Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 134–138, January 1991
Physiology/Pharmacology

Modulation of the general anesthetic sensitivity of a protein: A transition between two forms of firefly luciferase

(anesthetic target sites/hydrophobic binding pockets/cutoff effects)

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Communicated by Arnold Burgen, October 1, 1990

ABSTRACT **The activities of most proteins are relatively insensitive to general anesthetics. A notable exception is firefly luciferase, whose sensitivity to a wide range of anesthetic agents closely parallels that of whole animals. We have now found that this sensitivity can be controlled by ATP. The enzyme is insensitive at low (μM) concentrations of ATP and very sensitive at high (mM) concentrations. The differential sensitivity varies from anesthetic to anesthetic, being greatest (about a 100-fold difference) for molecules with large apolar segments. This suggests that anesthetic sensitivity is modulated by changes in the hydrophobicity of the anesthetic-binding pocket. Parallel changes in the binding of the substrate firefly luciferin, for which anesthetics compete, indicate that anesthetics bind at the same site as the luciferin substrate. These changes in the nature of the binding pocket modify not only the sensitivity to anesthetics but also the position of the "cutoff" in the homologous series of primary alcohol anesthetics; the cutoff position can vary from octanol to pentadecanol, depending upon the concentration of ATP. Our results suggest that particularly sensitive anesthetic target sites in the central nervous system may possess anesthetic-binding pockets whose polarities are regulated by neuromodulatory agents.**

One of the major problems regarding the molecular basis of general anesthesia lies in understanding why some proteins are sensitive to anesthetics whereas others are not. In fact, the majority of proteins that have been tested have been found to be relatively insensitive to most general anesthetics (1). Some proteins, however, are extremely sensitive. Firefly luciferase, for example, has been shown to be inhibited by a wide range of these agents at the concentrations that maintain general anesthesia in animals (2). (A few integral membrane proteins, including some ion channels, are affected by some anesthetics. However, interpretation is complicated by the presence of membrane lipid, in which these lipid-soluble agents readily dissolve, as well as by the possible role of regulatory proteins that might themselves be the primary anesthetic targets.) Firefly luciferase is thus one of the few simple and well-defined protein models of general anesthetic target sites, and previous work (2, 3) in this laboratory has established some of the features that account for its sensitivity to general anesthetics and its ability to mimic *in vivo* phenomena such as the cutoff effect, which is observed in homologous series of anesthetic compounds.

We have demonstrated (2) that anesthetics inhibit firefly luciferase by competing for binding with the hydrophobic heterocyclic substrate firefly luciferin and not by interfering with the catalytic mechanism of the light-producing reaction. In the course of our previous work (2, 3) which was mainly carried out at "saturating" levels of ATP, we made the chance observation that at low ATP concentrations the enzyme became much less sensitive to anesthetic inhibition. We have now followed up this observation by carrying out a detailed analysis of the behavior of the enzyme as a function of ATP concentration. Our results suggest that ATP induces a conformational change in the enzyme that makes its anesthetic- and luciferin-binding pocket more hydrophobic. This not only causes the enzyme to become more susceptible to anesthetic inhibition but also substantially shifts the position of the cutoff point in the homologous series of primary alcohols.

MATERIALS AND METHODS

D-Luciferin, desiccated firefly lanterns, ATP (grade I), sodium azide, and 1-tridecanol were purchased from Sigma. *N*-Glycylglycine, $MgSO_4$, chloroform, butanone, paraldehyde, and the primary alcohols from propanol to dodecanol were obtained from BDH. Ethanol was obtained from James Burrough and acetone was from May and Baker (Dagenham, U.K.). Benzyl alcohol and 1-tetradecanol were purchased from Aldrich. Halothane (as Fluothane) was a gift from I.C.I. All reagents used were of the highest purity available from the above suppliers and were used without further purification. Pure crystals of the enzyme firefly luciferase were obtained from lanterns of the North American firefly *Photinus pyralis* by using the purification method of Branchini *et al.* (4).

Luciferase assays were usually initiated by rapidly injecting (using an air-driven glass syringe) 2.5 ml of a buffered ATP solution into a glass vial containing 5 ml of a buffered solution of the enzyme, luciferin, and $MgSO_4$ (2). For experiments in which K_m values for ATP were measured, the injected solutions contained luciferin and $MgSO_4$, and the vial solutions contained the enzyme and ATP. For these latter experiments at the two highest luciferin concentrations used, the assay volumes were reduced (to conserve expensive luciferin) to 0.161 ml of injected solution and 0.5 ml of vial solution. When anesthetics were present, they were always preequilibrated with the enzyme in the reaction vial. Anesthetics were usually added directly to buffer, but the relatively insoluble primary alcohols larger than heptanol were added as ethanolic solutions; in these cases, the final concentration of ethanol never exceeded 24 mM (4% of the EC_{50} for the high-affinity form of the enzyme); nonetheless, the same concentrations of ethanol were present in the control assays. The buffer used was *N*-glycylglycine, titrated to pH 7.8 with NaOH. To avoid microbial growth, sodium azide was added to all ATP solutions (which were always made up on the day of the experiment) such that its concentration in the final reaction solutions was about 0.01%; this had no detectable effect on the assays. The final reaction solution concentrations were as follows: 1–10 nM enzyme

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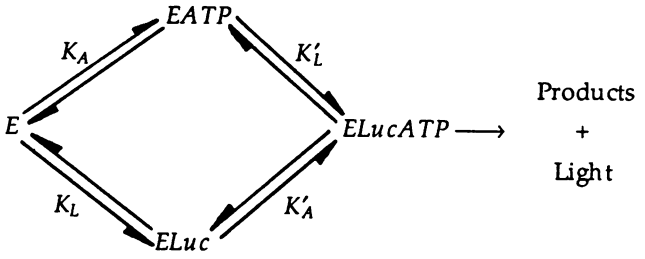
(luciferase), 0.05–1000 μM luciferin, 0.1–2000 μM ATP, 6.7 mM $MgSO_4$, and 25 mM *N*-glycylglycine. Experiments were carried out at room temperature, typically 24°C. Light output from the luciferase reaction was detected with a photomultiplier tube, and the signal was then amplified and recorded on a digital oscilloscope and chart recorder. The enzyme activity was taken as the peak of the light output.

The results presented in this paper (in the absence of anesthetics) were interpreted in terms of the following simple random binding model:



Scheme I

where E represents the free enzyme, EATP is the enzyme with ATP bound, ELuc is the enzyme with luciferin bound, ELucATP is the enzyme with both substrates bound, and the four *K* symbols represent dissociation constants. Since it is known (5) that the rate-limiting steps in the reaction occur after the formation of the bisubstrate complex ELucATP, all four forms of the enzyme were taken to be in equilibrium with each other and the substrates ATP and luciferin.

The Michaelis constant K_m for a given substrate is the concentration of that substrate at which half of the maximal reaction rate is achieved when the concentrations of the other substrates remain fixed. It can be shown that, for the above scheme, the two relevant Michaelis constants for ATP and luciferin, respectively, are

$$K_m^{ATP} = K_A \frac{1/K_L + 1/[Luc]}{1/K'_L + 1/[Luc]} \quad [1]$$

and

$$K_m^{Luc} = K_L \frac{1/K_A + 1/[ATP]}{1/K'_A + 1/[ATP]}, \quad [2]$$

with the constraint that

$$K_A K'_L = K_L K'_A. \quad [3]$$

The Michaelis constant (and its standard error) for each substrate was determined at various fixed concentrations of the other substrate by analyzing double-reciprocal plots of $(\text{rate})^{-1}$ versus $(\text{concentration})^{-1}$, using the method of weighted least squares, as described in detail elsewhere (6). Theoretical curves satisfying Eqs. **1–3** were then fitted to all of these experimental Michaelis constants, using only a single set of values of the four dissociation constants K_A, K'_A, K_L and K'_L (only three of which are independent). This set of values was determined from the K_m values at the highest and lowest substrate concentrations.

In the presence of anesthetic, the simple binding scheme above can be expanded to include competitive interactions of anesthetic with the luciferin-free enzyme forms E and EATP, which are assumed to sequentially bind each of one or more anesthetic molecules with inhibition (dissociation) constants K_i and K'_i, respectively. The analysis is simplified by introducing a function $f(I)$, defined (2) as the factor by which the apparent Michaelis constant for luciferin increases in the presence of inhibiting anesthetic at a free concentration (I). This function is given by

$$f(I) \equiv \frac{(K_m^{Luc})_{app}}{K_m^{Luc}} = \frac{v_0}{v_I} + \frac{[Luc]}{K_m^{Luc}}\left(\frac{v_0}{v_I} - 1\right), \quad [4]$$

where, at a given ATP concentration, v_I and v_0 are the reaction rates in the presence and absence of anesthetic, respectively, and K_m^{Luc} is the Michaelis constant for luciferin in the absence of anesthetic (see Eq. 2). The right-hand expression was used to convert the actual experimental reaction-rate data into values of $f(I)$, which were then analyzed as described below.

If *n* anesthetic molecules bind sequentially with dissociation constant K_i to E, and if *m* anesthetic molecules bind sequentially with dissociation constant K'_i to EATP, but only one molecule is required to block binding of luciferin to either form, then it is possible to show that

$$f(I) = \frac{K_A(1 + [I]/K_i)^n + [ATP](1 + [I]/K'_i)^m}{K_A + [ATP]} \quad [5]$$

Thus when only one anesthetic molecule can bind to the enzyme ($n = m = 1$), it follows that, at any ATP concentration [ATP], $f(I)$ is linear with [I] and

$$f(I) = 1 + [I]/K_i^{app}, \quad [6]$$

where

$$K_i^{app} = K_i \frac{K_A + [ATP]}{K_A + [ATP]K_i/K'_i} \quad [7]$$

When more than one anesthetic molecule can bind to the enzyme, however, this linear behavior disappears and a plot of $f(I)$ versus [I] becomes a quadratic that curves upward. For the frequent case when $[ATP] << K_A$ and two anesthetic molecules ($n = 2$) bind to the enzyme, the curve becomes a perfect square that obeys the relationship

$$\sqrt{f(I)} = 1 + [I]/K_i \quad [8]$$

For cases covered by Eqs. **6–8**, the inhibition constants K_i^{app} and K_i (and their standard errors) were determined from linear plots of $f(I)$ or $\sqrt{f(I)}$ versus [I], using the method of weighted least squares, as described in detail elsewhere (6). Typically, each plot was constructed using three control and six anesthetic data point determinations.

The EC_{50}, a convenient measure of anesthetic sensitivity at a given [ATP], is defined as the concentration [I] of anesthetic at which the rate of reaction is 50% of the uninhibited rate, when [Luc] is set at K_m^{Luc} (see Eq. 2). This latter proviso is necessary because the degree of inhibition depends upon the luciferin concentration, since anesthetics compete with luciferin for binding to the enzyme. It follows directly from Eq. 4 that, when $[I] = EC_{50}$, $f(I) = 3$. Thus EC_{50} concentrations can always be found by determining that anesthetic concentration [I] at which $f(I) = 3$. This was the procedure adopted when the anesthetic/enzyme stoichiometry varied with ATP concentration, a situation not covered by Eqs. **6–8**. For example, with halothane we found that two anesthetic molecules bind at high [ATP] but only one at low [ATP], and thus

solving Eq. 5 with $n = 1$, $m = 2$, [I] = EC_{50}, and $f(I) = 3$ yields:

$$EC_{50} = K_i' \left\{ \sqrt{\left(1 + \frac{K_i' K_A}{2K_i[ATP]}\right)^2 + 2\left(1 + \frac{K_A}{[ATP]}\right)} - \left(1 + \frac{K_i' K_A}{2K_i K_A[ATP]}\right) \right\}. \quad [9]$$

On the other hand, for cases covered by Eqs. 6–8, EC_{50} values were determined directly from the measured inhibition constants, using $EC_{50} = 2K_i^{app}$ (from Eq. 6) or $EC_{50} = 0.732\ K_i$ (from Eq. 8).

RESULTS

The reciprocal effects of ATP and luciferin on increasing the apparent binding affinities of each other to the enzyme firefly luciferase are illustrated in Figs. 1 and 2. Increasing concentrations of ATP decreased the K_m for luciferin, and increasing concentrations of luciferin similarly decreased the K_m for ATP. Our results are consistent with a simple random binding model (Scheme I) in which the bisubstrate complex ELucATP can be formed from the free enzyme E by either of two pathways: (*i*) ATP first binding to the free enzyme E (with a dissociation constant K_A) followed by luciferin binding to the resultant EATP complex (with a dissociation constant K_L') or (*ii*) luciferin first binding to E (with a dissociation constant K_L) followed by ATP binding to the resultant ELuc complex (with a dissociation constant K_A'). The curves drawn through the data points in Figs. 1 and 2 are the predictions (Eqs. 1–3) of this model using a single set of three independent dissociation constants. The insets to each figure give representative double-reciprocal plots in the two "plateau" regions; these plots were linear in all cases and were used for the determination of all of the Michaelis constants K_m (and their standard errors) plotted in Figs. 1 and 2.

Anesthetics inhibit luciferase at high ATP concentrations by competing with luciferin for binding to the protein (2). We found the same competitive behavior at low ATP concentrations. Fig. 3 *Inset* shows a representative series of double-reciprocal plots at low (0.6 μM) ATP for the anesthetic halothane. The inhibition is competitive only with respect to the luciferin substrate and not with respect to the ATP substrate (since as one approaches limiting high luciferin concentrations the anesthetic inhibition vanishes). If anesthetics and luciferin bind to the same site (2), one might expect a modulation by ATP of the anesthetic sensitivity of luciferase. This was in fact observed for all of the anesthetics tested (see, for example, Fig. 3 and Table 1), with some

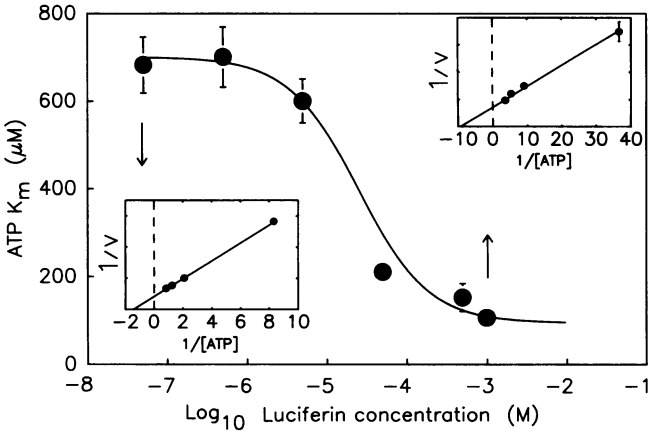


FIG. 2. Dependence of the K_m for ATP on luciferin concentration. K_m values and their standard errors were determined from weighted linear regression plots such as those shown in the insets. The smooth curve was calculated according to the predictions (Eq. 1) of a simple random binding model (Scheme I) and the parameter values listed in the legend to Fig. 1. (*Insets*) Double-reciprocal plots of reciprocal reaction velocities (arbitrary units) versus reciprocal ATP concentrations (mM^{-1}) at the luciferin concentrations indicated by the horizontal positions of the arrows; each point is the mean of multiple (typically $n = 3$) measurements. Where no error bar is shown, the standard error was less than the size of the symbol.

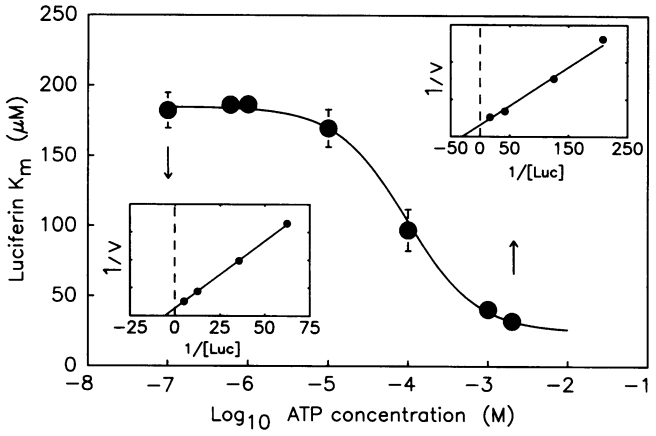


FIG. 1. Dependence of the K_m for luciferin on ATP concentration. K_m values and their standard errors were determined from weighted linear regression plots such as those shown in the insets. The smooth curve was calculated according to the predictions (Eq. 2) of a simple random binding model (Scheme I). This and all other theoretical curves in this paper were calculated using the following unique set of parameter values (only three of which are independent): $K_A = 700$ μM, $K_A' = 95$ μM, $K_L = 185$ μM, and $K_L' = 25$ μM. (*Insets*) Double-reciprocal plots of reciprocal reaction velocities (arbitrary units) versus reciprocal luciferin concentrations (mM^{-1}) at the ATP concentrations indicated by the horizontal positions of the arrows; each point is the mean of multiple (typically $n = 3$) measurements. Where no error bar is shown, the standard error was less than the size of the symbol.

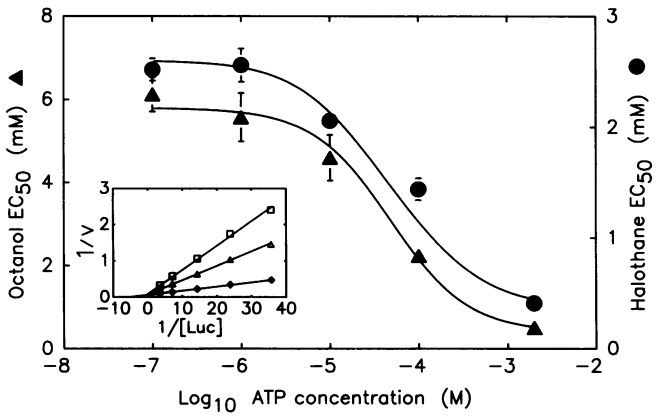


FIG. 3. Dependence of EC_{50} concentrations of halothane (●) and 1-octanol (▲) on ATP concentration and the competitive nature of anesthetic inhibition. The smooth curves are the predictions of a simple random binding model. The curve for halothane was calculated using Eq. 9 with $K_A = 700$ μM, $K_i = 1.3$ mM, and $K_i' = 0.5$ mM. The curve for octanol was drawn using Eq. 7 with $EC_{50} = 2K_i^{app}$, $K_A = 700$ μM, $K_i = 2.9$ mM, and $K_i' = 0.2$ mM. The error bars give the standard errors estimated from $f(I)$ plots. (*Inset*) Double-reciprocal plots, at a low (0.6 μM) ATP concentration, of reciprocal reaction velocity (arbitrary units) versus reciprocal luciferin concentrations (mM^{-1}) at the following concentrations of halothane: ◇, 0.0 mM; △, 2.5 mM; □, 4.9 mM. Each point is the mean of multiple (typically $n = 3$) measurements. There was no significant difference in the values of the intercepts on the ordinate axis. Where no error bar is shown, the standard error was less than the size of the symbol.

Table 1. EC_{50} concentrations for inhibiting luciferase at low and high ATP concentrations

Inhibitor	EC_{50}, mM	
	0.6 μM ATP	2.0 mM ATP
Acetone	750 ± 90 (2)	160 (2)
Butanone	192 ± 9 (2)	31 (2)
Paraldehyde	51 ± 5 (1)	27 (2)
Benzyl alcohol	14.7 ± 0.6 (2)	1.7 (2)
Chloroform	9.3 ± 0.2 (2)	1.4 (2)
Halothane	2.0 ± 0.2 (1)	0.39 (2)
Ethanol	1930 ± 300 (2)	600 (2)
1-Propanol	444 ± 45 (2)	48 (2)
1-Butanol	86 ± 5 (2)	9.6 (2)
1-Pentanol	39 ± 5 (2)	3.0 (2)
1-Hexanol	14 ± 2 (2)	0.99 (2)
1-Heptanol	12.4 ± 0.7 (2)	0.83 (2)
1-Octanol	6.6 ± 0.6 (1)	0.28 (1)
1-Decanol	0.980 ± 0.180 (1)	0.0056 (1)
1-Dodecanol	0.045 ± 0.004 (1)	0.00046 (1)
1-Tridecanol	0.017 ± 0.005 (1)	0.00030 (1)
1-Tetradecanol	0.021 ± 0.009 (1)	0.00029 (1)

EC_{50} concentrations and their standard errors at 0.6 μM ATP were determined. EC_{50} concentrations at 2.0 mM ATP are from Franks and Lieb (2, 3). Numbers in parentheses give the stoichiometry of the interaction between inhibitor and enzyme, as determined from the linearity of plots of $f(I)$ or $\sqrt{f(I)}$ versus [I]; where these numbers could not be clearly determined from the data, their estimated values are underlined. Inhibition constants K_i and K_i' can be calculated from the above data using Eq. 5 and K_A = 700 μM and $f(EC_{50})$ = 3.

anesthetics undergoing a change of >100-fold in binding constant.

Furthermore, the inhibition patterns can be accounted for quantitatively by a simple extension of Scheme I in which anesthetic binds weakly to the free enzyme E (with a dissociation constant K_i) but more strongly to the ATP–enzyme complex EATP (with a dissociation constant K_i'). This is shown for halothane and 1-octanol in Fig. 3, where their EC_{50} concentrations are plotted as a function of ATP concentration. The smooth curves are the predictions of the model using the same substrate dissociation constants as above plus values of the inhibition constants K_i and K_i' determined from the "plateau" EC_{50} concentrations at low and high ATP concentrations, respectively. For 1-octanol, plots of $f(I)$ versus [I] were linear at all ATP concentrations, which implies that only one octanol molecule can interact with the enzyme. For halothane, on the other hand, although plots of $f(I)$ versus [I] were linear at low ATP, they were parabolic at high ATP concentrations. This behavior is consistent with one molecule of halothane binding to the enzyme at low ATP levels but two molecules binding at high levels and suggests a conformational change in the anesthetic-binding site.

Table 1 lists EC_{50} values at low (0.6 μM) and high (2 mM) ATP concentrations for 15 general anesthetics and for two long-chain primary alcohols, tridecanol and tetradecanol, which are ineffective at anesthetizing animals (7). Next to each value is the number N of anesthetic molecules that can interact with the enzyme at the listed ATP concentration, as judged by the linear (N = 1) or parabolic (N = 2) behavior of the corresponding $f(I)$ versus [I] plots. By using these values of EC_{50} and N, one can readily calculate values (unlisted) for the inhibition constants K_i and K_i', using the procedure described in Table 1.

DISCUSSION

It might be supposed that a protein is either sensitive to general anesthetics, or it is not. Our results show, however, that a single protein can exist in both sensitive and insensitive states and that a transition between these states can be effected simply by the binding of a ligand. This has important implications for general anesthesia, in that it opens up a range of possible mechanisms. It suggests that the sensitivity of anesthetic target sites involved in general anesthesia and its various side effects may well be regulated (for example, by neuromodulatory agents). Furthermore, as we will show below, our data provide important clues as to what changes might account for such modulation of anesthetic sensitivity and how these changes relate to the cutoff effects seen in homologous series of anesthetic agents.

For firefly luciferase, the transition between the sensitive and insensitive states is controlled by the molecule ATP. At low concentrations of ATP, the enzyme is relatively insensitive to inhibition by general anesthetics, whereas at high ATP levels the enzyme is extremely sensitive to a wide range of general anesthetic agents, with EC_{50} concentrations that are essentially identical to those that produce general anesthesia in animals (2). An unresolved question in previous investigations (2, 3) was whether anesthetics and luciferin compete by binding to the same binding pocket on the enzyme or whether anesthetics bound to a separate site that then allosterically modified the luciferin-binding site. The fact that the ATP concentration dependence of modulation was similar for both anesthetics and luciferin supports the simple view that both anesthetics and luciferin bind to the same pocket on the enzyme.

Why is firefly luciferase so sensitive to anesthetic inhibition at high levels of ATP? A clue comes from the observation (see Table 1) that the most hydrophobic anesthetics undergo the largest percentage changes in potency as the ATP concentration is changed. This is shown most clearly for the homologous series of primary alcohols in Fig. 4, where we have plotted EC_{50} concentrations for both the insensitive (0.6 μM ATP) and sensitive (2 mM ATP) forms of the enzyme. For both states of the enzyme, there is a generalized increase in potency (decrease in EC_{50}) as one ascends the homologous series, as would be expected for a largely hydrophobic binding pocket. In addition, both curves show the same localized deviations from this general pattern, suggesting that the overall structure of the binding pocket is conserved. However, it is clear that the increase in potency with chain length is much steeper for the sensitive than for the insensitive form of the enzyme. This can be made quantitative by

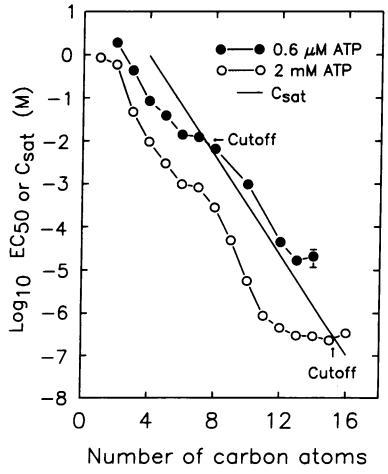


FIG. 4. Cutoff effect at low and high ATP concentrations for the homologous series of primary alcohols. The EC_{50} concentrations at 0.6 μM ATP (●) and at 2 mM ATP (○) are plotted against the number of carbon atoms in the alcohols. Values not listed in Table 1 are from Franks and Lieb (3). The straight line gives the maximum (saturated) aqueous concentration (C_{sat}) of the alcohols (8). The cutoffs correspond to the points where the EC_{50} curves intersect this straight line.

calculating from the data in Table 1 (using inhibition constants) the mean incremental standard Gibbs free energies $\Delta(\Delta G^0_{CH2})$ of transfer from buffer to enzyme of a methylene (CH_2) group. Between C_2 and C_{12}, these are, on average, $\Delta(\Delta G^0_{CH2}) = -3.7$ kJ/mol for the sensitive state but only -2.9 kJ/mol for the insensitive state. What this means is that each methylene group binds more tightly (on average by -0.8 kJ/mol) to the luciferase pocket on the sensitive than on the insensitive form of the enzyme. In other words, ATP increases the sensitivity of the enzyme by making its anesthetic-binding pocket more hydrophobic.

Some regions of the anesthetic-binding pocket when the enzyme is in its sensitive state are surprisingly hydrophobic, in the sense that they are capable of binding additional methylene groups unusually tightly. For example, between C_2 and C_6 and between C_8 and C_{11}, the binding free energies per methylene group are, on average -4.0 kJ/mol and -4.7 kJ/mol, respectively. These values are large even compared to that for the transfer of methylene groups from water to the completely apolar solvent hexadecane (9). Other examples of "enhanced hydrophobicity" have been reported, but for enzymes such as chymotrypsin (10, 11) and certain tRNA synthetases (12), which are required to recognize particular apolar amino acids. Such enzymes might be expected to have evolved binding sites that maximize dispersion forces between the particular substrates and the protein. For the anesthetic-binding site on firefly luciferase, which is capable of binding such a diverse range of compounds, such an explanation seems less likely, and the reason for the unusually large values of $\Delta(\Delta G^0_{CH2})$ is at present unclear.

Our results also have important implications for the cutoff effect in general anesthesia: as one ascends a homologous series of anesthetic agents, such as the primary alcohols, potencies increase until a point is reached above which higher members of the series are inactive. The cutoff point for primary alcohols is just after C_{12} for tadpoles; i.e., 1-tridecanol does not cause anesthesia (7). For a protein target site, the cutoff occurs (3, 13) when the EC_{50} concentration becomes greater than the saturated aqueous concentration. From Fig. 4, it is clear that the position of the cutoff is a function of ATP concentration, being just after C_7 at 0.6 μM ATP and C_{15} at 2 mM ATP, with intermediate positions predicted for intermediate ATP concentrations. For example, at an ATP concentration of 15 μM, the cutoff position would occur just after C_{12} (calculated using Eq. 5, Table 1, and K_A = 700 μM), which is identical to that for general anesthesia. In addition, the marked leveling off in the EC_{50} curves of Fig. 4 before cutoff occurs is then replaced by a gradual decline to cutoff, mimicking recent tadpole observations (7). Thus it is a mistake, when considering protein models of general anesthesia, to place undue attention on the exact cutoff positions (14) or on the detailed behavior on approaching cutoff (7), since these features can depend quite markedly on the concentrations of the modulating agents.

We have shown that the anesthetic sensitivity of a protein can be dramatically modulated by (presumably) an allosteric effect due to the binding of another ligand. The molecular mechanism underlying this effect was found to be an alteration in the hydrophobicity of the anesthetic-binding site. Our results suggest that other proteins, involved in general anesthesia and its side-effects, may also have their anesthetic sensitivities modified in a similar fashion. Such effects might be expected when the activity of multisubunit or multidomain protein targets (e.g., ion channels, receptors, or regulatory proteins) can be modulated by allosteric interactions with neurotransmitters, neuromodulators, or second messenger proteins, resulting in altered conformations due to covalent (e.g., phosphorylation) or noncovalent (e.g., binding to guanine nucleotide binding proteins) interactions (15). Experimentally, these effects would show up, and should be looked for, either as a change in the affinity of the protein to anesthetics in the presence of the modulator or as a change in the affinity of the protein to the modulator in the presence of anesthetic. Such modulations in protein sensitivity may be important in the allosteric inhibition and activation of certain membrane receptors, ion channels, and enzymes by general anesthetic agents.

This work was supported by grants from the National Institutes of Health (GM 41609), the Medical Research Council (U.K.), and the BOC Group.

1. Franks, N. P. & Lieb, W. R. (1982) *Nature (London)* **300**, 487–493.
2. Franks, N. P. & Lieb, W. R. (1984) *Nature (London)* **310**, 599–601.
3. Franks, N. P. & Lieb, W. R. (1985) *Nature (London)* **316**, 349–351.
4. Branchini, B. R., Marschner, T. M. & Montemurro, A. M. (1980) *Anal. Biochem.* **104**, 386–396.
5. DeLuca, M. & McElroy, W. D. (1974) *Biochemistry* **13**, 921–925.
6. Curry, S., Lieb, W. R. & Franks, N. P. (1990) *Biochemistry* **29**, 4641–4652.
7. Alifimoff, J. K., Firestone, L. L. & Miller, K. W. (1989) *Br. J. Pharmacol.* **96**, 9–16.
8. Bell, G. H. (1973) *Chem. Phys. Lipids* **10**, 1–10.
9. Franks, N. P. & Lieb, W. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5116–5120.
10. Dorovska, V. N., Varfolomeyev, S. D., Kazanskaya, N. F., Klyosov, A. A. & Martinek, K. (1972) *FEBS Lett.* **23**, 122–124.
11. Fastrez, J. & Fersht, A. R. (1973) *Biochemistry* **12**, 1067–1074.
12. Fersht, A. R., Shindler, J. S. & Tsui, W.-C. (1980) *Biochemistry* **19**, 5520–5524.
13. Franks, N. P. & Lieb, W. R. (1987) *Trends Pharmacol. Sci.* **8**, 169–174.
14. Miller, K. W., Firestone, L. L., Alifimoff, J. K. & Streicher, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1084–1087.
15. Maze, M. (1990) *Anesthesiology* **72**, 959–961.