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New Concepts

Receptor Desensitization by Neurotransmitters in Membranes: Are Neurotransmitters the Endogenous Anesthetics?†

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ABSTRACT: A mechanism of anesthesia is proposed that addresses one of the most troubling peculiarities of general anesthesia: the remarkably small variability of sensitivity within the human population and across a broad range of animal phyla. It is hypothesized that in addition to the rapid, saturable binding of a neurotransmitter to its receptor that results in activation, the neurotransmitter also acts indirectly on the receptor by diffusing into the postsynaptic membrane and changing its physical properties, causing a shift in receptor conformational equilibrium (desensitization). Unlike binding, this slower indirect mechanism is nonspecific: each neurotransmitter will, in principle, affect all receptors in the membrane. For proteins modeled as having only resting and active conformational states, time-dependent ion currents are predicted that exhibit many characteristics of desensitization for both inhibitory and excitatory channels. If receptors have been engineered to regulate the time course of ion currents by this mechanism, then (a) mutations that significantly alter receptor sensitivity to this effect would be lethal and (b) by design, excitatory receptors would be inhibited, but inhibitory receptors activated, so that their effects are not counterproductive. The wide range of exogenous molecules that affect the physical properties of membranes as do neurotransmitters, but that do not bind to receptors, would thus inhibit excitatory channels and activate inhibitory channels, i.e., they would act as anesthesics. The endogenous anesthetics would thus be the neurotransmitters, the survival advantage conferred by their proper membrane-mediated desensitization of receptors explaining the selection pressure for anesthesic sensitivity.

Anesthesia is an extremely unusual phenomenon for many reasons, perhaps the most notable being the remarkably broad range of molecular characteristics of inhalation anesthetics. In combination with the Meyer−Overton correlation and the additivity of anesthetic action, this would seem to implicate an indirect mechanism in which a change in bilayer properties, induced nonspecifically upon incorporation of a few mol percent of an anesthetic into cell membranes, alters the activity of one or more key membrane proteins, presumably ligand-gated ion channels (LGICs),[1] sufficiently to induce anesthesia (*1*, *2*). In opposition to such an indirect mechanism (and thus in support of a direct receptor binding mechanism), it has been argued that the readily measured structural and thermodynamic properties of membranes are only slightly altered by clinical concentrations of anesthetics (and in any

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[1] Abbreviations: GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; LGICs, ligand-gated ion channels; MAC, minimum alveolar concentration.

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case, the coupling between many membrane physical properties and protein activity is probably weak); furthermore, the existence of exceptions to the Meyer−Overton correlation and the small difference in potency of a few enantiomeric pairs would make such a bilayer-mediated mechanism seem far less likely (*3*, *4*). However, it has been counter-argued (*5*) that there is an important property of bilayers, the lateral pressure profile, that is strongly affected by anesthetic incorporation, exhibits a clear mechanical and thermodynamic coupling to protein conformational equilibria, predicts many of the anomalies to the Meyer−Overton correlation, predicts the additivity of anesthetic effects, and is not inconsistent with small differences in potencies of enantiomeric pairs.

Although less frequently mentioned, there are two other unusual characteristics of anesthesia that would seem to be far more difficult to reconcile with any molecular mechanism, whether indirect or direct. The first is the remarkably uniform sensitivity to inhalation anesthetics within the human population (and across a wide range of animal phyla). As has been discussed by Sonner (*6*), this is most naturally interpreted as evidence of strong selection pressure, either for the anesthetic state or to some closely linked trait. This would seem highly unlikely, given that no endogenous or environmental anesthetic has yet been identified (except for a few compounds, such as nitric oxide, that have partial anesthetic potency but exist at concentrations well below the levels needed to cause anesthesia), as it would imply a strong survival advantage for sensitivity to a broad range of molecules, none of which is ever encountered.

There is a second peculiarity of inhalation anesthetics that is relevant to the hypothesis proposed below. Anesthetics inhibit currents in excitatory LGICs and activate currents in inhibitory channels, so any endogenous analogues would presumably perform this pair of constructively opposite functions. Is this a result of the molecular characteristics of anesthetics or does this provide evidence of some related survival advantage to organisms with receptors engineered in this way?

Receptor Desensitization. Because modulation of synaptic transmission in the CNS occurs at clinical concentrations of anesthetics and because both inhibition of the activity of the postsynaptic excitatory channels and enhancement of the activity of the postsynaptic inhibitory channels that underlie synaptic transmission occur at clinical concentrations of most anesthetics, these ligand-gated ion channel receptors are the most likely group of anesthetic targets (*4*, *15*). These receptors exhibit a set of characteristic long-time responses to agonists described collectively as desensitization. While these phenomena can be quite complex, with multiple time scales and varied dependence on agonist concentration, the most common feature is a time-dependent change in ion current upon continued exposure to agonist; for excitatory channels the current decreases, the decay rate increasing with increasing agonist concentration (*7*). In some cases, at extremely high concentrations of agonist, no current is observed (*8*), which is to be expected if the rate of desensitization is sufficiently rapid that it competes successfully with the activation that results from neurotransmitter binding. For an inhibitory channel such as the $GABA_A$ receptor, the situation appears to be more complex: continuous application of GABA below saturating concentrations causes a gradual increase in current, but at high concentrations (well above binding saturation), the current decays (*9*).

There is another effect associated with desensitization: for excitatory channels, after washing the neurotransmitter from the aqueous phase, a second application of neurotransmitter results in a smaller initial current, the magnitude of the reduction decreasing with a longer wash time and increasing both with the duration of the initial pulse and the neurotransmitter concentration. By contrast, for inhibitory channels such as the GABA receptor, it is found that the current is prolonged during washout, although at saturating concentrations, subsequent application of a pulse of neurotransmitter results in a smaller initial current, as for excitatory channels (*9*, *10*).

These two characteristics of desensitization have been interpreted almost exclusively as evidence for the existence of one or more distinct desensitized conformational states that are nonconducting and to which the neurotransmitter is much more tightly bound, with relatively slow kinetics of the conformational change to and from this state. Kinetic schemes have been suggested, and with appropriate fitting of the rate and equilibrium constants, they can reproduce electrophysiological data for various receptors (*9*−*12*). However, there is little direct structural evidence for the existence of such desensitized conformational states within the superfamily of pentameric receptors likely to be involved in anesthesia, although recent studies on the tetrameric glutamate receptor do provide strong evidence of a transition to a distinct nonconducting conformational state (*13*). Thus, it is important to allow for the possibility that mechanisms that do not involve such putative states may contribute to some (although certainly not all) of the desensitization phenomena.

Hypothesis. Suppose that in addition to the direct effect of rapid, specific, and saturable binding of the neurotransmitter that results in activation, there is a second mechanism by which neurotransmitters can influence receptor conformational equilibria. Consider the possibility that on a slower time scale, the neurotransmitter diffuses into the membrane, presumably partitioning predominantly near the zwitterionic and/or charged headgroup region at the aqueous interface of the lipid bilayer. Its presence alters physical properties of the membrane that thereby induce a shift in the conformational equilibrium of its receptor and thus causes (or at least contributes significantly to) one or more of the changes in receptor activity described collectively as desensitization. Aside from the different time scale, this membrane-mediated effect would differ from agonist binding in that it is relatively nonspecific (i.e., significant membrane concentrations of one neurotransmitter would likely affect many different receptors in the membrane, and various neurotransmitters could affect each receptor). Also, since the membrane/aqueous partitioning is likely to remain linear to fairly high membrane concentrations, as will the dependence of the free energy difference of protein conformational states on membrane concentration of the neurotransmitter, saturation of this effect would not be expected to occur before reaching aqueous concentrations much higher than those corresponding to saturation of receptor binding.

If receptors have been engineered to use this mechanism to regulate some characteristics of the time course of ion currents, then there are two obvious consequences. First,

assuming that although its physiological significance is poorly understood, functional desensitization is nonetheless essential for proper function of the CNS, then mutations that significantly alter receptor sensitivity to this effect would presumably be lethal. Second, because of the nonspecificity (a given neurotransmitter could affect many different receptors), if excitatory receptors have been designed to be inhibited, then inhibitory receptors would have to be potentiated by this mechanism; otherwise, the effects would be counterproductive. The very wide range of exogenous molecules that affect the physical properties of membranes as neurotransmitters would be expected to do (the indirect mechanism being quite nonspecific), but that do not bind to receptors, would thus inhibit excitatory channels and activate inhibitory channels, i.e., they would act as anesthetics. The endogenous anesthetics would thus be the neurotransmitters, the survival advantage conferred by their proper membrane-mediated action on receptors explaining the selection pressure for anesthesic sensitivity. From this perspective, the fact that anesthetics inhibit excitatory channels but activate inhibitory ones is consistent with requirements of receptor design, not with some remarkable characteristics of the anesthetic molecules themselves.

THEORETICAL MODEL

To examine the consequences of this hypothesis, the time course of ion channel currents is predicted for both continuous and pulsed application of the neurotransmitter, using a simple model. It is assumed that the receptor exists either in an inactive (nonconducting) resting state (R) or an active (conducting) state (A). The neurotransmitter (S) can shift the equilibrium between these states via the two mechanisms described previously: by binding to the specific agonist binding sites on the receptor; and independently by entering the membrane, perturbing its physical properties, and thus modulating the protein conformational equilibrium. (Note that a desensitized conformational state is not incorporated within the model to determine which of the experimentally known characteristics of functional desensitization are predicted in its absence.) Let S_{aq} symbolize the neurotransmitter as an aqueous solute, and S_m as a solute in the membrane, with concentrations $[S_{aq}]$ and $[S_m]$, respectively. It is assumed that the application and washout of the neurotransmitter occurs sufficiently rapidly and accurately so that $[S_{aq}]$ is determined entirely by experimental design.

Conformational and Binding Equilibria. Although there are two ligand binding sites on postsynaptic LGICs, it is assumed for simplicity that the binding is sufficiently cooperative that the singly bound state can be ignored, or in any case that this complexity has no fundamental bearing on desensitization. Then, the binding equilibria can be written simply as

$$\begin{array}{lcll} R + 2S_{aq} & \rightleftarrows & RS_2 & K_R \\ A + 2S_{aq} & \rightleftarrows & AS_2 & K_A \end{array} \qquad (1)$$

It will be assumed that these equilibria are reached on a fast time scale, i.e., that the off and on kinetics are fast as compared to the rates of incorporation of neurotransmitter into the membrane. In the absence of neurotransmitter in the membrane, the equilibrium between the resting and the active states of the protein is characterized by the equilibrium constants $K°_u$ and $K°_b$, in the absence and presence of bound neurotransmitters, respectively

$$\begin{array}{lcll} R & \rightleftarrows & A & K°_u \\ RS_2 & \rightleftarrows & AS_2 & K°_b \end{array} \qquad (2)$$

The dependence of protein conformational equilibria on membrane concentration of the neurotransmitter may become quite complex at high concentrations. However, as has been discussed in detail elsewhere (*14*), at lower membrane concentrations, the properties of the membrane will vary linearly with $[S_m]$, so the effect on the free energy difference between the protein states will vary in proportion to $[S_m]$ as well. The equilibrium constant both without and with bound agonist will thus vary exponentially with $[S_m]$, so that $K°_u$ and $K°_b$ are replaced by $K_u = K°_u e^{\beta[S_m]}$ and $K_b = K°_b e^{\beta[S_m]}$, respectively. The constant β, a measure of the sensitivity of the conformational equilibrium to a change in membrane composition, can be positive (activation) or negative (inhibition), the value of which is assumed for simplicity to be independent of the ligation state of the protein. Defining $[S_m]^*$ to be the bilayer concentration at which $|\beta| = 1$ (i.e., at which the protein equilibrium has been shifted by a factor of e (activation) or 1/e (inhibition)) and defining a reduced membrane concentration $x = [S_m]/[S_m]^*$, then

$$K_u = K°_u e^{\pm x};\ K_b = K°_b e^{\pm x} \qquad (3)$$

where the (+) and (−) signs in the exponent correspond to activation and inhibition, respectively. Note that it is thus implicitly assumed that the approximation of linear response remains good to membrane concentrations high enough to generate a large shift in protein activity.

The pairs of equilibrium constants are related, so it will be useful to define a ratio of these constants as $C = K_A/K_R = K_b/K_u = K°_b/K°_u$. Note that in the absence of agonist (either in the membrane or the aqueous phase), almost all protein is in the resting state; if this fraction is about 1%, then $K°_u \approx 0.01$. With bound agonist (but before membrane-mediated desensitization, i.e., with no agonist in the membrane), the majority of the channels are active; in the calculations below, this fraction is taken to be 90%, which corresponds to $K°_b = 9$ and thus $C \approx 10^3$. In any case, the specification of these values does not significantly affect the character of the predictions described below.

Protein Activity. The ion current is presumed to be proportional to the fraction of protein f in the active state, independent of the ligation state of the protein. Then

$$f = \frac{[A] + [AS_2]}{[A] + [AS_2] + [R] + [RS_2]} = \frac{K}{1 + K} \qquad (4)$$

with an effective equilibrium constant K defined as

$$K = [(C^{-1} + K_R[S_{aq}]^2)/(1 + K_R[S_{aq}]^2)]K°_b e^{\pm x} \qquad (5)$$

It is useful to rewrite the expression for K in terms of dimensionless variables. Let $[S_{aq}]^\dagger$ represent the aqueous concentration at which half of the resting proteins are in the RS_2 and half in the R form (i.e., where $[RS_2]/[R] = K_R[S_{aq}]^{\dagger 2} = 1$) and define a dimensionless aqueous concentration $y =$

$[S_{aq}]/[S_{aq}]^{\dagger}$, in terms of which eq 5 becomes

$$K = [(C^{-1} + y^2)/(1 + y^2)]K^{\circ}_{b}e^{\pm x} \quad (6)$$

This expression simplifies in certain ranges of aqueous concentration. For $y < C^{-1/2}$, very little binding occurs, so $K \approx K^{\circ}_{u}e^{\pm x}$. For $y \gg C^{-1/2}$, which includes the intermediate range of the binding curve, eq 6 reduces to

$$K \approx (1 + y^{-2})^{-1}K^{\circ}_{b}e^{\pm x} \quad (7)$$

Finally, in the limit of binding saturation, $y \gg 1$, so $K \approx K^{\circ}_{b}e^{\pm x}$. In eqs 5−7, as in eq 3, the (+) and (−) signs in the exponent refer to activation and inhibition, respectively.

Note that although $y = 1$ corresponds to $[RS_2] = [R]$, the fraction of active protein depends both on K°_{b} and on $[S_m]$. For example, for $[S_m] = 0$ and $K^{\circ}_{b} = 9$, $f(y = 1) = (1 + 2/K^{\circ}_{b})^{-1} = 0.82$, and the value of y at which half the protein is active ($f = 0.5$) is $y_{1/2} = (K^{\circ}_{b} - 1)^{-1/2} = 0.35$.

Kinetics. To simplify the analysis, it is assumed that a shift in conformational equilibrium of the protein occurs instantaneously (whether in response to a change in protein ligation state or to a change in the membrane concentration of the neurotransmitter), as do any changes in the aqueous concentration of the neurotransmitter. It is thus assumed that the only time-dependent process is the diffusion of the neurotransmitter between aqueous and membrane domains (i.e., only $[S_m]$ varies gradually with time). This partitioning of S between the aqueous and membrane phases can be written as a pair of opposing steps

$$S_{aq} \rightleftarrows S_m \quad (8)$$

with first-order rate constants k_{in} and k_{out}. If the aqueous concentration is constant over some time period, $[S_m]$ varies exponentially with time

$$[S_m](t) = [S_m]_{eq} + ([S_m]_0 - [S_m]_{eq})e^{-k_{out}t} \quad (9)$$

where $[S_m]_0$ is the initial ($t = 0$) concentration, and the equilibrium ($t = \infty$) value for given $[S_{aq}]$ is $[S_m]_{eq} = [S_{aq}]k_{in}/k_{out}$. With $[S_{aq}]^*$ defined to be the aqueous concentration in equilibrium with the membrane at concentration $[S_m]^*$, and letting $\kappa = [S_{aq}]^{\dagger}/[S_{aq}]^*$, then eq 9 can be reexpressed in reduced units as

$$x(\tau) = \kappa y + (x_0 - \kappa y)e^{-\tau} \quad (10)$$

where $\tau = k_{out}t$, and $\kappa y = [S_{aq}]/[S_{aq}]^* = [S_m]_{eq}/[S_m]^* = x_{eq}$ is the membrane concentration (in reduced units) that would be obtained at equilibrium with an aqueous phase of neurotransmitter concentration $[S_{aq}]$. Note that the simplicity of eqs 9 and 10 arises from the implicit assumption, valid at low concentrations, that the partition coefficient is a constant. However, it will likely become inaccurate at high bilayer concentrations and could even exhibit saturation-like behavior if the activity coefficient of the neurotransmitter in the membrane were to rise sufficiently rapidly.

Only one independent parameter, $\kappa = [S_{aq}]^{\dagger}/[S_{aq}]^*$, needs to be specified. If significant membrane-mediated desensitization occurs at aqueous concentrations well below binding saturation, then $\kappa > 1$, whereas if desensitization only occurs for aqueous concentrations corresponding to saturation, then $\kappa < 1$. Regardless of the value of κ, in the limit of binding saturation ($y \gg 1$), the aqueous concentration appears in f only as κy, so κ provides a scaling factor; if the reduced aqueous concentration is redefined as $y^* = \kappa y = [S_{aq}]/[S_{aq}]^*$, then the equation for f has no independent parameters in the saturation limit.

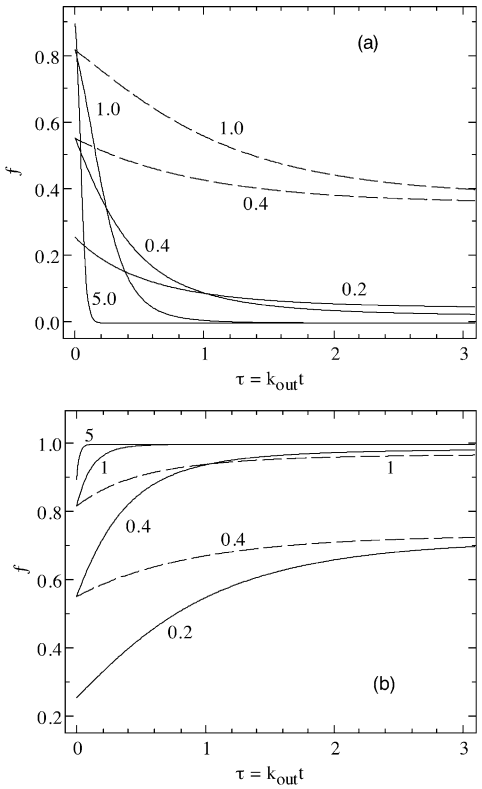

FIGURE 1: Predicted activities (ion current as a fraction of its maximum value) for continuous application of agonist, for $\kappa = 10$ (solid line) and $\kappa = 2$ (dashed line), for values of reduced aqueous concentration (y) as indicated in the figure. (a) Inhibition of excitatory channels. (b) Activation of inhibitory channels. In all cases, $K^{\circ}_{b} = 9$.

RESULTS

Predictions of the time dependence of protein activity, $f(\tau)$, are determined for a range of aqueous neurotransmitter concentrations. In a first set, $[S_{aq}]$ is taken to be constant. In a second set, the neurotransmitter is applied at a fixed concentration for a time τ_p (in reduced units) followed first by a wash ($[S_{aq}] = 0$) of duration τ_w and then by a pulse of agonist at the original concentration. As mentioned above, it is assumed that changes in $[S_{aq}]$ are effectively instantaneous.

Continuous Application. Initially, there is no neurotransmitter in the membrane, so $x(\tau) = \kappa y(1 - e^{-\tau})$, which, upon substitution into eqs 6 or 7, gives the explicit time dependence of K and thus of $f(\tau)$. Predicted current−time plots for the inhibition of excitatory channels are given in Figure 1. Note that in the binding saturation limit, both the rate and the degree of membrane-mediated desensitization are predicted to increase with increasing aqueous concentration and with increasing value of κ. For the excitation of inhibitory channels an increase in current is predicted, both at high and at intermediate aqueous concentrations, in qualitative agreement with experiment at concentrations near the EC_{50} value but in disagreement with results for the $GABA_A$ receptor at high (mM) aqueous concentrations (*9*).

After the membrane concentration of the neurotransmitter reaches its steady-state value ($t = \infty$), the activity is predicted to depend on the aqueous concentration as

$$f_\infty(y) = [1 + (1 + y^{-2})(K^\circ_b e^{\pm \kappa y})^{-1}]^{-1} \qquad (11)$$

For inhibition of excitatory channels (negative exponent), this steady-state activity has a maximum f_∞^* at a concentration y^* determined by the solution of $y^{*3} + y^* = 2/\kappa$. For example, for $\kappa = 5$ and $K^\circ_b = 9$, $y^* \approx 0.36$ and $f_\infty^* \approx 0.15$; the value of f_∞^* decreases with increasing κ. The existence of a maximum reflects the fact that at the concentrations at which binding saturates, the membrane-mediated influence on protein equilibria does not; once the agonist is present at high enough concentrations to bind to a significant fraction of the channels, further increases in agonist concentration serve only to raise the membrane concentration of the agonist and thus decrease protein activity. Note finally that for inhibitory channels, if the neurotransmitter is applied at concentrations well below saturation, in the presence of another molecule that partitions into the bilayer (such as an anesthetic or a different neurotransmitter), the steady state current rises significantly, as has been observed experimentally for the GABA receptor (*15*).

Pair of Pulses. The time dependence of the membrane concentration of the neurotransmitter is easily determined, from which K is obtained from eqs 6 or 7, and from which the activity, $f(\tau)$, can be obtained using eq 4. During the initial pulse, $x(\tau) = \kappa y(1 - e^{-\tau})$, at the end of which the neurotransmitter concentration in the membrane is $x(\tau_p) = \kappa y(1 - e^{-\tau_p})$. During the subsequent wash ($\tau_p < \tau < \tau_p + \tau_w$), the membrane concentration decreases as $x(\tau) = \kappa y(1 - e^{-\tau_p})e^{-(\tau - \tau_p)}$, at the end of which, $x(\tau_p + \tau_w) = \kappa y(1 - e^{-\tau_p})e^{-\tau_w}$. During the following pulse ($\tau > \tau_p + \tau_w$), $x(\tau) = \kappa y\{1 + [(1 - e^{-\tau_p})e^{-\tau_w} - 1]e^{-[\tau - (\tau_p + \tau_w)]}\}$. In Figure 2a,b is presented the predicted time dependence of protein activity for inhibition of excitatory channels. In qualitative agreement with typical experimental results, the attenuation of the initial current of the second pulse is predicted to increase both with decreasing wash time (Figure 2a) and with increasing duration of the first pulse (Figure 2b), as well as with increasing aqueous concentration (data not shown). In Figure 2c are presented results for the activation of inhibitory channels after a single pulse; the prolonging of the current is predicted to increase with both increasing pulse duration and aqueous concentration.

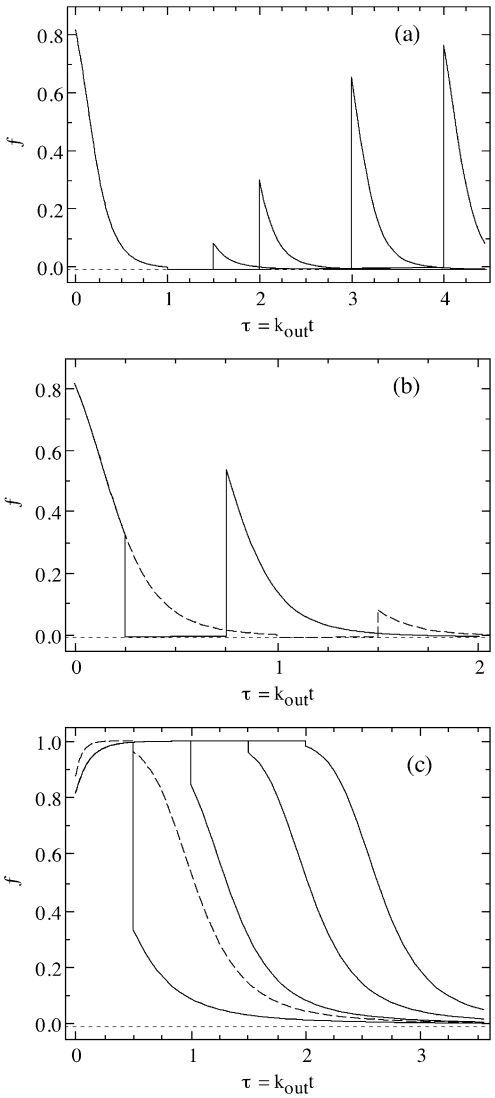

FIGURE 2: Predicted activities for a pulse of agonist of duration τ_p followed by washout of duration τ_w and a subsequent pulse of agonist. In all cases, $\kappa = 10$ and $K^\circ_b = 9$. (a) Effect of varying τ_w at fixed $\tau_p = 1$, for inhibition of excitatory channels; predictions are presented for $\tau_w = 0.5$, 1.0, 2.0, and 3.0. In all cases, $y = 1$. (b) Inhibition of excitatory channels for two different pulse lengths ($\tau_p = 0.25$, 1.0) for fixed $\tau_w = 0.5$; in both cases, $y = 1$. (c) Activation of inhibitory channels at varying pulse length ($\tau_p = 0.5$, 1.0, 1.5, 2.0) for a pulse followed by long washout for two different values of aqueous concentration: $y = 1$ (solid line) and $y = 2$ (dashed line).

DISCUSSION

The plausibility of this mechanism depends on the kinetics and thermodynamics of membrane partitioning of neurotransmitter and that it alters membrane properties similarly to anesthetics, sufficiently to modulate receptor conformational equilibria. Unfortunately, there exist no data on partitioning for either the charged or zwitterionic neurotransmitters. Given the chemical characteristics of, and particularly, the charge distributions within molecules such as acetylcholine, GABA, or glutamate, it is likely that bilayer partitioning occurs largely as adsorption into the lipid headgroup region adjacent to the aqueous interface. After the very rapid diffusion of the neurotransmitters across the synaptic cleft to the surface of the postsynaptic membrane, the kinetics of this adsorption into the headgroup region of the bilayer is thus likely to be quite fast, possibly even approaching the time scale of the binding process at the two agonist sites. With regard to equilibrium partitioning, since the closely related compounds acetylcholine and procaine both perturb the mobility of spin labels in membranes (*16*), the ratio of the magnitude of those effects at equal aqueous concentrations, along with the partition coefficient of procaine (*17*), provides an order-of-magnitude estimate of that of acetylcholine: a few mol percent in the membrane at millimolar aqueous concentrations, the concentration range in which acetylcholine has also been shown to perturb the surface potential of lecithin monolayer films (*18*). More generally, it has been shown (*19*) that charged organic molecules partition much more into membranes than might be expected, largely residing in or near the headgroup region. Neurotransmitters might be expected to partition similarly,

affecting lipid headgroup packing and presumably increasing the out-of-plane component of their dipole moments, which would result in large increases in repulsive interactions and which would thus be expected to cause a net redistribution of the lateral stresses in the membrane from the interior toward the aqueous interfaces, even at low membrane concentrations of neurotransmitters (*20*). If it is such changes in the lateral pressure profile that cause the shift in protein equilibria, then the overall effect of the neurotransmitters, as predicted by geometric models of protein shape changes (*21*), would be expected to be roughly similar to that of anesthetics—shifting pressure out of the membrane interior—although the details of the redistribution of the lateral pressures may be quite different. (As discussed below, these differences could result in considerable variability in the bilayer-mediated effect of a neurotransmitter on different receptors and of various neurotransmitters on a given receptor.) However, other properties, such as the depth-dependent order parameter profile of the lipid acyl chains, might be affected very differently, perhaps explaining why Forman et al. (*8*) did not observe changes in the order parameter profile at concentrations of agonist that caused self-inhibition of the acetylcholine receptor.

It has been assumed that anesthesia results from modulation of the activity of one or more postsynaptic LGICs, although the argument would be valid for any proteins that exhibit desensitization. For the reasons discussed in the introduction, these proteins are the most likely targets (*4*, *15*), although it is possible that other proteins, such as voltage-gated ion channels, may be involved. However, even among the LGICs, the effects of different volatile anesthetics on a given receptor do not always parallel their anesthetic potencies, although they may nonetheless contribute additively to anesthetic endpoints such as MAC (*23*). This is not inconsistent with the mechanism proposed here, which only implies that each of the LGICs that undergoes desensitization does so in a manner that depends on how the changes in bilayer properties that accompany the incorporation of its normal agonist into the bilayer couple to the conformational equilibrium of that receptor. Thus, given the range of molecular characteristics of neurotransmitters (amphiphilicity, charge distribution, size, flexibility, etc.), it would not be surprising if there were some variability of their effects on a given receptor (i.e., that the conformational changes of different receptors might well be properly tuned only for their normal agonist), resulting in different sensitivities to other neurotransmitters. Any such differences would then be consistent with a range of effects of an anesthetic on different receptors (or of various anesthetics on a given receptor), but if multiple LGICs contribute to anesthesia, these different anesthetics would still be predicted to make additive contributions to MAC.

A simple in vitro test of this hypothesis would be to adapt an electrophysiological procedure that measures the ability of an anesthetic to alter the activity of a particular receptor, but with the anesthetic replaced by a neurotransmitter that does not bind to that receptor, applied at concentrations corresponding to estimated peak concentrations at the postsynaptic membrane in the synaptic cleft. For example, using a membrane patch containing a single excitatory receptor (e.g., nAChR), preincubate the patch with a different neurotransmitter (e.g., GABA) and determine the time dependence of the ion current at various concentrations of the normal agonist (acetylcholine); if the proposed mechanism is valid, the presence of a different neurotransmitter, whether its receptor is inhibitory or excitatory, should inhibit the ion current significantly. Conversely, preincubation of an inhibitory receptor with a different neurotransmitter would be predicted to potentiate the response of the receptor to its normal agonist, and at high enough concentrations, to activate the channel in the absence of its agonist. (In essence, this would be a test of functional, nonspecific heterologous desensitization, which has been explored for G-protein coupled receptors such as opioid receptors but not for the LGICs likely to be involved in anesthesia.) The relevance to anesthesia will be strongly supported to the extent that the details of any such changes in the time course of the ion current are similar to those resulting from the presence of clinical concentrations of anesthetics. As an additional test, it will be useful to determine whether the effect of the different neurotransmitter on the response of the receptor to its normal agonist changes over long times (i.e., if there is any evidence of the development of tolerance, the absence of which is a well-known characteristic of volatile anesthetics).

If the desorption of the neurotransmitter from the membrane back into the synaptic cleft is relatively slow, then the neurotransmitter may diffuse within the postsynaptic membrane to distances of order microns, with accompanying drop in concentration that will be more gradual than in bulk since membrane diffusion is essentially two-dimensional. This process could thus cause significant spillover to both inhibitory and excitatory synapses, from which it follows that since excitatory receptors have been designed to be inhibited, inhibitory receptors would have to be potentiated by this mechanism or else their combined effects would be counterproductive.

Diffusion of the neurotransmitters into and within the presynaptic membrane could also influence synaptic transmission if the activity of relevant presynaptic proteins that undergo conformational transitions is modulated by changes in membrane properties. In particular, with an estimated 5000−10 000 neurotransmitter molecules encapsulated in a vesicle having an inner diameter on the order of 40−50 nm, the vesicle concentration of neurotransmitter is on the order of 250 mM, so that a significant amount would likely partition into the inner leaflet of the vesicle membrane and would thus diffuse laterally into the outer leaflet of the presynaptic membrane during vesicle fusion.

Many cells besides neurons may contain membrane proteins that undergo transitions between conformational states in which specific binding of a signaling molecule rapidly activates the protein, with inactivation occurring on longer time scales. As Sonner (*6*) has pointed out, the broadly conserved sensitivity to anesthetics across different animal phyla implies that the selection pressure is of very distant common ancestry. Thus, it is tempting to speculate that this double mechanism of neurotransmitter action—fast specific binding followed by nonspecific membrane-mediated modulation—may have been developed early in evolution as an efficient mechanism to tune the time dependence of the activity of such proteins, with the advantages that it requires only a single molecule and obviates the need for transitions to additional protein conformational states. If so, this generic

mechanism may have been implemented in many different contexts besides ion channels in excitable cells and could thus explain the sensitivity to anesthetics—albeit using varied behavioral endpoints—in animals not only with a much simpler nervous system but even in single-celled organisms (*22*).

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