A Molecular Description of How Noble Gases and Nitrogen Bind to a Model Site of Anesthetic Action

James R. Trudell, PhD*, Donald D. Koblin, PhD, MD†, and Edmond I Eger II, MD‡

Departments of Anesthesia, *Program for Molecular and Genetic Medicine, Stanford School of Medicine, Stanford; †Veterans Administration Hospital, San Francisco; and ‡University of California-San Francisco, San Francisco, California

How some noble and diatomic gases produce anesthesia remains unknown. Although these gases have apparently minimal capacities to interact with a putative anesthetic site, xenon is a clinical anesthetic, and argon, krypton, and nitrogen produce anesthesia at hyperbaric pressures. In contrast, neon, helium, and hydrogen do not cause anesthesia at partial pressures up to their convulsant thresholds. We propose that anesthetic sites influenced by noble or diatomic gases produce binding energies composed of London dispersion and charge-induced dipole energies that are sufficient to overcome the concurrent unfavorable decrease in entropy that occurs when a gas molecule occupies the site. To test this hypothesis, we used the x-ray diffraction model of the binding site for Xe in metmyoglobin. This site offers a positively charged moiety of histidine 93 that is 3.8 Å from Xe. We simulated placement of He, Ne, Ar, Kr, Xe, H_2, and N_2 sequentially at this binding site and calculated the binding energies, as well as the repulsive entropy contribution. We used free energies obtained from tonometry experiments to validate the calculated binding energies. We used partial pressures of gases that prevent response to a noxious stimulus (minimum alveolar anesthetic concentration [MAC]) as the anesthetic endpoint. The calculated binding energies correlated with binding energies derived from the *in vivo* (ln) data (RTln[MAC], where R is the gas constant and T is absolute temperature) with a slope near 1.0, indicating a parallel between the Xe binding site in metmyoglobin and the anesthetic site of action of noble and diatomic gases. Nonimmobilizing gases (Ne, He, and H_2) could be distinguished by an unfavorable balance between binding energies and the repulsive entropy contribution. These gases also differed in their inability to displace water from the cavity. **Implications:** The Xe binding site in metmyoglobin is a good model for the anesthetic sites of action of noble and diatomic gases. The additional binding energy provided by induction of a dipole in the gas by a charge at the binding site enhanced binding.

(Anesth Analg 1998;87:411–8)

The anesthetic properties of xenon and krypton (1, 2) have made noble and diatomic gases the subject of many studies of small molecule binding (3–7) and anesthetic mechanisms (8, 9). These and other noble gases have an elegant simplicity; they are spherically symmetric, uncharged, and have zero dipole moments. Such properties compromise theories of anesthetic action that demand a particular conformation, dipolar nature, or the ability to either make or break hydrogen bonds. These characteristics also magnify the problem of explaining how inert molecules, such as noble and diatomic gases, produce anesthesia.

The partial pressures of gasses that prevent movement in response to a noxious stimulus (minimum alveolar anesthetic concentration [MAC]) for Ar, Kr, Xe, and N_2, as well as the nonanesthetic (nonimmobilizing) properties of He, Ne, and H_2 measured or referenced by Koblin et al. (10) provide a self-consistent set of data that enables a test of a previous suggestion about sites of noble gas binding. Many theories of the mechanism by which inert gases produce anesthesia assume that binding determines and is essential to anesthetic action (11). Because inert gases are uncharged and nonpolar, there is no Coulombic component to binding. Two important components of the binding energy remain. The first is a charge-induced dipole term. This potential energy results from induction of a dipole in the gas molecule by a charged binding site. Although noble and diatomic gases are nonpolar, their diffuse clouds of negative electrons can be repelled from or attracted to negative or positive charges, respectively. The resulting distortion of these electron clouds (measured as polarizability) produces an induced dipole that is attracted toward the charge that induced it. The second is an

The research was supported by the Stanford Department of Anesthesia, the Beckman Program for Molecular and Genetic Medicine, and National Institutes of Health Grant PO1 GM47818.

Dr. Eger is a paid consultant to Ohmeda PPD.

Accepted for publication March 26, 1998.

Address correspondence to Edmond I Eger II, MD, Department of Anesthesia, University of California, San Francisco, CA 94143-0464.

0003-2999/98/$5.00

induced dipole-induced dipole term. This is the attractive energy (London dispersion energy) produced when the distribution of electrons in one molecule fluctuates to produce an instantaneous dipole in that molecule. This instantaneous dipole then produces a temporary induced dipole in a second molecule. The initiating instantaneous dipole could be in either the gas molecule or the binding site, and the responding induced-dipole could be in either molecule (12, 13).

These two terms can cause binding of nonpolar molecules to a putative site of anesthetic action. Sufficient binding energy is thought to be an essential component of the production of anesthesia because it assures a high probability of occupancy of the site by the anesthetic molecule and suggests a potential reciprocal alteration of the putative site. However, binding requires that the attractive energy terms (charge-induced dipole and London dispersion) overcome the repulsive terms resulting from confining the molecule to the binding site. The latter entropy terms result from restraining the rotational motion of diatomic gases and the translational motion of all gases (13, 14). The unfavorable entropy term can be thought of as the penalty for confining the statistical probability of a molecule to a particular region of space. The penalty is related to the coordinates of the molecule and is only weakly influenced by the mass of the gas. Because charge-induced dipole and London dispersion energies decrease steeply with molecular weight, whereas the disfavorable entropy terms decrease only slightly, we predicted that low molecular weight gases have a low probability of occupying an anesthetic binding site.

An additional component of binding energy results from the ability of hyperbaric pressure to increase the concentration, and thereby the apparent potency, of noble and diatomic gases. Hyperbaric pressure increases the concentration of gas molecules in all regions of the nervous system and, by the law of mass action, increases the probability that a gas molecule will occupy a site relevant to anesthetic action. In terms of the binding energies described above, the additional favorable binding energy provided by hyperbaric pressure (P) is RTln(P/1 atm), where R is the gas constant, T is the absolute temperature, and In represents *in vivo* (13). This contribution is substantial; 10 atm of pressure increases binding energy by 1.4 kcal/mol, an amount more than twice that available from thermal energy at 37°C.

Katz and Simon (15) used thermodynamic variables to assess and compare the binding properties of noble gases with those of clinical anesthetics. They studied data sets that included loss of righting reflex in mice, blockage of peripheral nerves, and depression of both the dentate gyrus and the olfactory cortex. They concluded that anesthetic sites influenced by noble gases must contain either a positive or negative charge that induces a dipole in the gas molecule needed to provide the binding energy and, thereby, the occupancy by a noble gas. However, they did not perform computational chemistry calculations to support their hypothesis.

The site of anesthetic action remains unknown (16). Candidate structures include lipid (17) and protein (18). In the present study, we used the *in vivo* data from Koblin et al. (10) to provide insights about the properties of a protein binding site relevant to anesthetic action. As our model, we chose the binding site for Xe in metmyoglobin because Xe occupies it at a partial pressure of 1 atm, approximately the same partial pressure as MAC in rats. In additional, this site is well characterized and has amino acid residues that provide both London dispersion energy and charge-induced dipole energy (11). Finally, experimental manometric values are available for binding of H_2, Ar, N_2 (19), and Xe (7) to metmyoglobin. The latter values were used to validate our calculations of binding energies.

Binding of Xe to metmyoglobin has been studied by x-ray diffraction (4, 5, 20) and molecular dynamics simulations (6, 21). Studies using nuclear magnetic resonance have helped to define the dynamics of Xe binding (22, 23). Xe binds to four sites in metmyoglobin, but only one is significantly occupied at 1 atm (5, 7). The present study considered the latter site both because it is occupied at a relevant anesthetic partial pressure and because it is the best characterized. We used literature values of London dispersion energies (20) and changes in entropy (7), our calculations of charge-induced dipole energies, and *in vivo* MAC values from Koblin et al. (10) to provide a self-consistent set of data. We tested whether the binding cavity in metmyoglobin supplies the attractive energy required to overcome the disfavorable entropy terms (7, 19, 24).

Methods

The free energy ($\Delta G°$) of anesthetic binding to an arbitrary site can be described in terms of the P of the anesthetic gas: $\Delta G° = RT\ \mathrm{In}(P/X)$, where X is the ratio of the number of moles of anesthetic molecules to the number of moles of the arbitrary anesthetic sites, and P = 1 atm is the standard state for all calculations. If the Meyer-Overton hypothesis applies, then X is a constant, and the equation can be rewritten as $\Delta G° = RT\ln(MAC) - RT\ln(X)$. The difference between RTln(MAC) values of any two gases of decreasing molecular weight (i.e., $RT\ln[MAC_1] - RT\ln[MAC_2]$) will be $\Delta\Delta G$ because the unknown constant RTln(X) is dropped. $\Delta\Delta G$ then provides the slope of the change in free energy.

We wanted to compare the $\Delta\Delta G$ derived above from *in vivo* data with thermodynamic calculations of binding to a hypothetical site of anesthetic action. We used

Table 1. Properties of Noble and Diatomic Gases

	H_2	He	Ne	N_2	Ar	Kr	Xe
ΔH_{London}	−1.98	−0.52	−0.97	−4.31	−4.06	−6.14	−10.00
ΔH_{charge}	−1.06	−0.28	−0.52	−2.31	−2.18	−3.29	−5.36
$T\Delta S_{translational}$	−3.94	−4.00	−4.16	−4.19	−4.23	−4.30	−4.34
$T\Delta S_{rotational}$	−0.62	0.00	0.00	−0.62	0.00	0.00	0.00
Polarizability (α)	0.8	0.21	0.39	1.74	1.64	2.48	4.04
MW (g/mol)	2.00	4.00	20.20	28.00	40.00	83.80	131.30
$\Delta G_{binding}$							
$\epsilon = 1$	1.51	3.20	2.68	−1.81	−2.01	−5.13	−11.02
$\epsilon = 2$	3.04	3.60	3.42	1.50	1.11	−0.42	−3.34
$\epsilon = 3$	3.54	3.74	3.67	2.61	2.15	1.15	−0.78
$\epsilon = 4$	3.80	3.80	3.79	3.16	2.67	1.94	0.50
$\epsilon = 5$	3.95	3.84	3.86	3.49	2.98	2.26	1.27
$\Delta H_{London} - T\Delta S$ ($\epsilon = 3$)	3.90	3.83	3.84	3.38	2.87	2.10	1.01
MAC (atm)				110	27	7.3	1.6
RTln(MAC/1 atm)				2.9	2.0	1.2	0.3
ΔG_{exp} (metmyoglobin)	2.79			2.55	1.95		0.27

Unless other units are given in parentheses, all values are kcal/mol with the convention that more negative ΔH or ΔG values indicate stronger binding to the anesthetic site, whereas more negative TΔS values result in weaker binding.

The enthalpies ΔH_{London} and ΔH_{charge} are calculated from the London dispersion energy and the charge-induced dipole energy, respectively. The energy terms that disfavor binding, $T\Delta S_{translational}$ and $T\Delta S_{rotational}$, are caused by restriction of translational and, in the case of diatomic gases, rotational motion, respectively. The polarizability (α) of each gas is in $(Å)^3$. The calculated free energy of binding ($\Delta G_{binding}$) = $(\Delta H_{London} + \Delta H_{charge})/\epsilon - (T\Delta S_{translational} + T\Delta S_{rotational})$, where the local apparent dielectric constant (ϵ) = 1, 2, 3, 4, or 5. The set of values labeled $\Delta H_{London} - T\Delta S$ are the same as $\Delta G_{binding}$ except that ΔH_{charge} was not included.

The partial pressures that prevent response to a noxious stimulus (minimum alveolar anesthetic concentration [MAC]) are from Koblin et al. (10).

The additional free energy of binding to an anesthetic site that is provided by increased pressure is calculated as −RTln(MAC/1 atm). MAC is divided by a 1-atm standard state partial pressure, and the important figure is the difference between neighboring values (ΔΔG°), rather than the absolute values of ΔG°. The values for ΔG_{exp} are from tonometry experiments (7, 19), they have been converted to a 1-atm standard state.

experimental data from binding of gases to an unoccupied cavity in metmyoglobin to calculate the difference between the free energies of binding of each gas of decreasing molecular weight (ΔΔG°) where $\Delta G° = \Delta H_{binding} - T\Delta S°$). To accomplish this, we estimated three terms for each gas molecule: 1) the charge-induce dipole energy (ΔH_{charge}); 2) the London dispersion energy (ΔH_{London}) (the sum of these two terms is $\Delta H_{binding}$); and 3) the disfavorable entropy term TΔS°. We used the convention that more negative ΔH or ΔG values indicate stronger binding to the anesthetic site, whereas more negative TΔS° values weaken binding.

We first calculated the attractive energy produced by a charge-induced dipole interaction (ΔH_{charge}). The metmyoglobin binding site contains a histidine residue with a single positive charge shared between two nitrogen atoms 4.13 and 3.47 Å from Xe (20). We treated this charged site as a single positive charge at a mean distance (r) of 3.8 Å from the center of each gas molecule. The field (E) at the gas molecule depends on the r between it and the positive charge (q): $E = q/r^2$. The dipole induced in each gas molecule equals E times the polarizability (α) of the gas. The α of a molecule is a measure of how much the electron cloud around a molecule is distorted when it is placed in an electric field. The values for α for the noble gases and diatomic gases were taken from the literature (25) and are shown in Table 1. Finally, the attractive energy (ΔH_{charge}) is the square of the vector product of the field at the center of the gas molecule times α divided by two times the local apparent dielectric (ϵ) (12, 26); that is: $\Delta H_{charge} = E^2\alpha/2\epsilon$. The value for ϵ is 1 in a vacuum, but values of 2–5 are considered to best approximate the effective screening in the interior of a protein cavity (27, 28). Calculations were performed with ϵ set equal to 1, 2, 3, 4, and 5.

The ΔH_{London} for Xe binding to metmyoglobin is approximately −10 kcal/mol (4). This energy is a linear function of α (13). Therefore, to provide a self-consistent set of data, ΔH_{London} was calculated for the other noble gases as $-10(\alpha_1/\alpha_2)$ kcal/mol. The α of diatomic gases is not equal in all directions; therefore, the mean value of α was used. Table 1 shows the α values and the results of the calculations.

An unfavorable decrease in entropy results from restriction of the translational and rotational motions of a small molecule in a binding site. This decrease in entropy decreases the probability that a gas will occupy that site. The TΔS° for Xe in metmyoglobin is 4.34 kcal/mol at 310°K (37°C) (7). To provide a self-consistent set of data, this value for Xe was scaled in proportion to the molecular weights (M) of the other gases to provide TΔS° values for those gases (Table 1). Entropy decreases because of restriction of translational motion in proportion to the M according to $\Delta\Delta S = \ln(M_1/M_2)^{3/2}$ (14). In addition to the restriction in translational motion, the diatomic gases are also restricted in their ability to rotate about their x and y axes. This disfavors binding by an additional 0.6 kcal/mol (13, 14). The assumption that the TΔS° term can be scaled in this manner requires that conformational

entropy caused by altered flexibility in the site secondary to gas binding be either small or constant. The x-ray diffraction structure supports this assumption. The overall effect of up to four Xe molecules bound to different sites is small; a root mean square deviation between metmyoglobin and metmyoglobin plus Xe of 0.28 Å for all atoms. This is thought to result from Xe occupying preexisting and unoccupied internal cavities (5). The most strongly bound Xe is in close contact with the positively charged histidine-93 and also interacts with phenylalanine-138 (5).

We calculated $\Delta G_{binding} = \Delta H_{London} + \Delta H_{charge} - T\Delta S°$ for each gas using the five choices of local apparent dielectric. However, results with a local apparent dielectric of 1 are not shown because they do not fit the scale of the figures. We considered only the difference in binding energies between successive members of the series ($\Delta\Delta G°$) by plotting RTln(MAC) versus the $\Delta G_{binding}$ values as shown in Table 1.

Results

If $\Delta G_{binding}$ contains the dominant contributions to the binding energy at a site important to anesthesia, then the graph of $\Delta G_{binding}$ versus RTln(MAC) will be a straight line with a slope of 1 (i.e., anesthetic potency will be directly proportional to $\Delta G_{binding}$). Figure 1 displays four sets of data points for available data (He, Ne, and H_2 are not anesthetics) when the local apparent dielectric equals 2, 3, 4, and 5. The graphs of $\Delta G_{binding}$ versus RTln(MAC) $\Delta H_{binding}$ have slopes ($\pm$ SD) of 1.88 $\pm$ 0.42, 1.30 $\pm$ 0.26, 1.01 $\pm$ 0.16, and 0.86 $\pm$ 0.08 (r^2 = 0.91, 0.93, 0.95, 0.98), respectively). The graph with local apparent dielectric of 1 would not fit the scale of Figure 1, but it has a slope of 3.58 $\pm$ 1.0 (r^2 = 0.86). To illustrate the contribution of ΔH_{charge}, a fifth pair of data points for only $\Delta G_{London} - T\Delta S°$ (i.e., excluding ΔG_{charge}), evaluated with a local apparent dielectric of 3, is included. This graph has a slope of 0.91 $\pm$ 0.10 (r^2 = 0.98). As emphasized in Methods, it is the slope of each line in Figure 1 that is important (15, 24, 29–31).

We compared the calculations of $\Delta G_{binding}$ with measurements of free energies of gas binding to metmyoglobin by tonometry. Data at 20°C are available for H_2, N_2, Ar (19), and Xe (7). The ΔG_{exp} value for Xe was calculated from Table 1 by converting the equilibrium constant of 200/M to a 1-atm standard state. Figure 2 displays four graphs of $\Delta G_{binding}$ evaluated with local apparent dielectrics of 2, 3, 4, and 5 versus the experimental free energies (ΔG_{exp}). The slopes of the lines are 2.38 $\pm$ 0.29, 1.62 $\pm$ 0.16, 1.25 $\pm$ 0.09, and 1.03 $\pm$ 0.05 (r^2 = 0.97, 0.98, 0.99, 0.99), respectively. The graph with local apparent dielectric of 1 would not fit the scale of Figure 2. For comparison, the data set for ΔG_{London} is included, evaluated with the local

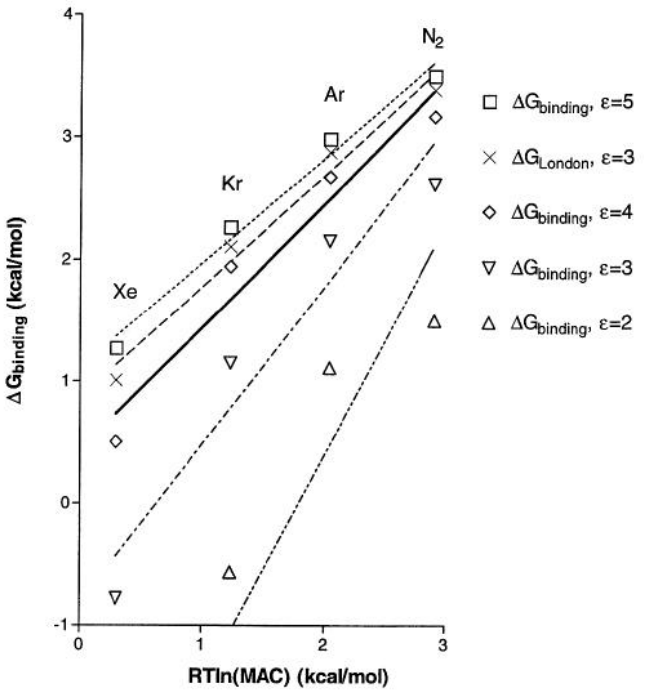

Figure 1. Graphs of calculated $\Delta G_{binding}$ versus RTln(MAC/1 atm) of data points for available data (He, Ne, and H_2 are not anesthetics) when the local dielectric constant (ϵ) was set to 2, 3, 4, and 5. The slopes of the lines are important. The intercepts must have an unknown quantity added to them (RTln[X]).

apparent dielectric of 3 that gave a slope of 0.91 in Figure 1. The latter graph has a slope of 1.10 $\pm$ 0.07 (r^2 = 0.99).

We evaluated the suitability of metmyoglobin as a model for an anesthetic binding site by comparing RTln(MAC) versus ΔG_{exp} for gas binding to metmyoglobin as measured by tonometry (Figure 3). The slope of the line is 0.89 $\pm$ 0.07 (r^2 = 0.99), which suggests that the Xe binding site in metmyoglobin suitably models a site of anesthetic action.

Why are nonimmobilizers (i.e., nonanesthetics) without anesthetic potency? Table 1 provides calculated binding energies of all gases in the series. Figure 4 displays $\Delta H_{binding}$ (evaluated with a local apparent dielectric of 4 [slope 0.86 $\pm$ 0.27; r^2 = 0.83], as well as $T\Delta S°$, evaluated at 37°C [slope 0.15 $\pm$ 0.13; r^2 = 0.46]) for each gas versus RTlnMAC). The favorable $\Delta H_{binding}$ term decreases more steeply than does $T\Delta S°$ as M decreases. Therefore, there is a decreasing probability that the site will be populated by the smaller gas molecules.

A second feature that may distinguish nonimmobilizers from anesthetics is their ability to compete with water for a binding site. We applied the calculations used to obtain the values in Table 1 to estimate $\Delta G_{binding}$ for a water molecule in the metmyoglobin cavity ($\Delta G_{binding}$ = 3.8 kcal/mol). The values used were: ϵ = 4, α = 1.45, M = 18, and a correction of 0.92 kcal/mol

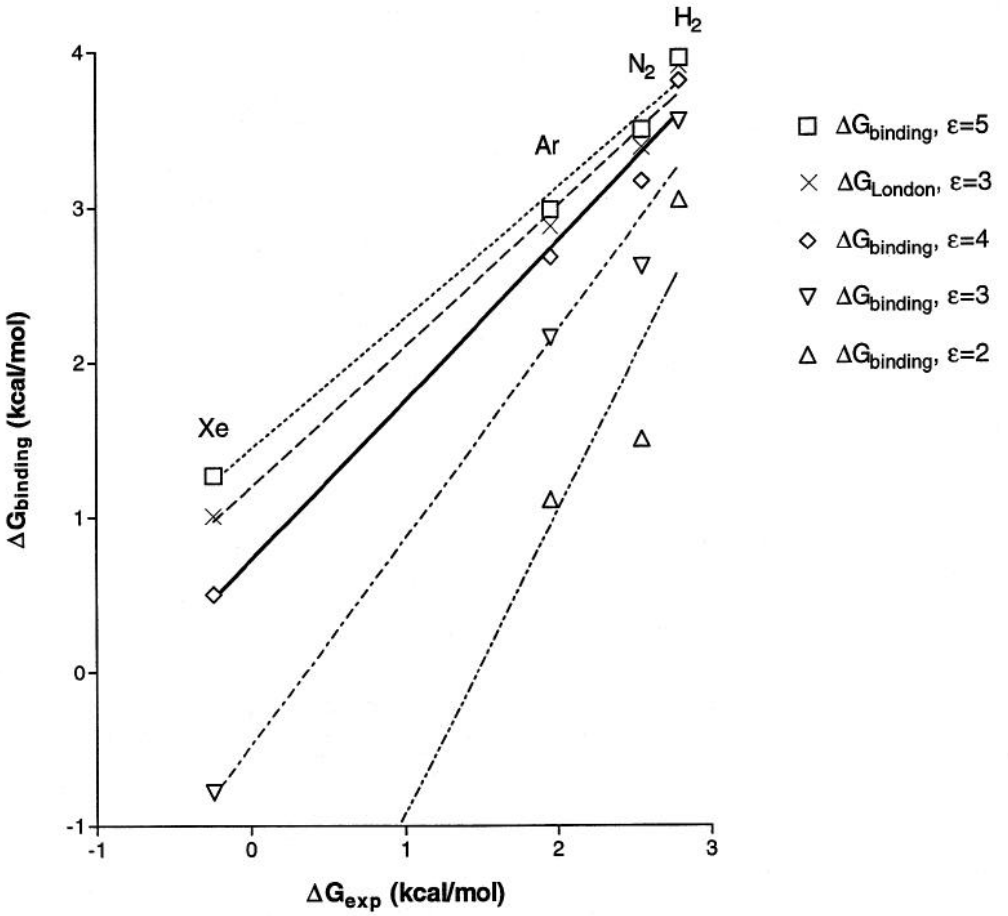

Figure 2. Graphs of calculated $\Delta G_{binding}$ and ΔG_{London} versus experimental free energies of gas (ΔG_{exp}) binding to metmyoglobin measured by tonometry. Data are only available for H_2, N_2, Ar, and Xe. The $\Delta G_{binding}$ was evaluated with local dielectric constants (ϵ) of 2, 3, 4, and 5. The ΔG_{London} was evaluated with a local dielectric constant of 3 because that value gave a slope near 1 in Figure 1.

for limiting three degrees of rotational freedom at 310°K (13,14,32). This calculation has a much greater error than the others because water has a dipole moment, it can form hydrogen bonds, and the polarizability tensor is not symmetric (33,34). Nevertheless, the calculation is approximately correct as evidenced by the failure of water to occupy the binding site in metmyoglobin (5,7). Water does not have sufficient attractive binding energy to overcome the unfavorable entropy terms for binding in metmyoglobin. At $\epsilon = 4$, the nonimmobilizers H_2, He, and Ne have $\Delta G_{binding}$ values of 3.79–3.80 (Table 1), values identical to water, a molecule known not to bind to the metmyoglobin cavity. The weak anesthetic N_2 (MAC 110 atm) has a $\Delta G_{binding}$ of 3.16 at $\epsilon = 4$.

Discussion

The present results provide binding energies of noble and diatomic gases in the binding site of metmyoglobin. We predicted and found that a graph of these binding energies plotted versus the MAC values from Koblin et al. (10) would have a slope near 1. We predicted that He, Ne, and H_2 have less binding energy than water, and therefore are unlikely to bind to the site in metmyoglobin, which is known not to bind water. We used the x-ray diffraction study of Xe bound to metmyoglobin (5, 20) as a model for a hypothetical anesthetic site because of the large amount of experimental data available. In our simple model, we considered a relatively close interaction with a single positive charge on histidine-93, as well as the ΔH_{London}, with all atoms in the binding site. In a protein binding site relevant to anesthetic action, the binding energy may be increased by the sum of interactions of many charges or decreased by ϵ (27). We calculated the binding energy of two diatomic molecules, H_2 and N_2, to provide continuity with the accompanying article by Koblin et al. (10) (Table 1), correctly predicting that N_2 should be a more potent anesthetic than H_2, which is thought to be a nonimmobilizer. Although Koblin et al. (10) also studied SF_6, its properties are so different from the noble gases

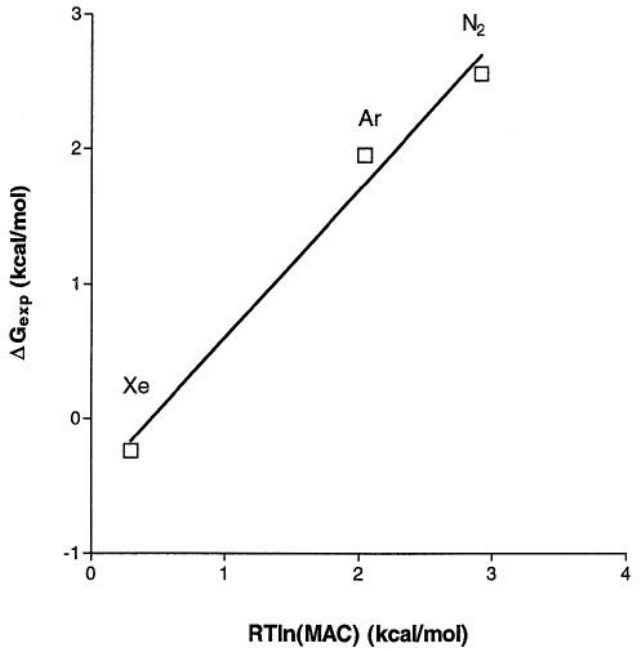

Figure 3. Graphs of experimental free energies (ΔG_{exp}) for gas binding measured by tonometry versus RTln(MAC) measured by Koblin et al. (10). Both sets of data are available for only N_2, Ar, and Xe.

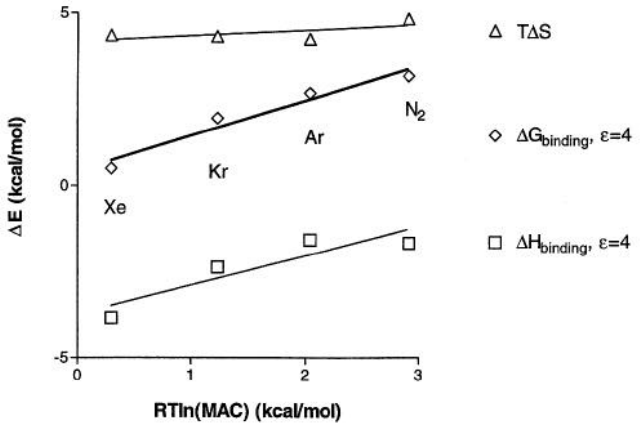

Figure 4. The favorable enthalpy of binding ($\Delta H_{binding}$), evaluated with a local dielectric constant (ϵ) of 4, was plotted versus RTln(MAC/1 atm) measured by Koblin et al. (10). The term that disfavors binding (TΔS), evaluated at 37°C, was also plotted versus RTln(MAC/1 atm). The favorable $\Delta H_{binding}$ term declines more rapidly than does TΔS as molecular weight decreases (Table 1). Therefore, their difference ($\Delta G_{binding}$), evaluated with a local dielectric constant of 4, has a slope near 1.

that we did not attempt calculations of its binding energies.

The graphs in Figure 1 exhibit good linearity, show the correct ordering of anesthetic potency versus calculated binding energy, and have slopes near 1 at the reasonable ϵ of 4. We conclude that a sufficiently hydrophobic internal cavity, which is not normally occupied by water molecules and which has a relatively low local apparent dielectric, suitably mimics a binding site relevant to anesthesia. The calculations leading to the slopes of the lines in Figure 1 do not assess the importance of including an induced dipole energy as predicted by Katz and Simon (15). Both the graph of the data including the ΔH_{charge}, evaluated with a ϵ of 4, and the graph of the data including only ΔH_{London}, evaluated with a ϵ of 3, have slopes of approximately 1.0. However, the values of $\Delta G_{binding}$ for Xe, evaluated at a ϵ of 3, are −0.78 with ΔH_{charge} and +1.01 kcal/mol with only ΔH_{London}. The difference is −1.79 kcal/mol (more attractive) when ΔH_{charge} is included (Table 1). The additional −1.79 kcal/mol of binding energy provided by ΔH_{charge} may provide the energy that allows Xe to occupy the cavity in metmyoglobin at 1 atm. An x-ray study of Xe binding in the pore of cartilage oligomeric matrix protein supports this point (35). At 1, 2, or 5 atm of Xe, only water occupied the internal pore. At 10 atm of Xe, eight Xe molecules displaced water and occupied the pore. The additional binding energy provided by 10 atm is −RTln(10 atm/1 atm) = −1.42 kcal/mol, a value near the −1.79 kcal/mol noted above for ΔH_{charge}.

Our comparison of calculated $\Delta G_{binding}$ with ΔG_{exp} of gas binding to metmyoglobin measured by tonometry (Figure 2) supports the view that the calculated energies are correct. A slope of 1.25 is obtained when $\Delta G_{binding}$ is evaluated with a ϵ of 4. This comparison suggests that the calculated $\Delta G_{binding}$ does include the most important terms of the binding energy. Again, inclusion of ΔH_{charge} results in absolute values near the experimental values (Table 1).

Figure 3 shows that the metmyoglobin binding site is a reasonable model for a site relevant to anesthesia. The slope of 0.89 in the graph of ΔG_{exp} for gas binding measured by tonometry versus RTln(MAC) suggests that metmyoglobin binds these gas molecules as strongly as the anesthetic site characterized by the MAC values. All data in Figure 3 are experimental values, and no assumption is made regarding a ϵ value.

Figures 1–3 provide a strong link among calculated $\Delta G_{binding}$, ΔG_{exp} of gas binding to metmyoglobin measured by tonometry, and RTln(MAC) values from Koblin et al. (10). This link supports our hypothesis that binding energies predict anesthetic potency and that metmyoglobin provides a relevant model of an anesthetic site of action. However, a second goal of this study was to explain or predict the lack of anesthetic activity of the nonimmobilizers Ne, He, and H_2. In the context of the metmyoglobin model, one reason for lack of activity is that these gases bind poorly because the entropy term that opposes binding stays large, whereas the free energy term that favors binding decreases rapidly with decreasing M (Figure 4). Nevertheless, low binding is insufficient to explain the

lack of anesthetic effect. We used the slopes and intercepts from Figure 1 to calculate predicted MAC values of approximately 290 atm for the three nonimmobilizers. The additivity studies performed by Koblin et al. (10) should have detected an anesthetic effect of these drugs if their MAC values were really 290 atm, and they did not.

One reason for the lack of potency of the nonimmobilizers is that, like water molecules, they cannot occupy regions that provide low binding energy. Calculations based on the variables that determine binding of water in hydrophobic cavities support this suggestion (21). We noted in Results that an estimate of the binding energy of water in metmyoglobin is slightly less than the weak anesthetic N_2 and is equal to the nonimmobilizers He, Ne, and H_2. Water does not occupy the binding site in metmyoglobin (5,7), and the same may be true of nonimmobilizers.

A second possible reason for lack of potency of nonimmobilizers is that, although such gases may bind to an anesthetic site, the site may not be affected. Although small molecules are known to bind to internal cavities (24), fairly specific binding sites (4,36), and interfacial sites (37), binding alone may not be sufficient to alter the stability of a conformer or interconversion between conformers. To cause anesthesia, such binding must also alter the function of neural sites essential to consciousness (38).

Calculated binding energies correlate closely with anesthetic binding calculated from *in vivo* measurements of MAC. Including a charge-induced dipole term provides the additional energy needed to overcome the penalty for constraining the molecular motion of the gas molecules, thereby causing them to occupy the cavity in metmyoglobin. The good correspondence between binding energies derived from tonometry experiments with RTln(MAC) suggests that the binding site in metmyoglobin shares many features with sites relevant to anesthetic action. The lack of efficacy of the nonimmobilizers may be a result of their inability either to bind to sites or to affect the sites while bound.

References

1. Lawrence JH, Loomis WF, Tobias CA, Turpin FH. Preliminary observations on the narcotic effect of xenon with a review of values for solubilities of gases in water and oils. J Physiol 1946;105:197–204.
2. Cullen SC, Gross EG. The anesthetic properties of xenon in animals and human beings, with additional observations on krypton. Science 1951;113:580–2.
3. Chin JH, Trudell JR, Cohen EN. The compression-ordering and solubility-disordering effects of high pressure gases on phospholipid bilayers. Life Sci 1976;18:489–97.
4. Schoenborn BP, Watson HC, Kendrew JC. Binding of xenon to sperm whale myoglobin. Nature 1965;207:28–30.
5. Tilton RF Jr, Kuntz ID Jr, Petsko GA. Cavities in proteins: structure of a metmyoglobin-xenon complex solved to 1.9 Å. Biochemistry 1984;23:2849–57.
6. Tilton RF Jr, Singh UC, Kuntz ID Jr, Kollman PA. Protein-ligand dynamics: a 96 picosecond simulation of a myoglobin-xenon complex. J Mol Biol 1988;199:195–211.
7. Ewing GJ, Maestas S. The thermodynamics of absorption of xenon by myoglobin. J Phys Chem 1970;74:2341–4.
8. Wulf RJ, Featherstone RM. A correlation of van der Waals constants with anesthetic potency. Anesthesiology 1957;18:97–105.
9. Miller KW, Paton WDM, Smith EB, Smith RA. Physicochemical approaches to the mode of action of general anesthetics. Anesthesiology 1972;36:339–51.
10. Koblin DD, Fang Z, Eger EI II, et al. Minimum alveolar concentrations of noble gases, nitrogen, and sulfur hexafluoride in rats: helium and neon as (nonimmobilizers) nonanesthetics. Anesth Analg 1998;87:419–24.
11. Eger EI II, Koblin DD, Harris RA, et al. Hypothesis: inhaled anesthetics produce immobility and amnesia by different mechanisms at different sites. Anesth Analg 1997;84:915–8.
12. Hirschfelder JO, Curtiss CF, Bird RB. Molecular theory of gases and liquids. New York: John Wiley & Sons, 1954.
13. Atkins PW. Physical chemistry. New York: WH Freeman, 1994.
14. McQuarrie DA. Statistical mechanics. New York: Harper Collins, 1976.
15. Katz Y, Simon SA. Physical parameters of the anesthetic site. Biochim Biophys Acta 1977;471:1–15.
16. Johansson JS, Eckenhoff RG. Minimum structural requirement for an inhalational anesthetic binding site on a protein target. Biochim Biophys Acta 1996;1290:63–8.
17. Trudell JR. A unitary theory of anesthesia based on lateral phase separations in nerve membranes. Anesthesiology 1977;46:5–10.
18. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994;367:607–14.
19. Mortimer RG, Bauer N. The affinity of legoglobin and other heme proteins for gaseous nitrogen, hydrogen and argon. J Phys Chem 1960;74:387–90.
20. Schoenborn BP, Nobbs CL. The binding of xenon to sperm whale deoxymyoglobin. Mol Pharmacol 1966;2:491–8.
21. Hermans J, Shankar S. The free energy of xenon binding to myoglobin from molecular dynamics simulation. Isr J Chem 1986;27:225–7.
22. Tilton RF, Kuntz ID. Nuclear magnetic resonance studies of xenon-129 with myoglobin and hemoglobin. Biochemistry 1982; 23:2857–65.
23. Xu Y, Tang P. Amphiphilic sites for general anesthetic action? Evidence from ^{129}Xe-[^{1}H]intermolecular nuclear Overhauser effects. Biochim Biophys Acta 1997;1323:154–62.
24. Morton A, Baase WA, Matthews BW. Energetic origins of specificity of ligand binding in an interior nonpolar cavity of T4 lysozyme. Biochemistry 1995;34:8564–75.
25. Miller TM. Atomic and molecular polarizabilities. In: Lide DR, ed. Handbook of chemistry and physics. Boca Raton, FL: CRC Press, 1992:10-194–10-200.
26. Van Belle D, Couplet I, Prevost M, Wodak SJ. Calculations of electrostatic properties in proteins: analysis of contributions from induced protein dipoles. J Mol Biol 1987;198:721–35.
27. Sharp KA, Honig B. Electrostatic interactions in macromolecules: theory and applications. Annu Rev Biophys Biophys Chem 1990;19:301–32.
28. Honig BH, Hubbell WL. Stability of "salt bridges" in membrane proteins. Proc Natl Acad Sci USA 1984;81:5412–6.
29. Morton A, Matthews BW. Specificity of ligand binding in a buried nonpolar cavity of T4 lysozyme: linkage of dynamics and structural plasticity. Biochemistry 1995;34:8576–88.
30. Weber G. Protein interactions. New York: Chapman and Hall, 1992.
31. Dickinson R, Franks NP, Lieb WR. Thermodynamics of anesthetic/protein interactions. Biophys J 1993;64:1264–71.
32. Dunitz JD. The entropic cost of bound water in crystals and biomolecules. Science 1994;264:670.

33. Rashin AA, Iofin M, Honig B. Internal cavities and buried waters in globular proteins. Biochemistry 1986;25:3619–25.
34. Wade RC, Mazor MH, McCammon JA, Quiocho FA. A molecular dynamics study of thermodynamic and structural aspects of the hydration of cavities in proteins. Biopolymers 1991;31: 919–31.
35. Malashkevich VN, Kammerer RA, Efimov VP, et al. The crystal structure of a five-stranded coiled coil in COMP: a prototype ion channel? Science 1996;274:761–5.
36. Dickinson R, Franks NP, Lieb WR. Can the stereoselective effects of the anesthetic isoflurane be accounted for by lipid solubility? Biophys J 1994;66:2019–23.
37. Peters KP, Fauck J, Frommel C. The automatic search for ligand binding sites in proteins of known three-dimensional structure using only geometric criteria. J Mol Biol 1996;256:201–13.
38. Leff P. The two-state model of receptor activation. Trends Pharmacol Sci 1995;16:89–97.