UNDERSTANDING how anesthetics act requires a synthesis of information from \textit{in vitro} (molecular, receptor, and neuronal systems) and \textit{in vivo} (whole animal) studies. Most investigators argue that only anesthetic concentrations required for clinical anesthesia (e.g., 0.2–2.0 minimum alveolar concentration [MAC]) are relevant to \textit{in vitro} studies of anesthetic mechanisms. In a previous report in this journal, Eckenhoff and Johansson\cite{1} supplied several arguments for potential relevance to effects produced by any concentration, even concentrations far above the clinical range. In the current article, we conclude that only concentrations close to the clinical range are relevant to \textit{in vitro} studies of anesthetic mechanisms. A Glossary of the several acronyms used in this article is supplied at the end of the article.

Eckenhoff and Johansson\cite{1} offered a simple model in which the additive effects of anesthetics on different target molecules produce anesthesia. They found that if 10 receptors each have an EC\textsubscript{50}\textsuperscript{a} of 1 unit (actual units are not relevant here) \textit{in vitro}, then the combined \textit{in vitro} effect occurs at a concentration of approximately 0.1 units, and the concentration–effect relation for the combined effect is steeper than for the individual \textit{in vitro} concentration–effect relations. They found that this steepness approaches the steepness for concentration–effect relations for MAC.\textsuperscript{2–6} Such steepness is well illustrated with halothane\textsuperscript{2–6}: approximately 90\% of patients move in response to incision at halothane concentrations below 0.72\%, whereas only 10\% move at concentrations exceeding 0.77\% (fig. 1).

The conclusions of Eckenhoff and Johansson\cite{1} may be questioned on two grounds. First, they assume that the total response of multiple effects is obtained by simple (parallel) addition. This has the perplexing result that the combined maximum effect is greater than 1.0. Second, their analyses fit a sigmoid curve to those data points less than 1.0 only. The resulting fit (forced to a maximum possible effect [Emax] = 1.0) poorly describes the data. In fact, if the data are normalized to Emax = 1, the slope equals that of a single receptor. The model in Appendix A considers sequential rather than parallel additivity and also predicts only small increases in steepness for multiple sites. Appendix B introduces the concept of threshold to better understand the relation between receptor and population sensitivities to anesthetics.

The present article considers the concentrations relevant to \textit{in vitro} studies of the mechanisms underlying one individual anesthetic effect, namely, immobilization. First, we examine the thesis that the additive effect of multiple receptors can produce concentration–effect responses similar to those found in the determination of MAC. We conclude that the steepness of the slope defining MAC results from the limited variations in individual responses to anesthetics and likely reflects a small number of target sites. Franks and Lieb\cite{7} reached a similar conclusion regarding the number of target sites based on results of studies of stereospecificity. Second, we consider whether \textit{in vitro} studies of receptor effects should restrict the anesthetic concentrations applied to those that are clinically relevant. We conclude that they should.

Receptor concentration–effect relations\textsuperscript{8–16} produce shallower curves than found for MAC determinations. For example, the concentration–effect relation for \textit{γ}-aminobutyric acid receptor type A (GABA\textsubscript{A}) and acetylcholine receptors goes from 10 to 90\% of the maximum effect in one to two orders of magnitude (10- to 100-fold) change in concentration (fig. 2).

How might these \textit{in vitro} shallow concentration–effect curves produce the \textit{in vivo} steep concentration–effect curves for MAC? Eckenhoff and Johansson\cite{1} suggested that “the most plausible explanation for such highly conserved sensitivity to general anesthetics (i.e., the steepness of MAC concentration–effect curves) is that there are multiple contributing systems, each of which might be influenced to only a small degree by the anesthetic.”\textsuperscript{11} The steepness of the population curves that underlie MAC might be “explained by progressive, simultaneous actions at many targets of comparable sensitivity.” Eckenhoff and Johansson\cite{1} suggested that we can convert the relatively shallow receptor–concentration effect relations for a single receptor to steeper concentration–effect relations by adding the receptor–concentration effects of several different receptors. Fur-
At a lower partial pressure than their individual EC50, then their combined effect will be to produce immobilization and if each equally could mediate anesthesia, molecular targets having the same anesthetic dose–effect curve, and if each equally could mediate anesthesia. Several receptor concentration–effect relations occur in the clinically relevant range. For example, the response of GABA_A or acetylcholine receptors to inhaled anesthetics occurs primarily in the range of 0.2–1.5 MAC of these anesthetics (fig. 2). If immobility results from an action on several molecular targets (GABAA, glycine, glutamate, and acetylcholine) have roughly the same shape and position (EC50 values near MAC) for anesthetic concentration–effect curves. Therefore, either their combined effect is irrelevant to anesthesia (i.e., MAC is too close in position to the EC50 values of the individual targets) or the original argument about multiple targets being responsible for anesthesia is wrong (only one or a very few specific targets are relevant, but multiple targets are not relevant). However, consistent with the Eckenhoff; Johansson1 argument, some in vitro systems respond only at concentrations several-fold greater than 1.5 MAC. Can the additive interaction of two systems, one of which responds in the clinically relevant range, the other of which requires a higher concentration, each materially influence anesthesia? Evaluation of this question requires the mathematical exploration of the additive interaction of two or more receptors.

In our analysis (Appendix A), as the number of involved receptors (m or n) increases, the concentration–effect relation shifts to the left and steepens (fig. 3). The magnitude of steepening may be quantified by fitting a sigmoid Emax model to the resulting concentration–effect relation. Although Eckenhoff and Johansson suggested that the presence of multiple equipotent receptors (each with an Ry of 1.0) markedly steepens the concentration–effect relation, our analysis produces a value for the Hill coefficient for the receptor concentration–effect relation (Ry) that approaches a maximum of approximately 1.5 as an infinite number of receptors are added. This value is far less than the values of 6–20+ for the Hill coefficient for population concentration–effect relation (Ry) found for MAC (fig. 1), i.e., it is a value far less than would be needed to reconcile the different steepnesses of receptor and MAC concentration–effect relations.

The large leftward shift also appears to present a conflict. The shift means that the 50% effect point defining MAC would be achieved at a concentration much below individual receptor EC50 values (R_EC50 values). Such a relation is unusual (e.g., see fig. 2). The R_EC50 for most receptor–concentration effect curves lies close to MAC, not an order of magnitude or more to the right. Therefore, the similarity of MAC to the R_EC50 for receptor systems, believed to be clinically important, speaks against anesthesia resulting from additive effects of multiple receptors with similar R_EC50 values.

Now consider receptors having different R_EC50 values. Consider the cooperation of two receptors, one with an R_EC50 of 0.3 mM, the other with an R_EC50 of 3.0 mM. Applying equation 1 from Appendix A to these data but allowing for the different values for R_EC50 results in a combined effect with an EC50 of 0.25 mM and an Ry of 1.1 (fig. 4). Although the receptor with the larger value for R_EC50 contributes to the combined effect (i.e., it decreases EC50 from the value for the other receptor from 0.3 mM to a combined value of 0.25 mM), the effect on position and steepness is small.

Another scenario requiring exploration involves individual receptors each having steeper concentration–effect relations than shown in figure 2, e.g., having an Ry value of 2.0. Applying a variation of equation 4 demonstrates that the additive combination of receptors yields a concentration–effect relation with an Ry larger than 2.0 (data not shown). However, although the combined Ry increases, the increase is small.

The preceding arguments regarding the interaction of receptors do not explain the steepness of the MAC relation. They are consistent with the notion that recep-
tors that might mediate anesthetic effects will be altered by clinically relevant concentrations of anesthetics.

As Eckenhoff and Johansson observed, the categoric (yes–no) nature of the measurement underlying MAC has been invoked to explain the steepness of the population concentration–effect relation. At MAC, visible movement in response to a noxious stimulus ceases. A graded depression of synaptic excitatory transmission to motor neurons underlies this quantal response. The translation of this graded depression into failure of impulse generation that occurs when the excitatory input falls below a threshold contributes to the abruptness of cessation of movement. Thus, shallow dose–response curves for individual receptors becomes steep curves for in vivo responses when a threshold is involved. Below the threshold concentration, subjects move in response to

Fig. 2. Both γ-aminobutyric acid (GABA) and acetylcholine act on ligand-gated ion channel receptors thought to be potential mediators of anesthetic action. The data for GABA were obtained for GABA applied to α,β, receptors in Xenopus oocytes. Similarly, the data for acetylcholine were obtained for neuronal acetylcholine receptors expressed in Xenopus oocytes. The concentration causing a 90% change in receptor function is 10- to 100-fold larger than the concentration causing a 10% change. That is, the anesthetic concentration–effect relations for these receptors (to enhance the action of GABA and depress the action of acetylcholine) are less steep than the population concentration–effect relation that underlies MAC (Fig. 1).

Fig. 3. The anesthetic concentration–effect relations seen in figure 2 for a receptor or for receptor combinations can be modeled by a curve defined by equation 4 (Appendix A). In the current example, all of the receptors have the same $R_{EC50}$ (0.3 mM). As n (the number of independent receptors) increases, the concentration–effect relation shifts to the left and steepens. The magnitude of steepening may be quantified by fitting a sigmoid Emax model to the resulting concentration–effect relation. This analysis produces a value for $R_g$ (the Hill coefficient) that approaches a maximum of approximately 1.5 as an infinite number of receptors are added. This value is far less than the $R_g$ values of 6–20 found for MAC (Fig. 1), i.e., it is a value far less than would be needed to reconcile the different steepness of receptor and MAC concentration–effect relations.

Fig. 4. Figure 3 assumed an identical $R_{EC50}$ (0.3 mM) and Hill coefficient (an $R_g$ of 1.0) for all of the different receptors whose effects were added to produce a combined effect. Here the combined effect of two receptors is illustrated, one with an $R_{EC50}$ of 0.3 mM and the other with an $R_{EC50}$ of 3.0 mM, both having an $R_g$ of 1.1. Thus, although the receptor with the larger value for $R_{EC50}$ contributes to the combined effect, the effect is minimal both in the shift of the $R_{EC50}$ and the steepening of the resulting curve.
stimulation, and above this concentration they do not move. The resulting concentration–effect relation for MAC for each individual will be infinitely steep regardless of the steepness of the concentration–effect curve for the receptor(s) mediating MAC, and if all individuals are identical, then the population concentration–effect relation would also be infinitely steep.

Two factors may decrease this steepness of the population concentration–effect relation. The first is measurement error. The analyzers used to determine anesthetic concentrations produce values that may err, usually by only a small amount. Rounding errors add to the inaccuracy (e.g., analyzers rounding to the nearest tenth of a percent may differ by several hundredths of a percent from the true value). End-tidal samples may not accurately reflect alveolar anesthetic partial pressures, and end tidal to arterial partial pressure differences can exist and can vary among populations. Such factors add variability to the response, thereby flattening the resulting MAC concentration–effect relation.

Variations within and among individuals provide a second factor that may decrease the steepness of the population concentration–effect relation that produces MAC. Antognini et al. reported findings consistent with variations in receptor sensitivity within an individual. They found that the vigor of muscular response to a noxious stimulus progressively decreases over the anesthetic concentration range below the concentration that causes the suppression of response. Differences among individuals also may alter individual responses. These include differences in age, body temperature, central nervous system sodium concentrations, hormonal changes, and circadian rhythms.

Variations within an individual or differences among individuals may reflect differences in receptor responses of individuals to anesthetics. Various receptors, one or more of which might mediate the effects of anesthetics, underlie the function of the central nervous system. Receptors of a given class (e.g., GABA<sub>A</sub> receptors) may all respond to a specific ligand, but the response may vary (even for a recombinant receptor expressed in relatively homogeneous cell populations, such as Xenopus oocytes) because of differences in cellular conditions. In addition, the effects of anesthetics may differ as a function of the subunits comprising the receptor. For example, oocytes injected with RNA prompting the production of acetylcholine receptors can vary in their response to acetylcholine and can alter the depression of this response by isoflurane administration (figs. 5A–C; figs. 5A and 5B are approximations to unpublished data from Tomohiro Yamakura). How might such variations affect the steepness of population responses?

Figure 5A illustrates the depression of the response to acetylcholine in a single oocyte. Assume that patient movement might occur in response to surgical stimulation up to some fixed fraction of the maximal depression (zero response to acetylcholine). For the present, assume this fraction to be 0.5 (i.e., 50% depression). Thus, all patients represented by this oocyte would move at anesthetic concentrations up to the concentration indicated by arrow “A,” and no one would move at higher concentrations. Although isoflurane progressively depresses the receptor, producing a relatively shallow relation (R<sub>y</sub> slightly greater than 1), the quantal concentration–effect curve is infinitely steep, approximated in the present example by a sigmoid curve with a Py of 100 (fig. 5D).

Examination of several oocytes possessing acetylcholine receptors reveals that depression produced by isoflurane varies (fig. 5B): the curves are parallel but differ in position. For a quantal analysis (i.e., Y-values > 0.5 produce movement; those < 0.5 do not), all oocytes (patients) respond to a stimulus (move) at concentration A, 67% at B, 33% at C, and none at D. A sigmoid curve fit to the plot of the percent responding versus concentration yields a value for Py of 10 (fig. 5D), a steepness observed in studies of MAC in humans. Thus, variability in receptor–effect response to anesthetics might be similar to that shown in fig. 5B. A larger interindividual variability (e.g., fig. 5C) produces a Py of 2.5 (fig. 5D), a smaller value than found in studies of MAC.

Different receptors with different concentration–effect relations might act together to produce anesthesia and thereby might produce the larger range illustrated in figure 5C. But again, the quantal–effect curve would be flatter than actually found for MAC. In addition, if effects of two or more receptors added to produce anesthesia, the resulting concentration–effect relation would incorporate the variability of each receptor. If figure 5B approximates such variability, the combined variability might be similar to that displayed in figure 5C. Thus, a quantal analysis involving several receptors would probably yield a shallower concentration–response relation and a lower Py than one involving a single receptor. This suggests that only one or two receptors (not several receptors) are likely to mediate anesthesia.

The semiquantitative analysis shown in figure 5 can be refined with numerical simulations (Appendix B) to supply further insights into what concentrations are relevant for studies of anesthetic mechanisms in receptors. The simulations assume that anesthetics bind to receptors to produce their effects. Anesthesia occurs in an individual when more than a certain fraction of receptors, T (the threshold), are bound. These assumptions and the Hill equation may be used to explore the relation between anesthetic concentration (DC) and anesthetic effects. For a given anesthetic concentration, the sensitivity of target receptors (R<sub>EC50</sub>) and the Hill coefficient (R<sub>y</sub>) determine the fraction of receptors occupied. Individuals may differ in their R<sub>EC50</sub> or their T. We quantify
the differences among individuals in REC50 and threshold by the standard deviation of REC50 (RSD) and T (TSD). This permits us to simulate how anesthetic concentration is related to anesthetic effect in receptors versus human populations.

The simulations compute the fraction of receptors occupied for each value of T and REC50 for all T and REC50 values in the population as specified by TSD and RSD, and then determine for each T and REC50 whether the computed fraction of receptors produces anesthesia (i.e., exceeds T). Thus, categoric data (yes anesthesia occurs—no it does not) are derived from the Hill equation for a range of anesthetic concentrations and T, REC50, and Ry values. Logistic regression is then used to compute the population response to anesthetic (i.e., we compute P_{ED50} and Py). The complex relation among the variables will be described in detail elsewhere.

What range of receptor REC50 values can account for human anesthesia? Can receptors with EC50 values different from MAC produce population responses similar to those observed in humans? A highly sensitive receptor (REC50, PEC50) can generate the human results only if the threshold, T, is high (fig. 6), and a receptor with low sensitivity (REC50..PEC50) can generate the human results only if T is low (fig. 6). What higher or lower T values are reasonable? With T less than 0.1 or greater than 0.9 and Ry (the receptor Hill coefficient) between 1 and 2, TSD becomes smaller (< 3–5%; results from simulations, not shown) than values normally encountered in biologic systems. Thus, at T less than 0.1 or greater than 0.9, Ry likely exceeds 2, again a larger value than normally encountered.

We suggest that T lies within 0.1–0.9. If Ry equals 1, REC50 may be 10 times higher or lower than the PEC50 for...
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1. As previously noted, for values of $T$ less than 0.1 or greater than 0.9, $R_y$ probably exceeds 2. Given these thoughts, we conclude that $P_{EC50}$ and $R_{EC50}$ likely lie within a factor of 3 of each other, as denoted by the dashed lines in figure 6. That is, relevant concentrations for studies of anesthetic effects on receptors that mediate anesthesia are not expected to differ from clinically relevant concentrations by more than a factor of 3.

There is an important caveat for our analyses. We assumed a linear relation between anesthetic interaction with the receptor target and the anesthetic response of the whole organism. There is an incredible complexity of neuronal architecture, circuitry, and structure that determines the impact of receptor effects of anesthetics on in vivo response (movement). Nonlinearities, including thresholds, amplification, and feedback, exist in biological systems and may obscure the true association between sensitivity of the receptor target and sensitivity of the organism to anesthetics. And interindividual variability will accompany each additional step in the association and will tend to decrease the steepness of the population concentration–response curve.

In summary, several observations support the intuitive view that relevant concentrations for receptor–concentration effects lie near those that produce anesthesia in vivo. Far larger concentrations would lie on the flat portions of receptor concentration–effect relations and thus would be more affected by variability in receptor responsiveness, producing concentration–effect relations different from those found for MAC (fig. 1). Similarly, steeper receptor concentration–effect relations minimize the effect of variability on the population concentration–effect curves underlying MAC, and steeper portions of receptor concentration–effect relations usually lie close to MAC (e.g., see fig. 2). In addition, some theoretical considerations that might convert a shallow receptor–concentration effect curve to a steep in vivo (MAC) curve have limitations. Adding anesthetic effects of several receptors to produce a combined effect only marginally steepens the combined receptor–effect curve (fig. 3). The combined concentration–effect curve for several receptors having the same $EC_{50}$ would shift to the left of the concentration–effect curve for MAC, suggesting that, at most, only a few receptor–effect curves might be combined to produce MAC. Finally, in a combined concentration–effect curve for receptors having disparate $EC_{50}$ values, the receptor having the lowest $EC_{50}$ value (the more sensitive receptor) dominates the combined effect (fig. 4).

We conclude that (1) relevant concentrations for studies of anesthetic effects on the receptors (or interneuronal pathways) that mediate anesthesia probably do not differ markedly from concentrations required to produce anesthesia; (2) relevant concentrations are more likely to be close to the steeper portions of receptor concentration–effect curves. Furthermore, if models of additivity of receptors reflect the interaction of anesthetic sensitive receptors: (3) only one or two receptors are likely to mediate a specific anesthetic effect (immobility, amnesia); and (4) in a combined concentration–effect curve for receptors having disparate $EC_{50}$ values, the receptor having the lowest $EC_{50}$ value dominates the combined effect.

Appendix A

This analysis was supplied by Dr. Robert Cantor (written communication, January 2000).

Initially, let us assume that each receptor is independent and has the same $R_{EC50}$. The concentration–effect relation is typically described using a sigmoid $Emax$ model:

$$ E/Emax = DC^{*7}/(R_{EC50}^{*7} + DC^{*7}) $$

where $E$ is effect, $Emax$ is the maximum possible effect (i.e., at an infinite drug concentration), $DC$ is drug concentration, and $R_y$ is the exponent of the equation (the Hill coefficient), governs the steepness (sigmoidicity) of the concentration–effect relation. Examination of this relation for several receptors suggests that the value for $R_y$ in vitro is approximately unity, and the sigmoid $Emax$ model reduces to a simpler (Emax) model:

$$ E/Emax = DC/(R_{EC50} + DC) $$

Using this model, the probability that a receptor is not activated is:

$$ 1 - E/Emax = 1 - [DC/(R_{EC50} + DC)] = R_{EC50}/(R_{EC50} + DC) $$

To determine the likelihood that one or more receptors are activated (regardless of how many are activated), determine the likelihood that none is activated and subtract that from 1.0. If the receptors act independently, then the probability that all $n$ (or $m$) receptors are unactivated is the probability for each receptor raised to the nth power = $(R_{EC50}/(R_{EC50} + DC))^n$. In turn, the net effect (i.e., the
probability that one or more receptors is activated) may be represented as

$$E/Emax = 1 - \left[ \frac{REC50}{REC50 + DC} \right]^n$$

(4)

or

$$E/Emax = 1 - \left[ \frac{REC50A}{REC50A + DC} \right]^n \left[ \frac{REC50B}{REC50B + DC} \right]^n$$

(5)

Appendix B

JP Dilger: A quantitative analysis of the effect of variability in drug binding and response threshold on the steepness of population concentration-effect curves (manuscript in preparation).

We make the following assumptions:

1. Anesthetics bind to some receptor target. We characterize the binding with a binding constant, $R_{EC50}$, and a Hill coefficient, $R_\gamma$, and described it by:

$$\text{Fraction bound} = DC^\gamma/(REC50^\gamma + DC^\gamma)$$

(6)

where DC is the anesthetic drug concentration.

2. The anesthetic affects the behavior of the receptor target (e.g., inhibition or potentiation) in proportion to the fraction bound.

3. Anesthesia results when a certain threshold level, $T$, of binding is attained. $T$ may range from 0 to 1 ($Emax$).

4. The distribution of $REC50$ and $T$ among individuals in a population is described by normal distributions with standard deviations of $R_{SD}$ and $T_{SD}$, respectively.

Numerical calculations were performed using a Visual Basic macro in Microsoft Excel. The distribution weights of $R_{EC50}$ and $T$ were accumulated in the running total at each concentration. The distribution weights of $REC50$ and $T$ were evaluated at 41 points in the range of mean $-3\sigma$ to mean $+3\sigma$. One hundred sixty values of concentration were used (0.01–100 times $REC50$). For each combination of $REC50$ and $T$, and DC, we calculated whether threshold was reached. If so, the appropriate weighting factor was accumulated in the running total at each concentration. The accumulated terms represent the fraction of the population for which the threshold is reached (i.e., anesthesia is achieved). The $P_{EC50}$ and steepness, $R_\gamma$, of the population curve may be calculated by a fit of the data to a logistic transformation in equation 7:

$$\text{Fraction anesthetized} = DC^\gamma/(P_{EC50}^\gamma + DC^\gamma)$$

(7)

A copy of the Excel worksheet is available from the author.

Glossary

DC Drug concentration.

E Effect of a given drug.

$E_{C50}$ The agonist drug or anesthetic concentration producing a 50% effect.

Emax The maximum effect produced by a given agonist.

MAC The minimum alveolar concentration of inhibited anesthetic required to suppress movement in 50% of subjects in response to a noxious stimulus (i.e., an anesthetic $E_{C50}$).

m or n Number of independent receptors.

$P_\gamma$ The Hill coefficient ($\gamma$) that describes the steepness of the concentration-effect relation for a population. The bigger the number, the steeper the relation. The $P_\gamma$s for MAC equal 6–30.

$P_{EC50}$ Concentration producing a 50% effect in a population ($EC50$).

$R_\gamma$ The Hill coefficient ($\gamma$) that describes the steepness of the concentration-effect relation for a receptor. The bigger the number, the steeper the relation. The $R_\gamma$s for most receptors equal 1–2.

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