Propofol and halothane binding sites on human serum albumin

Binding of the General Anesthetics Propofol and Halothane to Human Serum Albumin: High-Resolution Crystal Structures*

A. A. Bhattacharya, S. Curry+ and N. P. Franks+

From the Biophysics Section, The Blackett Laboratory, Imperial College of Science, Technology & Medicine, London, United Kingdom.

*This work was supported by grants from the Medical Research Council, London, UK and the Biotechnology and Biological Sciences Research Council, Swindon, UK.

+To whom correspondence should be addressed: Biophysics Section, The Blackett Laboratory, Imperial College of Science, Technology & Medicine, Prince Consort Road, London SW7 2BW, United Kingdom. Tel.: 004420-7594-7629; Fax: 004420-7589-0191; E-mail: s.curry@ic.ac.uk or n.franks@ic.ac.uk.

Copyright 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
SUMMARY

Human serum albumin (HSA) is one of the most abundant proteins in the circulatory system and plays a key role in the transport of fatty acids, metabolites and drugs. For many drugs, binding to serum albumin is a critical determinant of their distribution and pharmacokinetics, however there have, as yet, been no high resolution crystal structures published of drug-albumin complexes. Here we describe high-resolution crystal structures of HSA with two of the most widely used general anesthetics, propofol and halothane. In addition, we describe a crystal structure of HSA complexed with both halothane and the fatty acid, myristate. We show that the intravenous anesthetic propofol binds at two discrete sites on HSA in preformed pockets that have been shown to accommodate fatty acids. Similarly we show that the inhalational agent halothane binds (at concentrations in the pharmacologically relevant range) at three sites which are also fatty-acid binding loci. At much higher halothane concentrations we have identified additional sites that are occupied. All of the higher affinity anesthetic binding sites are amphiphilic in nature, with both polar and apolar parts, and anesthetic binding causes only minor changes in local structure.
Propofol and halothane binding sites on human serum albumin

How general anesthetics exert their effects in the central nervous system has remained a puzzle for over 150 years, but there is now a growing consensus that they act by binding directly to protein targets (1). The identity of these targets, however, remains uncertain, although a large body of evidence is accumulating on the functional effects of general anesthetics on a variety of possible candidates (1, 2). Most of these data come from electrophysiological measurements, coupled more recently with the techniques of molecular genetics. While these approaches are crucial in understanding the actions of general anaesthetics, they give, at best, only indirect information on the forces that are involved in anesthetic-protein interactions and virtually no information on the molecular architectures of anesthetic binding sites.

The lack of direct structural information is due, at least in part, to the fact that the most likely targets for general anesthetics are thought to be neuronal ion channels. These are, of course, integral membrane proteins and have proven to be exceptionally difficult to crystallize in a form that is suitable for high-resolution X-ray diffraction analysis. However, there are several soluble proteins to which anesthetics are known to bind and studies with these proteins have provided valuable information on the nature of anesthetic binding sites. Most of this work has been done with serum proteins and luciferase enzymes, but so far the only example of an anesthetic-sensitive protein for which there is also high-resolution structural data is firefly luciferase (3).

Perhaps the most extensively studied anesthetic-binding protein is serum albumin, and there have been numerous attempts to characterise the binding sites involved (4-8), none of them, however, using direct structural techniques. This protein is not only amenable to high-resolution structural
Propofol and halothane binding sites on human serum albumin

analysis, but more importantly, is known to play a key role in the pharmacological actions of several general anesthetics.

The importance of serum albumin in anesthetic pharmacology derives from its high concentration in the circulatory system (approximately 0.6 mM in plasma) and from its ability to bind an extraordinarily diverse range of drugs (including most anesthetics), metabolites and fatty acids [for reviews see refs. (9-11)]. In several cases more than 50% of a clinically administered general anaesthetic will be bound to serum albumin, and in some cases, such as the intravenous agent propofol, approximately 80% is bound (12). Consequently, any changes in the interactions between an anesthetic and serum albumin, either by fatty acids or other drugs competing for binding, or by genetic modifications in the protein itself, could result in significant changes in the pharmacologically active concentration of the anesthetic.

Although a high-resolution structure of human serum albumin was published some years ago (13), the unavailability of the three-dimensional co-ordinates did not encourage others to extend this work. Curry et al. subsequently published (14) a high-resolution structure of the protein that identified the principal fatty acid binding sites, and this was followed by the publication of an independent determination of the native structure (15). The protein is heart-shaped and contains 585 amino acids. It is organised into three homologous domains (labelled I-III) and each domain consists of two sub-domains (A and B) which share common structural elements (Fig. 1). In this paper we have used X-ray crystallography to provide high-resolution information on the nature and locations of the principal binding sites for two of the most widely used general anesthetics - the intravenous agent propofol and the inhalational agent halothane (see below).
Propofol and halothane binding sites on human serum albumin

Propofol

Halothane
**EXPERIMENTAL PROCEDURES**

*Protein purification* - Most experiments were carried out using fat-free recombinant HSA, prepared by charcoal treatment (16) at low pH. This was supplied at a concentration of 250 mg ml\(^{-1}\) in 145 mM NaCl by Dr. John Woodrow of Delta Biotechnology Limited (Nottingham, UK). The halothane-myristate complex was formed using protein which, in addition, originally contained 40 mM octanoate (C8:0) and 15 mg litre\(^{-1}\) Tween-80. In both cases the protein was further purified on a Superdex S75 gel-filtration column (Amersham Pharmacia Biotech, Buckinghamshire, UK) with a phosphate running buffer (50 mM potassium phosphate, 150 mM sodium chloride, pH 7.5) to remove dimers and polymers of HSA, exactly as previously described (14). After combining the appropriate fractions, the running buffer was exchanged with a storage buffer (50 mM potassium phosphate, pH 7.0) and the protein was concentrated using an Amicon 30 kDa molecular weight cut-off centrifugal concentrator (Millipore, Watford, Hertfordshire, UK) to greater than 80 mg ml\(^{-1}\) and stored at 4°C. All chemicals were obtained from Sigma Chemical Company (Poole, Dorset, UK) unless otherwise stated.

*Crystallization and complex formation* - Crystals of native HSA were grown by vapour diffusion at 4 °C using the sitting drop configuration. Crystals were first grown with a reservoir of 28 - 30% (w/v) PEG 3350, 50 mM potassium phosphate, pH 7.0. After 2 - 3 months large stacked plates were observed in some drops, but these crystals were rarely single and diffracted poorly. However, using these crystals as seeds and equilibrating with a lower concentration of PEG 25 - 26% (w/v), crystals were obtained with dimensions of approximately 0.2 mm × 0.3 mm × 0.2 mm in 4 – 6 weeks. These crystals diffracted to high resolution (2.1 Å). For the propofol complex an identical crystallization procedure was followed except that a saturating concentration of propofol (approximately 4 mM in 25 – 26% PEG) was maintained.
Propofol and halothane binding sites on human serum albumin throughout. The propofol was a gift from Zeneca Pharmaceuticals, Alderley Park, Macclesfield, UK. Co-crystallisation with propofol generally resulted in larger crystals than those obtained in the absence of propofol. Native propofol-free crystals could be readily obtained by back-soaking in solutions which contained progressively less propofol, while at the same time progressively increasing the PEG concentration up to 32% (w/v).

Complexes with halothane were prepared by exposing native crystals to chosen partial pressures of halothane in 1-mm sealed glass capillaries at room temperature. The partial pressure was set by using mixtures of halothane and hexadecane at defined mole ratios. To the extent that halothane and hexadecane mix ideally, the vapour pressure of halothane above such a mixture can, according to Raoult’s Law, be taken to be proportional to its mole fraction. The maximum partial pressure of halothane that could be used with native crystals before a significant deterioration in the diffraction patterns was observed was 15% of the saturated vapour pressure (SVP) which would correspond to a partial pressure of 5% atm, or 2.6 mM in free aqueous solution. To prepare the halothane-myristate complex, crystals with myristate were first prepared (14) before exposure to halothane, as described above. In the presence of myristate we found that a much higher concentration of halothane could be used (60% of the SVP, which would correspond to a partial pressure of 20% atm, or 10.5 mM in free aqueous solution) before lattice disorder in the crystals reduced the resolution of the diffraction patterns.

Data collection and processing - Data were collected to high resolution at the synchrotrons in Daresbury (SRS, UK) and in Hamburg (DESY, Germany). At Daresbury (beamline 9.6), short exposure times (2 – 3 seconds) were used in order to minimise radiation damage which was evident with longer exposures. In Hamburg (beamline X-11) the exposure times were 20 – 30
seconds. All data was processed using MOSFLM (Andrew Leslie, personal communication). Details of the data collection are given in Table 1.

Structure determination and model refinement - The structure of native HSA was determined using molecular replacement with the program AMoRe (17). The co-ordinates of the search model were those of “molecule A” in the 2.5 Å structure of HSA (Brookhaven accession number 1AO6) recently determined by Sugio and colleagues (15).

Rigid-body refinement was carried out using the program X-PLOR (18) followed by restrained least squares crystallographic refinement. For the structure containing both halothane and myristate the HSA co-ordinates of the previously determined HSA-myristate structure (14) were used prior to rigid-body refinement. The co-ordinates for propofol were taken from the Cambridge Structural Database (19) and those for halothane were calculated assuming standard stereochemistry. At the resolution of our data the two enantiomers of halothane would have been indistinguishable and we arbitrarily chose to model the R enantiomer.

After the addition of water molecules, and fatty acid and anesthetic molecules where appropriate, all of the refined models had good stereochemistry (Table 2) with no main-chain dihedral angles lying in disallowed regions of the Ramachandran plot (not shown). Co-ordinates and structure factors have been deposited in the Protein Data Bank; ID codes are given in Table 2.
RESULTS

In the absence of fatty acids HSA crystallized in a P1 space-group with unit cell dimensions (Table 1) that have not been observed before despite the fact that our crystallization conditions were similar to those used by others (13, 15). The native HSA structure that we have determined is essentially identical to those previously published with only minor differences in the flexible subdomain IIIB (Fig. 1A), due no doubt to differences in crystal packing. For comparison, Fig. 1B shows the HSA structure in the presence of myristate (14, 20) and the locations of eight fatty acid binding sites.

For the crystals containing propofol, the quality of the difference electron density allowed the positions and orientations of two propofol molecules to be unambiguously determined. One molecule (PR1) binds in subdomain IIIA, and the other (PR2) in subdomain IIIB (Fig. 2). The propofol molecule in IIIA (Fig. 2B and Table 3) binds in an apolar pocket with the phenolic hydroxyl group making a hydrogen bond (3.1 Å) with the main-chain carbonyl oxygen of Leu 430, and with the aromatic ring of the anesthetic being sandwiched between the side-chains of Leu 453 and Asn 391. One of the two isopropyl groups makes numerous apolar contacts at one end of the pocket while the other is exposed at the aqueous entrance, although it too makes close contacts with several sidechains (Asn 391, Leu 407, Arg 410 and Tyr 411). The mouth of the binding pocket opens onto a network of five hydrogen-bonded water molecules that are further stabilised by interactions with Ser 489, Arg 410 and Tyr 411. The electron density for this solvent-exposed isopropyl group is much better defined (indicating a higher degree of order) than that of the isopropyl group which is deeper in the pocket. The only conformational adjustment that takes place on propofol binding to this pocket is a $120^\circ$ rotation of the side-chain of Val 433 which moves to accommodate the inner isopropyl group. Comparisons with structures which contain fatty acids (20) suggest that this propofol molecule would compete for ligand binding at fatty acid binding site FA3 and also
disrupt the binding of fatty-acid at site FA4 (via interactions with Arg 410 which co-ordinates the fatty acid carboxyl group).

The second propofol molecule (Fig. 2C, Table 3) binds in a cavity located in sub-domain IIIB that is mainly lined by aromatic residues (Phe 502, Phe 507, Phe 509 and Phe 551). The anesthetic is sandwiched between the sidechains of Phe 502 and Leu 532, which make close contacts with the propofol aromatic ring. The aliphatic portion of Glu 531 and the side chain of His 535, situated approximately 4 Å from the base of the propofol molecule, close off this end of the pocket. The hydroxyl group of Ser 579 makes a hydrogen bond (2.9 Å) with the propofol hydroxyl. The entrance to the binding pocket is quite polar, with several well-ordered water molecules and a number of polar residues in close proximity. As with the first propofol site, there are only a few minor local conformational changes on binding, the most marked of these being a 90º rotation about the Cα-Cβ bond of Phe 507, which moves the side chain away from the centre of the binding pocket (there are also minor movements in the aromatic rings of Phe 502 and Phe 509). Superposition of the fatty acid structures (20) indicates that the binding of this propofol molecule could be prevented by ligands that bind to fatty-acid binding site FA5. It is probable that the first of the two propofol binding sites (PRI in sub-domain IIIA) has the highest affinity because during one experiment where the crystals were partially back-soaked and the propofol concentration was reduced, the electron density for the second propofol molecule PR2 disappeared while that for the first molecule was easily interpretable (data not shown).

When crystals of HSA were exposed to halothane vapour we found that a maximum concentration of around 15% of the saturated vapour pressure (SVP) could be used before there was a noticeable deterioration in the resolution of the diffraction pattern. With myristate-containing crystals a significantly higher concentration could be used (60% of the SVP) before this occurred.
the lower concentration and in the absence of fatty acid the difference electron density showed three “high-affinity” halothane binding sites (molecules HAL1, HAL2 and HAL3; Fig. 3A, Table 4). (While the position of the electron-dense bromine atom was always clear, there was some ambiguity about the relative positions of the chlorine atom and the CF₃ group. In most cases the shape of the density was used to guide positioning of the slightly bulkier CF₃ group, but because the data are limited to 2.4 Å resolution and the model B-factors are relatively high, the orientations modelled cannot be regarded as definitive.) Two of these halothane molecules (HAL1 & HAL2) bind within a solvent-exposed trough at the interface between subdomains IIA and IIB which can also bind a fatty acid molecule (FA6). At the higher halothane concentration a third molecule (HAL4) also binds in the trough (see Figs. 3B and 4A), adjacent to HAL1 and HAL2. The strongest density was observed for the central halothane molecule HAL1 which binds in an amphiphilic environment formed on the one side by the polar groups of Arg 209 and Glu 534 which interact via a salt bridge (that also involves Asp 324) and on the other side by the aliphatic portion of Lys 212 and the side chains of Ala 213 and Leu 327. The second molecule (HAL2) is in a predominantly apolar environment (Ala 213, Leu 347, Ala 350 and the aliphatic portion of K351) although a polar interaction is provided by Arg 209. The third molecule (HAL4) in the trough only binds at much higher concentrations and makes relatively few interactions with neighbouring side chains. Even at the higher halothane concentration there was, within experimental error, no significant change in the local structure, despite the competitive displacement of myristate.

At the lower halothane concentration, in addition to the two molecules HAL1 and HAL2 at the IIA/IIB interface, a third “high-affinity” molecule (HAL3) is present in subdomain IIIA (Fig. 3A & 4B). This molecule binds in a site that overlaps with the methylene tail of the fatty acid bound in site FA3 and with the first
propofol molecule (PF1). HAL3 makes numerous close, mainly apolar, interactions within the binding pocket (Table 4 and Fig. 4B). The bromine atom interacts with the sulphur of Cys 438, the main chain of Gly 434 and makes additional (hydrophobic) contacts with Phe 403 and the side-chain of Asn 391 (Fig. 4B).

At the higher halothane concentration, electron density appears for molecules HAL5 and HAL6 within a binding site in subdomain IIA that can also bind fatty acid FA7. These two halothane molecules (see Fig. 3B & 4C) lie adjacent to one another in a predominantly apolar environment, although both molecules also interact with polar groups. The main-chain carbonyl oxygen of Arg 257 contacts halothane HAL5, while its charged guanidinium side-chain interacts with the bromine atom of the anesthetic. Similarly, the bromine atom of HAL6 is close to the guanidinium of Arg 222. HAL6 is also within 5 Å of Trp 214 that has been implicated in halothane binding to HSA (7).

With the HSA structure in the presence of myristate and at the higher halothane concentration, we observed strong electron density for two more halothane molecules (HAL7 and HAL8). One of these (HAL7) binds at the interface between subdomains IA and IIA (Fig. 3B & 4D) in a cavity that is formed as a consequence of the fatty acid-induced conformational change [ref. (14) and Fig. 1]. This conformational change rotates domain I relative to domain II to create a largely apolar cavity that is flanked on one side by the methylene tail of the fatty acid bound to FA2. The bromine atom is co-ordinated by several polar interactions (Tyr 30, His 67, Asn 99 and Asp 249). Binding of HAL7 displaces the myristate from site FA8. The other halothane molecule HAL8 present in the HSA-myristate crystals (Fig. 3B) binds in a solvent-exposed niche which is formed by the parallel side chains of Lys 136, Lys 159 and Lys 162 (not shown). The orientations of these side chains that
Propofol and halothane binding sites on human serum albumin

form the hydrophobic cavity are determined, very largely, by interactions with a symmetry-related HSA molecule in the crystal suggesting that the binding site for halothane HAL8 is a crystallographic artefact.

DISCUSSION

A number of general statements can be made about the nature of the propofol and halothane binding sites on HSA, and the effects these anesthetics have on the protein structure. First, only a relatively small number of discrete sites are involved. In all cases these are pre-formed pockets or clefts on the protein which are, in almost all cases, capable of binding natural ligands (i.e. fatty acids). Second, the only changes we observed in local structure were two side-chain conformational changes on propofol binding (see Results), and there was no evidence, in the pharmacologically relevant range of concentrations (see below), of global changes in protein structure. In the case of propofol, there were no generalized changes in structure even at saturating concentrations of the drug, while the same was true for halothane at concentrations up to 5% atm. in the absence of fatty acid and up to 20% atm. in the presence of fatty acid. Only above these concentrations did we see evidence of crystal disorder, but this could have been a consequence crystal contacts being disrupted rather than due to a conformational change in the protein.

It has been shown (21) that inhalational anesthetics shift the denaturation temperature of BSA to higher temperatures (presumably as a consequence of the anesthetics binding to the folded rather than the unfolded state) and it has also been shown (22) that the fluorescent anisotropy of two tryptophan residue in BSA are increased in the presence of anesthetics. On the basis of these two observations it has been proposed (22) that anesthetics may exert their effects on proteins at the molecular level by attenuating the movement
Propofol and halothane binding sites on human serum albumin

of the local amino-acid side chains which is, in turn, postulated to stabilize certain protein conformations and hence affect function. One prediction would be that amino acids that line anesthetic binding sites should show reduced crystallographic temperature factors when anesthetics bind. However, although the HSA/myristate/halothane structure does have an average temperature factor that is significantly lower than the structure with myristate alone, the amino acids directly in contact with the anesthetics have temperature factors reduced to the same extent as those amino acids which do not contribute to binding interactions.

Propofol binds at two sites, one in subdomain IIIA and one in subdomain IIIB. In both cases the aromatic ring lies within an apolar pocket, with the phenolic hydroxyl group making a hydrogen bond, in the one case (IIIA) with a main-chain carbonyl oxygen and in the other case (IIIB) with a serine hydroxyl (Fig. 2). Both propofol molecules would compete for fatty-acid binding: FA3 and possibly FA4 for the molecule in IIIA, and FA5 for the molecule in IIIB. The propofol molecule in IIIB not only binds weaker than the molecule in IIIA (because electron density for this molecule was the first to disappear when the propofol concentration was reduced) but it also binds in a site that almost certainly accommodates the most tightly binding fatty acid (23, 24). For these reasons one can safely conclude that, at pharmacologically relevant concentrations of propofol in the blood (which are many times lower than the concentrations present in our crystals), only a single propofol-binding site would be occupied (the site in subdomain IIIA). This site in subdomain IIIA has previously been identified crystallographically (13) as one of the two most important drug-binding sites [termed “site II” by Sudlow et al. (25, 26)], and one that can also accommodate diazepam, ibuprofen and other aromatic drugs.
Propofol and halothane binding sites on human serum albumin

Our data with propofol showing only two discrete binding sites, even at saturating concentrations, is very difficult to reconcile with some recent binding studies which have concluded that propofol binds to a large number (around 15) of saturable sites (27) or that propofol causes protein unfolding which results in the absence of any saturable sites (12). It is possible that these binding studies were somehow confounded by the presence of fatty acids (no particular precautions were taken to exclude them) and more work is clearly needed to resolve the apparent discrepancy between these binding studies and our crystallographic results.

At the “low” halothane concentration and in the absence of fatty acid, only three halothane binding sites were well-occupied (HAL1, HAL2 and HAL3). However, we could not discern any key features of these binding sites that distinguished them from the lower-affinity sites that were occupied at the higher halothane concentration. All of the binding sites were predominantly apolar, although most also showed evidence of significant polar interactions between charged or polar amino acids and the polarisable halogen atoms, particularly the bromine. The possible importance of polar interactions between proteins and halogenated compounds has been noted before (3, 28) and the likelihood that general anaesthetic binding sites are amphiphilic in nature has been stressed by our group (29, 30) and others (31-33).

Interestingly, as was the case with propofol, all of the halothane molecules bound within pre-formed pockets or clefts. Furthermore (leaving aside halothane HAL8, whose binding site was artefactually formed by crystal contacts) all of the binding sites were also binding sites for fatty acids. Indeed, in the crystal structure at the high halothane concentration, and in the presence of myristate, the fatty acid has clearly been displaced in sites FA6 (by HAL1, HAL2 and HAL4), FA7 (by HAL5 and HAL6) and FA8 (by HAL7). This is entirely consistent with the work of Dubois and Evers (5, 6) on the
related protein BSA that showed halothane and other volatile anesthetics competed with fatty acids for binding. In addition, the two halothane molecules HAL5 and HAL6 bind within a site which has been identified (13) as a key drug-binding locus on HSA [“site I” of Sudlow et al., (25, 26)].

Although halothane binding to HAL7 can displace myristate bound to FA8, this site is not occupied by fatty acids with longer chains (20) which are much more prevalent in normal plasma (34). Thus, under normal physiological conditions, the binding of HAL7 would be expected to increase rather than decrease due to the presence of fatty acids whose binding is responsible for the formation of the cavity within which HAL7 binds. This observation supports an early suggestion (35) that anesthetics might act by stabilising certain conformational states of a protein simply because binding sites appear fortuitously in that state. Thus even anesthetics that bind intrinsically very weakly to proteins could exert their effects by shifting the equilibria between functionally distinct conformational states (e.g. the open and closed states of an ion-channel).

Which of the halothane sites are pharmacologically relevant? This is a difficult question to answer with certainty. The “low” halothane concentration we used (5% atm.) was still significantly higher than the maximum concentration likely to be used for maintenance of anesthesia, so those binding sites which were only populated at the higher concentration (HAL4, HAL5 and HAL6) are most unlikely to be important. However, all three of the halothane molecules which bind at the lower concentration (HAL1, HAL2 and HAL3) are potentially displaceable by fatty acid, and between 0.1 and 2 molecule of fatty acid is thought to bind under normal physiological conditions. The halothane molecules HAL1 and HAL2 are probably less susceptible to displacement than HAL3 because in the myristate structure the halothane molecules HAL1 and HAL2 were able to displace the
fatty acid FA6 while, in contrast, the fatty acid FA3 was able to prevent the binding of halothane HAL3. In addition, other evidence (20) suggests that FA3 binds more tightly than FA6. Finally, it might be that there is sufficient fatty acid in the blood to induce the conformational change that results in the formation of the binding site for HAL7 which would also make this site (in addition to those for HAL1, HAL2 and HAL3) potentially relevant pharmacologically.

Because of the promiscuous nature of HSA-drug interactions, the possibility that the free, pharmacologically active, concentrations of co-administered drugs could be affected by their competing for common binding sites on the protein has often been considered (11). For example, the volatile anesthetic enflurane has been shown (36) to displace diazepam from HSA in vitro, and the in vivo pharmacokinetics of thiopental are known to be significantly affected (37) by the presence of halothane. Our finding that propofol binds with highest affinity to a site in subdomain IIIA that can also bind a benzodiazepine (13) suggests that there might be a significant interaction between these drugs (which are often co-administered). However, a common binding site does not guarantee a pharmacologically relevant interaction. While a high percentage of both drugs may be bound to HSA, for either drug the percentage of HSA molecules that are involved in binding could still be very small (because the plasma concentration of HSA is very much greater than the total drug concentration). Indeed, a brief report (12) concluded that diazepam did not displace bound propofol; nonetheless this potential interaction has yet to be extensively studied.

Perhaps paradoxically, it is the relatively weaker-binding drugs, such as the volatile general anesthetics, that might be more effective at competing with other drugs for binding to HSA. This is because they are present at sufficiently high concentrations to interact, at least potentially, with a large
Propofol and halothane binding sites on human serum albumin

fraction of the HSA molecules. From our data we can conclude that halothane (and perhaps other volatile anesthetics) could compete for the binding of propofol in subdomain IIIA. We are not aware of any binding studies that have investigated this possibility. Similarly, it is possible that halothane molecules HAL5 and HAL6 might displace so-called “site I” drugs. However, this seems much less likely because these halothane molecules clearly bind rather weakly (electron density only appears at higher halothane concentrations) and available binding data show that a variety of volatile anesthetics are relatively ineffective at displacing phenytoin and warfarin (38, 39) which are classed as “site I” drugs.

In summary, we have shown that two widely used general anesthetics, propofol and halothane, bind to a small number of discrete sites on HSA in the pharmacologically relevant range of concentrations. These sites are preformed amphiphilic pockets or clefts on the protein, and anesthetic binding causes only very minor changes in local structure.

Acknowledgements – We thank Delta Biotechnology Ltd for purified recombinant HSA and the staff at Daresbury SRS (UK) and at DESY Hamburg (Germany) for help with data collection. We wish to acknowledge the use of the EPSRC’s Chemical Database Service at Daresbury and we are very grateful to Peter Brick for helpful comments on the manuscript and Bill Lieb for many stimulating discussions. AB acknowledges the award of a PhD studentship from the MRC. This work was funded by grant support from the MRC and the BBSRC.
Propofol and halothane binding sites on human serum albumin

Table 1 Data collection details and unit cell parameters

<table>
<thead>
<tr>
<th></th>
<th>Native HSA</th>
<th>HSA-propofol</th>
<th>HSA-halothane</th>
<th>HSA-myristate-halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>X-ray source</strong></td>
<td>Daresbury 9.6</td>
<td>Daresbury 9.6</td>
<td>Hamburg X11</td>
<td>Daresbury 9.6</td>
</tr>
<tr>
<td><strong>Wavelength (Å)</strong></td>
<td>0.870</td>
<td>0.870</td>
<td>0.909</td>
<td>0.870</td>
</tr>
<tr>
<td><strong>Space Group</strong></td>
<td>Triclinic P1</td>
<td>Triclinic P1</td>
<td>Triclinic P1</td>
<td>Monoclinic C2</td>
</tr>
<tr>
<td><strong>a (Å)</strong></td>
<td>54.8</td>
<td>55.4</td>
<td>54.6</td>
<td>188.9</td>
</tr>
<tr>
<td><strong>b (Å)</strong></td>
<td>55.6</td>
<td>55.6</td>
<td>55.0</td>
<td>39.1</td>
</tr>
<tr>
<td><strong>c (Å)</strong></td>
<td>120.3</td>
<td>120.5</td>
<td>120.0</td>
<td>96.7</td>
</tr>
<tr>
<td><strong>α</strong></td>
<td>81.2</td>
<td>81.1</td>
<td>81.4</td>
<td>-</td>
</tr>
<tr>
<td><strong>β</strong></td>
<td>91.1</td>
<td>90.6</td>
<td>90.8</td>
<td>105.4</td>
</tr>
<tr>
<td><strong>γ</strong></td>
<td>64.3</td>
<td>65.5</td>
<td>65.5</td>
<td>-</td>
</tr>
<tr>
<td><strong>Resolution range (Å)</strong></td>
<td>36.3 - 2.6</td>
<td>29.9 - 2.2</td>
<td>15.0 - 2.4</td>
<td>46.0 - 2.4</td>
</tr>
<tr>
<td><strong>Independent reflections</strong></td>
<td>37,956</td>
<td>62,870</td>
<td>48,001</td>
<td>26,988</td>
</tr>
<tr>
<td><strong>Multiplicity</strong></td>
<td>2.0 (2.0)</td>
<td>1.9 (1.6)</td>
<td>1.9 (1.8)</td>
<td>3.5 (3.4)</td>
</tr>
<tr>
<td><strong>Completeness (%)</strong></td>
<td>97.5 (97.3)</td>
<td>96.1 (93.4)</td>
<td>95.7 (87.7)</td>
<td>99.1 (98.6)</td>
</tr>
<tr>
<td><strong>R_{merge} (%)</strong></td>
<td>4.5 (25.1)</td>
<td>4.6 (29.6)</td>
<td>4.9 (26.7)</td>
<td>4.9 (27.8)</td>
</tr>
<tr>
<td><strong>I/σ₁</strong></td>
<td>4.0 (1.3)</td>
<td>7.6 (2.2)</td>
<td>8.1 (2.2)</td>
<td>8.6 (2.6)</td>
</tr>
</tbody>
</table>

1Values for the outermost resolution shell are given in parentheses

2\( R_{merge} (%) = 100 \times \frac{\sum_h \sum_j |I_{hj} - I_h|}{\sum_h \sum_j I_{hj}} \) where \( I_h \) is the weighted mean intensity of the symmetry related reflections \( I_{hj} \)
## Table 2 Model refinement

<table>
<thead>
<tr>
<th></th>
<th>Native HSA</th>
<th>HSA-propofol</th>
<th>HSA-halothane</th>
<th>HSA-myristate-halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDB ID</td>
<td>1aaa</td>
<td>1bbb</td>
<td>1ccc</td>
<td>1ddd</td>
</tr>
<tr>
<td>Modelled amino acids</td>
<td>5 – 582</td>
<td>5 – 582</td>
<td>5 – 580</td>
<td>3 – 584</td>
</tr>
<tr>
<td>Number of water molecules</td>
<td>60</td>
<td>120</td>
<td>57</td>
<td>27</td>
</tr>
<tr>
<td>Resolution range (Å)</td>
<td>36.3 - 2.6</td>
<td>29.9 - 2.2</td>
<td>15.0 - 2.4</td>
<td>46.0 - 2.4</td>
</tr>
<tr>
<td>R&lt;sub&gt;model&lt;/sub&gt; (%)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24.7</td>
<td>24.8</td>
<td>27.8</td>
<td>23.0</td>
</tr>
<tr>
<td>R&lt;sub&gt;free&lt;/sub&gt; (%)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>27.7</td>
<td>27.2</td>
<td>29.8</td>
<td>28.1</td>
</tr>
<tr>
<td>RMS deviation from ideal bond lengths (Å)</td>
<td>0.006</td>
<td>0.007</td>
<td>0.009</td>
<td>0.007</td>
</tr>
<tr>
<td>RMS deviation from ideal bond angles (°)</td>
<td>1.1</td>
<td>1.2</td>
<td>1.7</td>
<td>1.18</td>
</tr>
<tr>
<td>Average B-factor (Å²)</td>
<td>75.4</td>
<td>59.9</td>
<td>76.3</td>
<td>51.3</td>
</tr>
</tbody>
</table>

<sup>1</sup><sub>R</sub><sub>model</sub> (%) = 100 × ∑<sub>hkl</sub> | F<sub>obs</sub> - F<sub>calc</sub> | ∑<sub>hkl</sub> F<sub>obs</sub> where F<sub>obs</sub> and F<sub>calc</sub> are the observed and calculated structure factors respectively.

<sup>2</sup><sub>R</sub><sub>free</sub> (%) is the <sub>R</sub><sub>model</sub> (%) calculated using a randomly selected 5% sample of reflection data omitted from refinement.
Propofol and halothane binding sites on human serum albumin

Table 3 Propofol binding sites

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Binding Location</th>
<th>Interactions with hydroxyl</th>
<th>Residues lining cavity walls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol 2</td>
<td>IIIB (FA5)</td>
<td>S579</td>
<td>F502, F507, F509, A528, E531, L532, H535, V547, F551, V576, Q580</td>
</tr>
</tbody>
</table>
### Table 4 Halothane binding sites

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Binding Location</th>
<th>Residues lining cavity walls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane 1</td>
<td>IIA-IIB (FA6)</td>
<td>R209, A210, A213, L347, A350, K351, E354, K212</td>
</tr>
<tr>
<td>Halothane 2</td>
<td>IIA-IIB (FA6)</td>
<td>R209, K212, A213, V216, D324, L327, L331</td>
</tr>
<tr>
<td>Halothane 3</td>
<td>IIIA (FA3 &amp; 4)</td>
<td>I388, N391, F403, L407, L430, V433, G434, C438, A449, L453</td>
</tr>
<tr>
<td>Halothane 4</td>
<td>IIA-IIB (FA6)</td>
<td>V216, F228, S232, V235, V325</td>
</tr>
<tr>
<td>Halothane 7</td>
<td>IA-IIA (FA8)</td>
<td>A26, Y30, L66, H67, F70, N99, D249, L250, L251</td>
</tr>
<tr>
<td>Halothane 8</td>
<td>IA-IB</td>
<td>A21, L135, K136, L139, L155, A158, K159, K162</td>
</tr>
</tbody>
</table>
REFERENCES

Propofol and halothane binding sites on human serum albumin

Propofol and halothane binding sites on human serum albumin

FIGURE LEGENDS

FIG. 1  **The structure of HSA and the locations of fatty acid binding sites.**  A) The native structure of HSA and B) the structure of HSA in the presence of myristate, showing the locations of eight fatty-acid binding sites (20). Fatty acids FA4 and FA8 are shown in a darker shade of grey for clarity of presentation. For further details on the fatty-acid binding sites see ref. (20). The domains are color-coded as follows: red, domain I; green, domain II; blue, domain III. The A and B sub-domains within each domain are depicted in dark and light shades respectively. The fatty acids are represented by space-filling models colored by atom type (carbon, grey; oxygen, red). All figures were prepared using Bobscript and Raster3D (40-42).

FIG. 2  **The propofol binding sites on HSA.**  A) HSA with propofol showing the locations of the two propofol binding sites. B) Site PR1, which is within sub-domain IIIA and C) site PR2, which is within sub-domain IIIB. The dashed lines represent hydrogen bonds. The difference electron density (orange) is an F_o – F_c omit map calculated at 4 \( \sigma \). The amino-acid side chains that are close to the propofol molecules are shown as ball and stick models (a complete list is given in Table 3).

FIG. 3  **The halothane binding sites on HSA.**  A) HSA with halothane at a “low concentration” showing three halothane binding sites. B) HSA with halothane at “high concentration” and myristate showing seven halothane binding sites and five fatty acid binding sites. The anesthetics and fatty acids are represented by space-filling models colored by atom type (carbon, grey; oxygen, red; bromine, brown; chlorine, dark green; fluorine, light green).
FIG. 4 Details of halothane binding sites. A) Halothane binding sites at the interface between subdomains IIA and IIB. B) Halothane site in subdomain IIIA. C) Halothane sites in subdomain IIA. D) Halothane site at the interface between subdomains IA and IIA. The difference electron density (orange) is an $F_0 - F_c$ omit map calculated at $4 \sigma$. Some of the amino-acid side chains that are close to the halothane molecules are shown as ball and stick models (a complete list is given in Table 4). Note that in D) only 11 of the 14 carbon atoms of myristate are shown because, due to disorder, the terminal carbons were not observed in the electron density map.
Binding of the General Anesthetics Propofol and Halothane to Human Serum Albumin: High-Resolution Crystal Structures
A. A. Bhattacharya, S. Curry and N. P. Franks

*J. Biol. Chem.* published online August 11, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M005460200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts