Commentary

A Revised View of Local Anesthetic Action: What Channel State Is Really Stabilized?

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A relatively large group of compounds, which includes local anesthetics, antiarrhythmics, and anticonvulsants induce voltage- and/or frequency-dependent block of sodium and other ion channels. Current block increases as the stimulus voltage and frequency of depolarization is increased. These voltage- and frequency-dependent blocking properties have been recognized for over 40 yr (Weidmann, 1955), and the voltage- and use-dependent properties of a wide range of molecules have been described in detail.

A characteristic set of features describes local anesthetic block, but the prevalence (or presence) of any one of these characteristics varies with drug structure and physical properties (Butterworth and Strichartz, 1990; Hille, 1992), which in turn affects the qualitative characteristics of current block. In quiescent cells with a very negative resting potential, even relatively high local anesthetic concentrations have little effect on electrical activity. However, when depolarizing pulses arrive at high frequency, the peak sodium current elicited by each subsequent depolarization becomes smaller until the current reaches a new steady level. The degree of this use dependent or phasic block will increase with increasing depolarization frequency. Local anesthetics also respond to steady depolarizations. When the membrane is depolarized before the test depolarization that elicits the sodium current, this will increase the fraction of the current that is blocked by a local anesthetic. When this effect of earlier depolarization on local anesthetic block is measured as a function of voltage using a voltage-clamp protocol designed to measure the voltage dependence of channel inactivation or availability, local anesthetics shift the voltage dependence of channel availability to more hyperpolarized potentials. At any particular potential, a smaller fraction of the channels is available for activation by depolarization. Finally, when local anesthetic block is increased by depolarization, the recovery from block upon repolarization is slow and may not be complete before the next depolarization. Local anesthetics that cause slow recovery from block after depolarization produce use-dependent block. When recovery from block on repolarization occurs rapidly, use dependence is not observed. Whether or not a given local anesthetic produces use-dependent block thus will vary with the binding kinetics of the local anesthetic and the stimulus frequency.

Although much of this basic phenomenology was established over time, it was during the 1970's that many of the key features of block by local anesthetics were outlined and their mechanisms of action explored intensively. Studies of tertiary amine and quaternary local anesthetics led Hille (1977) and Hondeghem and Katzung (1977) to propose a model, termed the modulated receptor hypothesis, that explained many features of block by local anesthetic-like compounds. This model built on explorations of state-dependent block by quaternary ammonium blockers of potassium channels by Armstrong (1969). This work provided insights into the key feature of block by local anesthetics, namely that steady state inactivation is enhanced and recovery from inactivation is slowed. The modulated receptor hypothesis, as originally proposed, suggested that local anesthetics bind with different affinities to different conformational states of the channel. In particular, the drug affinity for depolarized conformations of the channel is higher than for hyperpolarized conformations. Allosteric coupling, in turn, causes the high affinity drug binding to depolarized channels to stabilize these conformations relative to the conformations having the low drug affinity. Finally, if the high-affinity, depolarized conformation were the inactivated state, this would further enhance the effect of a blocking drug.

Inactivated channels do appear to be stabilized by local anesthetics, as steady state inactivation curves are shifted toward negative potentials. In addition, recovery from drug block after repolarization resembles a slowed recovery from channel inactivation, as if local anesthetic-bound channels have difficulty in recovering from inactivation (and deactivating). Thus, local anesthetic-like molecules were proposed to bind to depolar-

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ized channels and perhaps stabilize the inactivated state (Hille, 1977; Hondeghem and Katzung, 1977). A key experimental test of the model was provided by testing local anesthetics on channels in which inactivation had been removed by the protease pronase. In such channels, use-dependent block by local anesthetics was lost (Cahalan, 1978). Another test made use of the fact that sodium channel inactivation immobilizes a fraction of the charge associated with channel gating (Armstrong and Bezanilla, 1977). Local anesthetics also immobilize a fraction of gating charge, and this charge immobilization seemed to occlude charge immobilization by inactivation (Cahalan and Almers, 1979). This finding suggested that the charge immobilized by local anesthetics was the same component of charge that was immobilized by channel inactivation (Cahalan and Almers, 1979). These results were expected if local anesthetics acted by stabilizing the inactivated state.

In the 20 yr that have passed, many local anesthetics and related compounds have been studied in many different tissues on many different sodium channel molecules. Each compound and tissue displays its own idiosyncrasies of block. Nevertheless, perhaps the most often repeated statement in these various studies is that these compounds cause use- or voltage-dependent block by virtue of their ability to stabilize the inactivated state.

The article from Vedantham and Cannon (1999) in this issue of *The Journal of General Physiology* provides a new experimental test of this statement—with a surprising result. Vedantham and Cannon made use of the observation that the loop connecting the homologous domains III and IV of the sodium channel is critical for inactivation and is proposed to be the inactivation gate (Vassilev et al., 1988; Stühmer et al., 1989). A phenylalanine residue in a hydrophobic triplet approximately one third of the distance from the NH_2 terminus of that loop is critical for inactivation (West et al., 1992). When this phenylalanine is replaced with a cysteine and the intracellular surface of the channel is exposed to the water-soluble methane thiosulfonate reagent, methane thiosulfonate ethyltrimethylammonium (MTSET), the cysteine is modified and inactivation is blocked. By examining its ability to react with MTS derivatives, this loop was found to change conformation when the channel inactivates (Kellenberger et al., 1996; Vedantham and Cannon, 1998). In the hyperpolarized, noninactivated channel, the cysteine at this position is highly reactive, but if the channel is depolarized, the same cysteine becomes unreactive. Furthermore, the voltage dependence of the reaction rate tracks the voltage dependence of inactivation. Thus, the reaction rate of this substituted cysteine is a measure of the number of sodium channels with open inactivation gates.

Vedantham and Cannon (1999) have cleverly made use of the accessibility of this cysteine residue as an indicator of the position of the inactivation gate. Using this readout, they followed the position of the inactivation gate during depolarization-dependent block by the local anesthetic, lidocaine. They got the unexpected result that the inactivation gate reopens with virtually unchanged kinetics after a depolarizing pulse whether or not lidocaine is present. Current through the channels, however, still recovers extremely slowly after depolarizations in the presence of lidocaine. This leads to the conclusion that drug binding is linked only tenuously to the molecular machinery that causes fast inactivation. The drug molecule remains tightly bound in the channel but the inactivation gate reopens. Because recovery of the inactivation gate and recovery from depolarization-induced lidocaine block have drastically different time courses, the slowly recovering lidocaine block does not appear to depend on the stability of the inactivated state.

Despite the surprising nature of their basic result, and its intrinsic contradiction of the idea that local anesthetics stabilize the fast-inactivated state, Vedantham and Cannon (1999) find that many key predictions of the modulated receptor model concerning the stabilization of the inactivated state are verified. At a potential of −100 mV, where the inactivation gate was open (and readily modifiable) in the absence of lidocaine, lidocaine (binding) causes the gate to close and become unavailable for modification. Lidocaine thus seems to stabilize the inactivation gate in the closed position. Likewise, lidocaine shifts the voltage dependence of inactivation gate closure toward more negative potentials. These results are in agreement with both basic tenets of the modulated receptor hypothesis.

Vedantham and Cannon's findings also indicate that anesthetic-dependent block can occur without movement of the inactivation gate. As the lidocaine concentration is increased, up to 30% of the channels become blocked even though there has not been any movement of the inactivation gate as judged by the SH reactivity. There must be a component of the lidocaine-dependent block that does not involve closure of the inactivation gate. Also, the rate of modification does not drop as steeply as the current magnitude as lidocaine concentration is increased. Both results are expected if channels can be blocked by binding drug molecules with no closure of the inactivation gate. The modulated receptor hypothesis provides that fully resting channels bind local anesthetics, although the binding affinity is lower than that for binding to depolarized channels.

Even increased lidocaine block in response to depolarization can occur without movement of the inactivation gate. In the absence of lidocaine, channel availability for modification tracks the inactivation curve for

the channel closely. The modification rate thus reports the position of the inactivation gate. In the presence of 1 mM lidocaine, inactivation gate closure is not detected until the channel is depolarized beyond −100 mV. At this membrane potential, however, 1 mM lidocaine has blocked substantial current that was available for activation at −140 mV. That is, although there had been enough of a conformational change in the channel molecule (in response to depolarization) to increase the lidocaine block and reduce current, this conformational change was not due to, or accompanied by, closing of the inactivation gate. This change in conformation in response to depolarization without movement of the inactivation gate separates movement of the inactivation gate from the primary effect of depolarization on the lidocaine-induced block.

This dissociation between depolarization and inactivation gate closure may be relatively subtle, but the events occurring on repolarization to the holding potential after a depolarization are not. Whereas the inactivation gate becomes fully accessible to modification (reopens) at the normal rate by, at most, 30 ms after repolarization, the channels take ~1 s to recover completely from the voltage-dependent block induced by lidocaine during a 20-ms conditioning depolarization. That is, lidocaine blocks the current for at least 100× longer than it takes for the inactivation gate to reopen. Something else must be stabilizing the lidocaine molecule in its receptor besides the closed inactivation gate.

The findings of Vedantham and Cannon (1999) distinguish multiple voltage-dependent processes that occur in response to repolarization. Immediately upon repolarization, the channels close. Presumably, channel closure occurs with approximately normal kinetics. This is the first voltage-dependent conformational change. The reopening of the inactivation gate occurs with a slower time course and, in the absence of drug, would be expected to be associated with reversal of the charge immobilization that accompanies channel inactivation (Armstrong and Bezanilla, 1977). This process is highly voltage dependent (e.g., Kuo and Bean, 1994). It is assumed that the voltage dependence of these conformational changes remains invariant in the presence of lidocaine. However, this remains to be tested. A third voltage-dependent time process is the reversal of block by the local anesthetic, in this case lidocaine. Recovery of channel availability at different membrane potentials in the presence of local anesthetics also is highly voltage dependent (Bean et al., 1983; Kuo and Bean, 1994). This suggests that part of the voltage-sensing apparatus of the channel is stabilized by the drug and undergoes an additional voltage-dependent conformational change in response to repolarization long after the inactivation gate has reopened. Taken together, these results lead to the conclusion that there are at least three distinct voltage-dependent events that occur in succession upon repolarization in the presence of local anesthetic block: channel closure, recovery from inactivation, and deactivation of local anesthetic blocked channels.

What do these results mean for the modulated receptor model? The basic model remains intact. What changes is the role of the inactivation gate. Whereas the inactivation gate previously was considered to be central for local anesthetic block, the results of Vedantham and Cannon (1999) suggest that it may play a more peripheral role. Nevertheless, local anesthetics still bind with higher affinity to depolarized conformations of the channel. The closed configuration of the inactivation gate is still stabilized in the presence of local anesthetics, but its stabilization seems to be an allosteric one, secondary to stabilization of other depolarized configurations of the channel. Vedantham and Cannon (1999) explain these findings by proposing that local anesthetics bind more avidly to, and stabilize, activated (depolarized) conformations of the channel. These same depolarized conformations favor inactivation. Stabilizing the channel in an activated configuration by virtue of local anesthetic binding will, in turn, favor a configuration with a higher affinity for the inactivation gate, as was measured in their study. The rapid opening of the inactivation gate on repolarization, however, means that this reciprocal stabilization somehow breaks down upon repolarization. Additional quantitative measurements of charge and protein movement under a broader array of experimental paradigms will be necessary to further refine this aspect of the model.

The literature that examines block by local anesthetics and related compounds is large, and many members of the ion channel biophysics community have contributed to the current knowledge. This rich literature contains many findings (or conclusions) that are difficult to reconcile completely with the present findings and bear reexamination and reinterpretation. One finding that is particularly perplexing in view of the present results is the loss of depolarization and use-dependent block when the inactivation gate is disabled by proteases (Cahalan, 1978) or by generation of the IFM → QQQ mutation, which also prevents inactivation (Bennett et al., 1995; Balser et al., 1996). Vedantham and Cannon (1999) explain this by proposing a loss of inactivation gate stabilization by the depolarized channel—an idea that needs to be further developed. Whatever the final interpretation of the results of Vedantham and Cannon (1999), they remind us once again of the power of measures of conformational change, such as changes in cysteine or fluorescence accessibility to challenge, and test and refine biophysical hypotheses that propose changes in channel protein conformation.

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