $B_{\max}$), and $[\text{propofol}]_0$ is the mobile phase concentration of propofol. The elution profile was fitted to a Boltzmann sigmoid curve, with T_{50} being the time at which half of the maximum signal was obtained (see Figure 3A). V_{50} is obtained by multiplying T_{50} by the flow rate (0.4 ml/min).

Hydrogen–tritium exchange

Ligand binding should alter protein stability, and amide-hydrogen exchange is a sensitive method of measuring these changes [8,9]. We used this method to determine the free-energy change and relative binding-site distribution in HSA for propofol. Isolated domains of HSA and intact HSA (1 mg/ml) were incubated with 5–10 mCi of $^3\text{H}_2\text{O}$ in 1 M guanidinium chloride/0.1 M NaH_2PO_4 buffer, pH 8.5, for at least 18 h at 25 $\pm$ 1 °C for ^3H exchange inwards (exchange-in). Free $^3\text{H}_2\text{O}$ was removed, and the buffer was exchanged with a PD-10 gel-filtration column (Sigma), thereby initiating exchange outwards (exchange-out) with 0.5 M guanidinium chloride/0.1 M NaH_2PO_4 buffer, pH 7.4. After recovery from the column, the protein solution was immediately transferred to pre-filled Hamilton (Reno, NV, U.S.A.) gas-tight syringes containing 0.01–0.3 mM propofol for intact HSA, or 0.5 mM propofol for the domains, in exchange-out buffer, and then equipped with repeaters. Aliquots were precipitated with 2 ml of ice-cold 20% (w/v) trichloroacetic acid at timed intervals over at least 6 h. The precipitated protein was rapidly vacuum-filtered through Whatman GF/B filters, and washed with 8 ml of ice-cold 2% trichloroacetic acid. ^3H retained by the protein was determined by liquid scintillation counting. Exchange-out buffer conditions were adjusted to focus on the slowest 5–10%

Figure 1 Binding of HSA to halothane and propofol observed by ITC

The titration of 10 mM halothane into 30 μ M HSA (left panels), and of 0.5 mM propofol into 60 μ M HSA (right panels), at 20 °C in buffer containing 130 mM NaCl and 20 mM NaHPO₄, pH 7.0, is shown. Three portions of sequential titration were performed for both halothane and propofol. Programmed injection was performed in the first portion of titration for propofol to obtain the isothermal curve at a lower molar ratio. The interactions of halothane and propofol with HSA are both exothermic processes. The derived parameters of the ITC experiments are presented in Table 1.

of hydrogens over a 6 h period, to ensure that global unfolding events were being monitored.

Protection factor ratios (PFR) for given hydrogens were determined from the exchange-out curves, as described previously [10], and the changes of free-energy change ($\Delta\Delta G$) were determined [$\Delta\Delta G = -RT \ln(\text{PFR})$]. Negative values reflect stabilization (slower exchange-out).

Molecular properties and ligand–protein contact

The molecular properties of propofol and halothane were calculated using Molecular Analysis Pro (ChemSW, Inc., Suisun City, CA, U.S.A.; <http://www.chemsw.com/13052.htm>). Dipole moment and partial charges were calculated using MPEOE (modified partial equalization of orbital electronegativity) [11,12]. Atomic co-ordinates of the HSA–halothane (1E7B) and HSA–propofol (1E7A) complexes were obtained from the Protein Data Bank (<http://www.rcsb.org/pdb>). Ligand–protein contacts (LPCs) were derived with LPC software (<http://bioinfo.weizmann.ac.il:8500/oca-bin/lpccsu>) [13].

RESULTS

ITC

Both halothane and propofol interactions with HSA are exothermic processes (Figure 1). The final heat data for halothane and propofol fitted well to a one-binding-site model, and the derived

Table 1 Thermodynamic parameters derived from the ITC experiment

Larger deviation estimates were observed for the halothane–HSA interaction. Because the number of sites for halothane has been reported as seven (Protein Data Bank structures 1E7C and 1E7B) [3], we refitted the data by fixing $n = 7$. The isotherm fitted well ($\chi^2 = 8.9$) with $n = 7$ and a confident reasonable ΔH close to the known ΔH (−1.9 kcal/mol) [24] for the halothane–albumin (bovine) interaction. 1 cal $\approx$ 4.182 J.

	n	K_a (1/M)	ΔH (cal/mol)	ΔS (cal/mol per K)
Halothane (variable n)	7.6 ± 5.7	613 ± 88	-1319 ± 1031	8.3
Halothane (fixed n)	7	604 ± 21	-1445 ± 31	7.8
Propofol	1.7 ± 0.1	15280 ± 2330	-5199 ± 424	1.4

parameters are presented in Table 1. Propofol data were fitted to a model for two equivalent binding sites of 65 μ M affinity, whereas halothane had seven equivalent sites of 1.6 mM affinity. Propofol had a larger negative enthalpic component, whereas halothane had the larger entropic component.

Elution chromatography

The zonal retention time was 5.3 min for halothane and 6.5 min for propofol on the control glycine column, and 6.4 min for halothane and 20.8 min for propofol on the HSA-coupled column (Figure 2). The retention time of propofol increased slightly with an increase in the salt concentration on the control column, but dramatically increased on the HSA column (Figure 3).

Figure 2 Comparison of halothane and propofol binding to HSA by zonal elution chromatography

The retention time was 5.27 min for halothane (C-halo) and 6.46 min for propofol (C-PFL) in the control glycine column, 6.43 min for halothane (HSA-Halo) and 20.8 min for propofol (HSA-PFL) in the HSA column. Halo, halothane; PFL, propofol.

Figure 3 Effect of ionic strength on the binding of HSA to propofol observed by zonal elution chromatography

Although the retention time of propofol increased slightly with the increase of the salt concentration of the mobile phase in the control column, the retention time in the HSA column increased dramatically. C-0 mM, C-130 mM and C-500 mM represent data obtained in the control glycine column in 0 mM, 130 mM and 500 mM NaCl respectively. HSA-0 mM, HSA-130 mM and HSA-500 mM represent data obtained in the HSA column in 0 mM, 130 mM and 500 mM NaCl respectively.

Continuous elution experiments on the HSA-coupled columns produced a sigmoid curve for each propofol concentration, as shown in Figure 4(A). The V_{50} (i.e. the volume of eluate at which the gel matrix is half-saturated) derived from these curves is presented in Figure 4(B). On the HSA column, the expected inverse relationship between V_{50} and propofol concentration was noted. The reciprocal of the regression line slope is the functional capacity of the column (M_T), which provides an estimate of binding stoichiometry of approx. 1.8:1 for propofol:HSA when divided by the mol of protein immobilized on the gel (Figure 4B). The y-axis intercept is defined as $K_{M/H}/M_T$, allowing a derivation of 0.17 mM as the HSA/propofol K_d , assuming equivalence of the two sites. This K_d value probably represents an upper limit on the basis of the shape of a slight curve presented in Figure 4(B). Similar experiments for halothane in serum albumin (bovine) have been reported [7]. More dramatic curvature was interpreted as representing two classes of binding sites. Fitting two lines to the data suggested that there were at least two high-affinity sites and up to 20 weaker sites [7].

Figure 4 Propofol-HSA binding determined by continuous elution chromatography

(A) Continuous (frontal) elution profiles for the HSA column, using mobile phase containing a range of concentrations of propofol from 10 to 100 μ M, is presented. (B) The plot of $1/(V_{50}-V_0)$ as a function of propofol concentration, based on V_{50} values from elution profiles such as those in (A), is shown. M_T is the total capacity for propofol binding within the column; $K_{M/H}$ is the apparent dissociation constant of the protein-propofol complex. The calculated stoichiometry for propofol is 1.8 in HSA.

Hydrogen-tritium exchange

The three domains of HSA were all stabilized by propofol and halothane, but not equally. The $\Delta\Delta G$ values for propofol at a concentration of 0.5 mM were -0.28 kcal/mol (1 cal $\approx$ 4.184 J) for domain I, -0.40 kcal/mol for domain II and -1.30 kcal/mol for domain III. The $\Delta\Delta G$ values for halothane at 7 mM were -0.9 kcal/mol for domain I, -1.4 kcal/mol for domain II and -0.9 kcal/mol for domain III.

Molecular properties and ligand-protein contacts

The calculated molecular volume of propofol [$191 \text{ \AA}^3$ (1 $\text{\AA}$ = 0.1 nm)] exceeds that of halothane ($91 \text{ \AA}^3$), but both have a similar dipole moment {1.82 debyes for halothane and 1.60 debyes for propofol [using the MPEOE method; 1 debye (or D) $\approx 3.336 \times 10^{-30} \text{ C} \cdot \text{m}$]}. In the same HSA domain III binding site, propofol makes more surface contact with lining amino acid residues than halothane does. For example, propofol has hydrophobic interactions with 13 residues, whereas only seven residues were found for halothane. Both halothane and propofol contact Asn³⁹¹ with surface areas of $37 \text{ \AA}^2$ and $43 \text{ \AA}^2$ respectively, and propofol also contacts Arg⁴¹⁰ with a surface area of $34 \text{ \AA}^2$. A putative hydrogen bond (3.1 $\text{\AA}$) between the propofol (PFL2001) hydroxyl hydrogen and the backbone carbonyl oxygen of Leu⁴³⁰

Table 2 Comparison of residues in the same binding site in domain-III of HSA (Protein Data Bank structures 1E7A and 1E7B) contacting halothane and propofol

Contact property	Halothane	Propofol
Total contact surface area ($\text{\AA}^2$)	254	381
Number of contacting residues	13	18
Specific interaction		
Hydrogen bond	0	1 (Leu ⁴³⁰)
Hydrophobic	7	13
Aromatic	0	1 (Phe ⁴⁰³)
Hydrophobic/hydrophilic	3	8

was noted. No potential hydrogen bonds were found for halothane (Table 2).

DISCUSSION

Our study has shown that propofol binds to HSA at fewer sites but with a higher binding affinity than halothane. Hydrophobicity, van der Waals contacts and hydrogen bonding all contribute to propofol's relatively strong binding to HSA. Contrary to our prediction, charged residues do not contribute significantly to either propofol (the present study) or halothane [6] binding. The information derived from this study has both pharmacological and clinical significance. For example, the relatively strong binding of propofol to HSA should competitively release drugs bound to this site, transiently increasing their free concentration and physiological effects. Given the very high concentration of HSA in blood (≈ 0.6 mM), the change in free concentration of other compounds could be significant. The analysis and comparison of binding mechanisms also has provided information on how propofol might interact with target proteins in the CNS (central nervous system) to produce anaesthesia.

Number of binding sites and affinity

The X-ray crystal study of HSA and propofol revealed only two binding sites, whereas halothane has seven binding sites [3]. However, the physiologically unfavourable solution conditions necessary for protein crystallization require validation of ligand binding, especially when binding affinity is weak relative to the extent possible under more biologically relevant solution conditions. Our solution binding results are consistent with the previously published crystal data. ITC revealed seven or eight sites for halothane, and one or two sites for propofol. Since the number of sites for propofol has been reported to be more than 20 [14], we investigated the number of binding sites further by continuous elution chromatography, which also revealed only two sites. However, the data suggested the presence of additional low-affinity sites, on the basis of the slight curve in the plot of $1(V_{50}-V_0)$ against [propofol] shown in Figure 4(B), which is consistent with the hydrogen-exchange data showing stabilization of domain I and II. Previous continuous elution chromatography experiments also suggested at least two classes of sites for halothane [7], but the affinity of the two classes of sites for halothane is approx. 10-fold lower than for propofol.

Although solution experiments may more closely represent physiological conditions than X-ray crystallographic experiments, it is difficult to determine the location of the binding sites. Fluorescence quenching has been used to locate halothane-binding sites in some proteins [15], but this method is not suitable for propofol, since propofol itself has fluorescent properties. Instead, we expressed isolated domains of HSA and used hydro-

gen exchange to monitor specific binding interactions at the domain level. Using the magnitude of free energy change derived from a combined measure of affinity and stoichiometry, the major propofol-binding sites appear to be located in domain III, where the largest free-energy change was observed by hydrogen exchange, and the additional electron density attributed to propofol was found in the crystal-structure complex (1E7A). Likewise, halothane stabilized primarily domain II, where four of the seven sites were found in the crystal-structure complex (1E7C).

Although there is good agreement between the HSA-anaesthetic-complex high-resolution structure and our solution experiments in terms of stoichiometry and the location of binding sites, the X-ray crystallographic study provided little energetic information. The data from both the zonal and frontal elution chromatography studies indicated that the binding affinity of the propofol-HSA complex is much stronger than that of either halothane-HSA binding. ITC experiments indicated an overall 25-fold-higher affinity for propofol than for halothane. This difference is in accordance with the difference in clinical potency. The total 'anaesthesia' EC_{50} concentration of propofol in the blood is $50 \mu\text{M}$ [14]. Since HSA represents the predominant high-affinity carrier for propofol in blood, our results allow an estimation of the free propofol concentration at this total EC_{50} concentration. Using a K_d of $65 \mu\text{M}$ (from the ITC experiments), a total HSA concentration of $600 \mu\text{M}$, and a Hill coefficient of 1.5 for the HSA-propofol interaction, we estimate the free concentration of propofol to be approx. $7 \mu\text{M}$. The free concentration of halothane is $250 \mu\text{M}$, which is approx. 35-fold higher than that of propofol. A comparable difference in magnitude between HSA affinity and clinical EC_{50} for both halothane and propofol suggests that the HSA-binding site architecture bears a resemblance to that of pharmacologically relevant sites in the CNS.

Binding forces

The binding forces between halothane and HSA have been investigated and characterized previously [6,16,17]. These data are helpful to explore propofol binding forces by comparison, since propofol and halothane share a binding site in domain III of HSA [3].

Hydrophobic interaction

The hydrophobicity effect is clearly a dominant force in halothane binding to HSA [6], consistent with the ITC finding that the halothane-HSA interaction was accompanied by a small negative enthalpy change (-1.4 kcal/mol) and a relatively large entropy change: the typical energetic signature of hydrophobic interactions [18]. Propofol, which is larger and more hydrophobic than halothane, should have stronger hydrophobic interactions with HSA than halothane. The direct relationship between affinity and salt concentration in the elution chromatography experiments rules out important electrostatic interactions, leaving hydrophobic interactions as the dominant feature of propofol-HSA interactions. Furthermore, the structural analysis indicated that propofol has more hydrophobic contact with the side chains of lining amino acid residues in HSA-binding domains than has halothane. In fact, the additional $120 \text{\AA}^2$ of contact area (Table 2) could easily explain the 25-fold difference in affinity, using $20 \text{ cal}/\text{\AA}^2$ of buried surface to estimate the energetic contribution [19,20]. However, in contrast with halothane, ITC studies of the propofol-HSA interaction revealed that the major contributor to binding is the enthalpic component, suggesting that forces other than the hydrophobic effect contribute to binding.

Hydrogen bonds

Although halothane may act as both a hydrogen bond acceptor and donor [21], no putative hydrogen bond was found in any of the halothane-binding sites in HSA. In the case of propofol, however, the aromatic ring lies within an apolar pocket, with the phenolic hydroxy group in close proximity for establishing a hydrogen bond, in the one case (domain IIIA; 3.1 Å) with a main-chain carbonyl oxygen of the Leu⁴³⁰ residue, and in the other case (domain IIIB, 2.9 Å) with the hydroxy group of Ser⁵⁷⁹ [3]. It is likely that this relatively strong non-covalent bond contributes to the enhanced enthalpic change relative to halothane.

van der Waals interactions

Given the structures of halothane and propofol, which lack formal charges and have small dipole moments, van der Waals forces must play an important role in the stabilizing energetics that govern their binding to a protein [16]. van der Waals forces are derived from short-range, transient electrostatic interactions, and can be predicted to be larger for propofol, since its volume is twice that of halothane and a larger surface contact was also noted in the structural analysis. Therefore, in addition to hydrogen bonds, van der Waals forces must also contribute to the larger enthalpic change and the higher affinity of the propofol–HSA complex as compared with the halothane–HSA complex.

Electrostatic interactions with charged residues

The calculated dipole moment of halothane is similar to measured values [22], but experimental values for propofol have not been reported. On the basis of our calculations, propofol has a similar dipole moment of halothane, in addition to the aromatic quadrupole moment. The π electrons of the propofol aromatic ring contribute partial negative charges that can interact with cations and other partial positive charges [23]. It is unlikely that partial charge interactions in the protein matrix are sensitive to salt concentration, but our elution chromatography experiments, in this case, rule out a potentially important contribution to anaesthetic binding of full charges in a cavity. Similar results have been reported for halothane [6].

Conclusion

Propofol binding to HSA is an exothermic and enthalpy-driven process. Relative to the halothane–HSA complex, the stronger propofol–HSA interactions might be due to the combination of hydrogen bonding, stronger van der Waals and hydrophobic interactions at two principal sites and, perhaps, several less specific ones. Fully charged interactions hinder propofol binding to proteins, reaffirming the dominance of hydrophobicity in the structure–activity relationship for general anaesthetics. In addition to the obvious pharmacokinetic implications of drug competition at HSA sites, these data suggest that HSA might be a suitable platform for further characterization of the general anaesthetic structure–activity relationship through mutagenesis, solution binding studies and structural characterization.

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