Inactivation of Voltage-Gated Cardiac $K^+$ Channels

Randall L. Rasmusson, Michael J. Morales, Shimin Wang, Shuguang Liu, Donald L. Campbell, Mulugu V. Brahmajothi, Harold C. Strauss

Abstract—Inactivation is the process by which an open channel enters a stable nonconducting conformation after a depolarizing change in membrane potential. Inactivation is a widespread property of many different types of voltage-gated ion channels. Recent advances in the molecular biology of $K^+$ channels have elucidated two mechanistically distinct types of inactivation, N-type and C-type. N-type inactivation involves occlusion of the intracellular mouth of the pore through binding of a short segment of residues at the extreme N-terminal. In contrast to this “tethered ball” mechanism of N-type inactivation, C-type inactivation involves movement of conserved core domain residues that result in closure of the external mouth of the pore. Although C-type inactivation can show rapid kinetics that approach those observed for N-type inactivation, it is often thought of as a slowly developing and slowly recovering process. Current models of C-type inactivation also suggest that this process involves a relatively localized change in conformation of residues near the external mouth of the permeation pathway. The rate of C-type inactivation and recovery can be strongly influenced by other factors, such as N-type inactivation, drug binding, and changes in $[K^+]_o$. These interactions make C-type inactivation an important biophysical process in determining such physiologically important properties as refractoriness and drug binding. C-type inactivation is currently viewed as arising from small-scale rearrangements at the external mouth of the pore. This review will examine the multiplicity of interactions of C-type inactivation with N-terminal–mediated inactivation and drug binding that suggest that our current view of C-type inactivation is incomplete. This review will suggest that C-type inactivation must involve larger-scale movements of transmembrane-spanning domains and that such movements contribute to the diversity of kinetic properties observed for C-type inactivation. (Circ Res. 1998;82:739-750.)

Key Words: human ether-a-go-go–related gene ■ long QT syndrome ■ antiarrhythmic drug binding ■ C-type inactivation ■ $\beta$ subunit

In response to a depolarizing change in potential, voltage-gated $K^+$ channels undergo an activation transition in which the channel enters an open, or conducting, conformation. After activation, most voltage-gated $K^+$ channels can pass into a stable nonconducting, or inactivated, state through a process termed inactivation. The process of inactivation, however, is extremely variable in many of its properties and, presumably, mechanisms. Many advances have been made in understanding the molecular mechanisms of inactivation, primarily from studies of cloned Shaker $K^+$ channels. This article will briefly review a subset of these advances and discuss their functional implications for mammalian channels and drug binding.

Several voltage-gated $K^+$ channels contribute to repolarization in cardiac muscle.1 The component currents and the underlying molecular basis can differ depending on tissue and region, and differences can exist even between cells in a given region. The fast inactivating voltage-gated $K^+$ current, or $I_{Kr}$, probably has Kv1.4, Kv4.2, and Kv4.3 as its molecular bases, depending on the region of the heart, and could potentially involve other $K^+$ channel $\alpha$ subunits associated with inactivation-promoting $\beta$ subunits.2–6 Inactivation of this current is important in determining its prominent role in the early stages of repolarization and in limiting its role in late repolarization. Inactivation plays the opposite role in determining the contribution of $I_{Kr}$ in repolarization. Inactivation of $I_{Kr}$ and of the various splice variants of its underlying molecular basis, HERG,7–9 is rapid relative to activation. Therefore, during the initial phases of the action potential, $I_{Kr}$ is largely inactivated. Only during the late phase of repolarization does recovery from inactivation permit conduction through this channel. Therefore, inactivation limits $I_{Kr}$ until late repolarization.

The rate at which channels recover from inactivation is also an important determinant of their role in repolarization. In the case of $I_{Kr}$, inactivated channels that have not recovered

Received June 25, 1997; accepted January 6, 1998.
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This manuscript was sent to William A. Catterall, Consulting Editor, for review by expert referees, editorial decision, and final disposition.
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will not be available to open during subsequent beats. However, recovery from inactivation may also be important for determining the frequency dependence of K⁺ channels, such as those that underlie the ultrarapid delayed rectifier K⁺ current, which are typically thought of as noninactivating. Kv1.5 is thought to form the molecular basis for this current,10–12 although other α subunits may contribute.13 Although the inactivation rate of these channels is slow relative to the duration of the action potential, recovery is also slow. Therefore, increases in heart rate can cause this inactivation to accumulate during successive depolarizations. Thus, cumulative inactivation can determine the contribution to repolarization of K⁺ currents that are active during the plateau phase of the action potential. In summary, inactivation is an important property of most of the K⁺ channels that govern repolarization. Therefore, understanding inactivation at a molecular level is crucial in understanding the physiological and pathophysiology of repolarization.

The best understood inactivation mechanism is N-type inactivation, which is well described by a “ball-and-chain” model14,15 similar to one originally proposed for the squid axon sodium channel.16 The molecular basis for this model was first demonstrated in Shaker β channels to be dependent on a small group of amino acids in the NH2 terminus that bind to the activated channel and occlude the intracellular mouth of the channel.14,15 No sequence similarity has been found among the NH2 termini of the various N-type inactivating Shaker β channels and mammalian Kv1.4 or Kv3.3 channels.17,18 Only weak structural similarity for the N-termini of these channels, as determined using nuclear magnetic resonance spectroscopy,19 has been reported. Despite this apparent structural diversity, all of these channels have been demonstrated to have an N-type inactivation mechanism.17,18,20,21

In addition to the N-type mechanism, another type of inactivation, termed C-type, has been identified in Shaker β channels (see Reference 22 for review). This mechanism is visible in some Shaker splice variants that lack N-type inactivation and can be revealed in others when the NH2 terminus is deleted from those channels that exhibit N-type inactivation.23 Thus, the two types of inactivation can exist simultaneously in Shaker β channels. It has been proposed that C-type inactivation occurs by a mechanism in which the external mouth of the channel becomes occluded during sustained depolarization23 and involves conformational changes that involve cooperativity between the four subunits forming the functional K⁺ channel.25,26 Despite being potentially a more widespread mechanism of K⁺ channel inactivation than N-type, C-type inactivation is less well understood.

The basic biophysical mechanisms underlying C-type inactivation in voltage-gated K⁺ channels may also be applicable to other channel types. In Na⁺ channels, slow inactivation occurs via a mechanism that resembles C-type inactivation of K⁺ channels.27,28 Ca²⁺ channel inactivation has also been suggested to occur via a mechanism that is similar to C-type inactivation.29 Thus, C-type inactivation may be a general gating mechanism with application to a broad number of channels of physiological importance. The present review will focus on our current understanding of the physical conformational changes that control C-type inactivation. It will describe a larger and more complex picture of C-type inactivation involving conformational changes that span both sides of the membrane. These conformational changes will in turn be discussed as mechanisms for the modification of current kinetics by ion channel blockers.

Properties and Mechanism of N-Type Inactivation

N-type inactivation, which occurs on the order of milliseconds to tens of milliseconds, was frequently referred to as fast inactivation. N-type inactivation was shown to be mediated by a “tethered-ball” mechanism in which a segment of ∼20 amino acids in the N-terminus of the channel protein binds at the intracellular mouth of the channel pore.14,15,30–32 This process is shown in Figure 1A. This physical picture gives rise to many predictions concerning the functional properties of channels that inactivate by this mechanism, and these properties, in turn, are usually taken as defining N-type inactivation of a particular channel.

Some of these defining properties are listed here: (1) N-type inactivation is lost after N-terminal deletion14,15 and restored by exogenous application of short peptides derived from the N-terminal.15,33 In other words, removal of the “ball” domain from the channel removes the “plug” for the open channel.14 Similarly, deletions that reduce the length of the “tether” region should increase the rate of inactivation as the effective diffusional distance is decreased. However, analysis of such mutations in Shaker and Kv1.4 K⁺ channels does not show such a clear trend. Some shortening of the tether actually slow inactivation, suggesting that rather than being a simple tether,20,33 this region may have some secondary or tertiary structure that constrains the movement and permissibility of the inactivation ball. (2) Events, such as drug binding, which occur at the extracellular mouth of the pore, do not alter N-type inactivation, whereas those that occur at the intracellular mouth of the pore do alter N-type inactivation.34 Accordingly, one test for development of N-type inactivation is insensitivity to extracellular TEA⁺ but sensitivity to intracellular TEA⁺.34,35 For presumably similar reasons, development of N-type inactivation is unaffected by changes in extracellular K⁺.36,37 (3) Although N-terminal regions contain basic (presumably positively charged amino acid residues) lysines and arginines, binding of the N-terminal domain is presumably near the channel surface and does not traverse much of the transmembrane electrical field. Consequently, N-type inactivation is voltage insensitive at positive potentials.38 (4) Finally, N-type inactivation is insensitive to point mutations at the outer mouth of the channel pore and the outer region of S6.14,36,37 This last point is important in that it is a key molecular property that helps to
define the other type of inactivation, namely, C-type inactivation.

Properties and Mechanism of C-Type Inactivation

The type of inactivation that is insensitive to N-terminal deletion was termed “C-type” and is depicted in Figure 1B. It was given this name because its rate was dependent on the particular C-terminal splice variant of the Shaker K+ channel being studied.22 It is sometimes referred to as slow inactivation; this term was in use mainly before mutagenesis experiments in Shaker K+ channels elucidated the molecular basis of inactivation. Analysis using domain swaps and site-directed mutagenesis demonstrated that it was not the cytoplasmic portion of the C-terminal splice variant of Shaker that was involved in mediating this type of inactivation. Instead, it was found that the critical regions lay in the extracellular side of S614 (region 2 of Figure 2). Later analysis showed that a specific residue in the extracellular H5-S6 loop (position 449) was also importantly involved36 (region 1 of Figure 2). Functional data also indicated that C-type inactivation was slowed by increasing concentrations of the external permeant ion (ie, K+)36 and by the application of extracellular, but not intracellular, TEA+.34 Thus, C-type inactivation does not involve a second tethered ball on the C-terminus but involves closure of the extracellular mouth of the pore and results in the criteria that define it, as listed in the legend of Figure 1B.
With C-type inactivation. Despite the apparent simplicity of this conformational change, the C-type inactivation conformational change is large enough to encompass external TEA⁺ and [K⁺]o interactions at multiple sites along the permeation pathway.⁴⁰,⁴¹

C-type inactivation as described in Shaker and Kv1.4 channels has additional properties. Some of these properties are functionally similar to N-type inactivation despite arising from a completely different biophysical mechanism. For example, the rate of development of C-type inactivation is voltage insensitive at potentials for which activation is almost exclusively from the Drosophila Shaker K⁺ channels; few studies have addressed the role or mechanism of slow inactivation in mammalian channels. Extrapolation of mutational studies in Shaker K⁺ channels raised questions concerning the mechanism of slow inactivation in Kv1.4.⁴⁴ Mutation of threonine to lysine at position 449 in an NH₂ terminal–deleted mutant of Shaker B produces a rapid C-type inactivation time constant of <100 milliseconds.⁴⁶ However, Kv1.4 has a lysine at the analogous position (532)⁴⁵ yet has a slow inactivation time constant (on the order of seconds) when its NH₂ terminal is deleted.⁴³ However, the N-terminal–deletion mutant of Kv1.4 has inactivation properties that are slowed by elevation of [K⁺]o, altered by mutations at position 532. Therefore, it fulfills the criteria for C-type inactivation.⁴³

Inactivation that persists after the removal of the N-terminal has been labeled as C-type inactivation in a wide variety of channel types. In some cases, slow inactivation has been assumed to occur via a C-type mechanism. Thus, C-type inactivation has been ascribed to a diverse group of voltage-gated K⁺ channels,⁴⁶ such as Kv1.1,⁴⁷,⁴⁸ Kv1.2,⁴⁹ Kv1.4,⁵⁰,⁵¹ and Kv1.5.⁵⁰ However, among these closely related channels, there are distinguishing characteristics with respect to their recovery processes, the number of exponential components in their development, their sensitivity to β-subunit expression, and their competition with drug binding.

### General Attributes of C-Type Inactivation

<table>
<thead>
<tr>
<th>Region</th>
<th>Equivalent Residue</th>
<th>Location</th>
<th>Intervention</th>
<th>Properties</th>
<th>Hypothesized Mechanism(s) of Action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shaker B K⁺ channel family (Kv1)</td>
<td></td>
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<tr>
<td>1</td>
<td>447–451</td>
<td>Pore/extracellular vestibule</td>
<td>↑ [K⁺]o, ↑ τᵣ, ↓ τᵦec</td>
<td>“Foot in door” mechanism</td>
<td>14, 36, 42, 44</td>
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<td></td>
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<td></td>
<td>“Foot in door” mechanism</td>
<td>14–16, 23, 80</td>
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<td></td>
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<td></td>
<td>Change in ΔG of pore mouth closure</td>
<td>23–26, 36, 37</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Localized changes in conformation</td>
<td>39, 40</td>
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<tr>
<td>2</td>
<td>457, 458</td>
<td>S6-extracellular</td>
<td>Mutations</td>
<td>Changes τᵣ, τᵦec</td>
<td>Changes stability of pore mouth closure</td>
<td>14</td>
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<tr>
<td>3</td>
<td>476</td>
<td>S6-intracellular</td>
<td>Mutations</td>
<td>Changes τᵣ, τᵦec</td>
<td>Hypothesis not proposed</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>371</td>
<td>S4</td>
<td>Mutations</td>
<td>Changes steady-state inactivation and τᵦec</td>
<td>Coupling between S4 and pore mouth closure</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>6–46/395</td>
<td>N-terminus/inner vestibule</td>
<td>ΔN-terminus</td>
<td>Binding accelerates C-type inactivation</td>
<td>Restricts K⁺ flow through pore</td>
<td>63, 83</td>
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<tr>
<td>β Subunit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Immobilizes channel and thus stabilizes inactivatable state</td>
<td>37, 74, 75</td>
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<td>Ether-a-go-go–related gene (erg)</td>
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<td>Changes stability of pore mouth closure</td>
<td>57</td>
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<td></td>
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<td></td>
<td>Hypothesis not proposed</td>
<td>51</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>“Foot in door” mechanism</td>
<td>51, 52</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>?</td>
<td>S6?-unknown</td>
<td>Intrinsic voltage dependence of erg inactivation</td>
<td>Movement of transmembrane-spanning domains stabilize/destabilize pore mouth closure</td>
<td>54, 57, 62</td>
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τᵣ and τᵦec indicate time constants of inactivation and recovery, respectively; ΔG indicates Gibbs free energy change.
Inactivation in voltage-gated K⁺ channels that are much more distantly related to Shaker than the Kv1 family may also show C-type inactivation. An important example is the HERG channel, which is the basis for the rapidly activating delayed rectifier, $I_{\text{Kr}}$, and has been linked to a form of the familial long QT syndrome. The HERG channel has a rapid inactivation mechanism that is sensitive to 100 mmol/L extracellular TEA, permeant ions, and mutations near the exterior mouth of the channel. However, HERG inactivation shows several properties that are biophysically distinct from “classic” C-type inactivation (Table). The rate of recovery from C-type inactivation is generally thought to increase with increasing $[K^+]_o$. In contrast, the rate of recovery of HERG inactivation decreased with an increase of $[K^+]_o$. The rate of development of C-type inactivation is voltage insensitive at potentials positive to the threshold for activation; HERG inactivation is voltage sensitive at such potentials.

**Figure 2.** K⁺ channel structure and regions involved in C-type inactivation. Membrane topology showing hydrophobic membrane-spanning domains (S1 to S6) and both the amino- and the carboxyl-terminal regions (N and C, respectively; not drawn to scale) on the cytoplasmic side. Four such subunits are required to form a functional K⁺ channel (see Reference 98 for review). The S4 sequence carries basic residues at every third position and forms the voltage sensor of the channel. The amino-terminal region contains residues that are involved in N-type inactivation, which involves electrostatic and lipophilic interactions between the amino terminus (“inactivation ball”) and its receptor. The sequence between the S4 and the S5/H4 (the S4-S5 loop) functions as part of the receptor for the fast inactivation gate. The S4-S5 segment is involved in formation of the permeation pathway. The numbers label specific regions or residues that have been identified as playing a role in C-type inactivation or in modifying its properties. Region 1 indicates residues at the external mouth of the H5 pore loop that have been shown to move during C-type inactivation. Region 2 refers to a specific residue on the extracellular end of S6 that modulates the rate of C-type inactivation. Region 3 indicates a residue thought to line the pore on the intracellular end of S6 that alters the rate of C-type inactivation. Region 4 is the S4 voltage sensor; mutation of the middle charged residue of this region has been shown to alter the voltage dependence of C-type inactivation. Region 5 indicates the sites of interaction between the N-terminus and the intracellular mouth of the pore. N-type inactivation speeds the rate of development of C-type inactivation. Region 6 is physically undefined but represents the domains that may move and give rise to the voltage dependence of C-type inactivation in HERG. Specific roles for each of these regions are listed in the Table.

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Thus, the initial reports on members of the Kv4 family labeled this inactivation mechanism as C-type. However, like HERG, members of the Kv4 family all show rapid recovery from inactivation. The residues in the pore region and the extracellular half of S6, which are important in determining Shaker and Kv1.4 C-type inactivation, do not appear to be consistent with a C-type inactivation mechanism. Thus, others have called the C-type inactivation mechanism into question for members of the Kv4 family. Jerng and Covarrubias performed a series of N- and C-terminal deletions on the Kv4.1 channel and saw changes, but not removal, of inactivation of this channel. On this basis, they proposed a model in which there is considerable structural interaction between both the N- and C-termini and for which both the N- and C-termini contained N-type inactivation domains. The failure to completely remove inactivation through N- and C-terminal truncation can be explained through structural domains in the “tether” region, which can act as substitute N-type inactivation domains after deletion. Such an effect has been demonstrated for different length deletions of the N-terminal domain of Kv1.4. Their physical model remains controversial, since the N-type inactivation paradigm predicts that there will always be a truncation mutation that completely abolishes fast inactivation. Thus, the inactivation mechanism of the Kv4 family, and therefore the major component of human \( I_{Kr} \), remains controversial.

The diversity in properties associated with what has been broadly labeled C-type inactivation in various channels suggests that this type of inactivation involves multiple conformational changes; the data to support the involvement of specific regions in C-type inactivation are summarized in the Table. Figure 3B shows an expanded schematic indicating the putative involvement of movement of S4 voltage sensor and the potential involvement of movement of S6 and possibly other regions (eg, those involved in voltage dependence of C-type inactivation of \( I_{Ks} \) and HERG). Although these regions have been implicated in contributing energetically to the conformational changes resulting in C-type inactivation, many questions remain concerning the involvement of these regions in modulating the kinetic and pharmacological properties of C-type inactivation.

### Interactions Between N- and C-Type Inactivation

#### Permeation and Allosteric Modification

A relationship between N-type and C-type inactivation was first suggested by Hoshi et al for Shaker K⁺ channels. They
noted that the recovery rate in N-type inactivation was greatly slowed in channels in which mutations that enhanced the rate of development of C-type inactivation had been introduced. On the basis of these observations, they concluded that C-type inactivation could determine the rate of recovery from inactivation in the presence of N-type inactivation. Furthermore, the duration of the depolarization was insufficiently long to allow much development of C-type inactivation at the rate observed in the absence of the N-terminus. Therefore, they concluded that N-type inactivation must have been enhancing the development of C-type inactivation.

These observations and hypotheses have only recently been explored in more detail. Two distinct mechanisms have been proposed to explain the interaction between N-type and C-type inactivation. One group working with Shaker channels has proposed that changes in ion occupancy that occur after N-type inactivation promote C-type inactivation. Another group working with Kv1.4 channels has proposed that channel immobilization or other steric factors associated with N-type inactivation promote C-type inactivation. These two competing hypothesized mechanisms are not necessarily mutually exclusive and may potentially coexist in the same channel. Thus, depending on the channel type and/or the physiological or experimental conditions, one mechanism may predominate, or both mechanisms may jointly control the development of C-type inactivation after N-type inactivation. Therefore, it is important to understand the experimental basis for each of these hypothesized mechanisms.

Permeation Modification
Baukrowitz and Yellen showed that N-type inactivation promoted the development of C-type inactivation in Shaker K⁺ channels. In the absence of extracellular K⁺, the ability of N-type inactivation to speed development of C-type inactivation was sensitive to [K⁺]. From these data, they concluded that N-type inactivation promoted C-type inactivation by decreasing the occupancy of the pore by K⁺. This led to the putative model for N- and C-type inactivation interactions as shown in Figure 4A. After the intracellular mouth of the pore is occluded by binding of the N-terminal domain, no further K⁺ entry can occur. When the last ion leaves the pore, there is no K⁺ ion blocking the closure of the external mouth of the
pore, and so the channel rapidly makes a transition to the C-type inactivated conformation. Binding of the N-terminal domain increases C-type inactivation rate indirectly by decreasing \( K^+ \) entry from the intracellular domain. It was further suggested that channel immobilization and steric factors were uninvolved in the promotion of C-type inactivation by N-type inactivation in the presence of intracellular \( K^+ \). This permeation hypothesis has not been compared with predicted values of channel occupancy in the presence of physiological levels of \( [K^+]_o \) when channel occupancy by ions might not be strongly modulated by movement of ions from the intracellular side. Thus, this mechanism may not account for all of the observed coupling between N- and C-type inactivation.

Allosteric Modification

The inactivation of an N-terminal deletion mutant of Kv1.4 (Kv1.4Δ2-146) occurs via a mechanism fulfilling the criteria for C-type inactivation\(^{37} \) as described for Shaker \( K^+ \) channels. Similarly, this study has also reported that N-type inactivation promotes rapid development of C-type inactivation of these channels, although minor quantitative differences in the properties of C-type inactivation of Kv1.4 relative to Shaker were noted.\(^{37} \) Rasmusson et al. also examined the importance of movement of the voltage sensor in the development of N-type and C-type inactivation. Mutation of an arginine at position 454 in Kv1.4 to a glutamine reduces the charge on the S4 sensor and shifts both N-type and C-type inactivation of Kv1.4Δ2-146 in a parallel fashion. This charge neutralization shifts the threshold for inactivation and strongly slows recovery from inactivation. These observations led to two hypotheses: (1) Both N-type and C-type inactivation require similar movement of the activation sensor to proceed. (2) Factors that immobilize the S4 voltage sensor in the activated conformation also stabilize C-type inactivation (suggested by the slowing of recovery). These data suggested that promotion of C-type inactivation by N-type inactivation was mediated through channel immobilization (eg, as has been described for gating currents in Shaker channels\(^{60-64} \)) and through allosteric effects resulting from N-terminal binding,\(^{37} \) particularly since deactivated or resting channels do not undergo C-type inactivation. In other words, binding of the N-terminus “freezes” the channel in a fully activated conformation, which is conducive to C-type inactivation. In addition, this freezing continues as long as the N-terminal has not unbound (ie, recovery from N-type inactivation has not occurred). Therefore, it can continue to promote the development of C-type inactivation even after the membrane is repolarized. This “allosteric” mechanism of N-type acceleration is shown schematically in Figure 4B as an interaction between the N-terminus and the transmembrane-spanning domains that are energetically linked to the development of C-type inactivation (see Figure 3B).

Role of \( \beta \) Subunits in Inducing and Modulating Inactivation

A recently discovered class of ancillary (\( \beta \)) subunits can dramatically increase the rate of inactivation of the membrane bound Kv1 \( \alpha \) subunits.\(^{67-71} \) This class of subunits alters inactivation primarily through different amino terminal splice variants.\(^{72} \) One \( \beta \) subunit, Kv1.1, has been shown to increase the rate of inactivation through interaction of its N-terminus with the channel pore to cause rapid inactivation.\(^{67} \) Kv1.2, which except for a unique N-terminal 78–amino acid leader is identical to Kv1.1, can also increase the rate of inactivation but shows kinetically different behavior.\(^{73} \) Kvβ1.1 was the first ancillary voltage-gated \( K^+ \) channel subunit that was demonstrated to increase the rate of inactivation of Kv1 \( \alpha \) subunits.\(^{67} \) As shown in Figure 5A, Kvβ1.1 results in a very rapid and complete inactivation of an NH\( _2 \) terminal deletion mutant of Kv1.4 (Kv1.4Δ2-146). This was demonstrated to occur through an N-type mechanism by N-terminal deletion and reinduction of inactivation with application of exogenously applied N-terminal peptide to the intracellular face of a torn-off macropatch.\(^{67} \)

Kvβ1.2 was subsequently shown to modify inactivation gating.\(^{68,69,71} \) However, the increase in the rate of inactivation for this subunit was much smaller than that for other N-type inactivation domains (Figure 5B). Chimeric constructs containing the unique N-terminal amino acids of Kvβ1.2 attached to the Kv1.4Δ2-146 construct of Kv1.4 demonstrated that the functional properties of coexpression of this subunit were also conferred by the N-terminal domain.\(^{74} \)
of the β subunit. Furthermore, the effect on inactivation rate was specific only to certain α subunits. The mechanism underlying these properties was resolved through two key observations: (1) The rate of inactivation was dependent on the intrinsic C-type inactivation in the α subunit.56,74 In other words, Kvβ1.2 appeared to be modulating development of C-type inactivation. (2) There was a rapid and incomplete development of inactivation that had the properties of a rapid open-channel blocker.73,75 Thus, it appears that rapid incomplete N-type inactivation by Kvβ1.2 leads to a net increase in the rate of development of C-type inactivation.

The Role of C-Type Inactivation in Drug Binding and Use Dependence

C-type inactivation plays an important, but still poorly understood, role in the use dependence and binding of local and general anesthetic drugs. C-type inactivation appears to be an important determinant of the high-affinity binding of the methanesulfonamidol drugs doxetilide and E-4031 to the HERG channel.56,77 C-type inactivation of HERG is also important in determining the affinity of the HERG channel for the drug haloperidol.78 The presence of C-type inactivation is critical for the block of a variety of K⁺ channels by the general anesthetics ketamine and halothane.79 Thus, the interactions between drug binding and C-type inactivation are of widespread importance for many clinically relevant situations.

Competition between extracellular TEA⁺ and inactivation has served as a criterion for differentiating C-type inactivation from N-type inactivation.36 The amino acid (position 449 in Shaker) that confers or disrupts sensitivity to TEA⁺ also strongly modulates the rate of development and recovery from C-type inactivation.36 These properties of external TEA⁺ binding were critical in establishing the external pore closure mechanism of C-type inactivation. This external site appears to be specific for TEA⁺ relative to lipophilic quaternary ammonium compounds with longer side chains.45 However, recent studies examining the accessibility of thiol reducing agents to engineered cysteines in the external region of the pore have established that large-scale rearrangements of the outer mouth accompany the C-type inactivation conformational change. Such changes suggest that C-type inactivation may be an important determinant of the action of drugs that bind at or near the extracellular mouth of the pore. For example, the action of chloramine T modifies C-type inactivation through covalent modification of methionine 448 in Shaker K⁺ channels. An exciting new externally acting blocker of Kv1.3, 1-benzyl-4-pentylimino-1,4-dihydroquinoline, also requires C-type inactivation for high-affinity binding42 and may be the harbinger of new and more specifically acting compounds that use the C-type inactivated state as a substrate.

The intracellular side of the channel is a more common site of action for channel blockers and antiarrhythmic drugs. As with the interaction of N-type inactivation with promotion of C-type inactivation, there are two putative modes of action that are not necessarily mutually exclusive. One hypothesized mechanism is that extracellular pore mouth closure occurs indirectly as a consequence of a block of the entry of intracellular K⁺ into the pore from the intracellular space, leading to an empty and therefore rapidly inactivating channel (permeation mechanism).83 The other hypothesized mechanism is that channel immobilization and steric hindrance of conformational changes occur directly as a result of the binding of intracellular blockers (allosteric mechanism). These mechanisms are identical to those proposed for intracellular binding of the N-terminal and the resulting coupling between N- and C-type inactivation. The main additional complication to be considered with drug binding is that there may be a diversity of intracellular drug binding sites.34 Thus, drug binding may have a diversity of interactions with C-type inactivation if the allosteric mechanism is present. In general, the data suggest that both the permeation mechanism and allosteric mechanisms exist and can modulate the properties of C-type inactivation. However, it remains unclear which mechanisms are dominant under physiological conditions.

A study by Baukrowitz and Yellen83 has recently demonstrated that channel block by compounds that act at an intracellular site modulate C-type inactivation. Increased C-type inactivation and its associated slow recovery, not trapping or high-affinity binding, accounted for the use-dependent properties of a blocker. It was proposed that this modulation was solely the result of decreased occupancy of the permeation pathway due to block of entry of ions from the intracellular space.83 Allosteric mechanisms were suggested to be unimportant. These findings were based on experiments performed in Shaker K⁺ channels with zero extracellular K⁺. Similarly, they found that removal of intracellular K⁺ greatly reduced the ability of drug binding to increase the rate of C-type inactivation. If this hypothesis is a general mechanism, the use-dependent action of all K⁺ channel blockers on any channels expressing C-type inactivation will be determined only by dwell time and the properties of endogenous C-type inactivation for that channel.

However, several studies suggest that these findings do not generalize to all modes of channel inactivation ascribed to the C-type mechanism for other compounds. Block by 4-AP is an important case in point. Although it acts from an intracellular and not an extracellular site,45,65–67 it clearly competes with, rather than increases, C-type inactivation of Kv1.1 and Kv1.2 channels. 4-AP also competes with development of C-type inactivation of Shaker K⁺ channels from an intracellular site.47 Since the kinetics of association and dissociation of 4-AP are slow (>100 milliseconds), the competitive effect suggests that the structure of the blocking compound may be important, in addition to its dwell time. Dofetilide has also been noted to compete with development of C-type inactivation at positive potentials in HERG.58,89 Data from native myocytes suggest that common antiarrhythmic compounds, such as quinidine, flecainide, and propafenone, show different degrees of competition with inactivation and different patterns of use dependence and recovery.90 These data are contradictory to the effects predicted for the permeation mechanism and suggest that other factors may also influence such interactions between drug binding and C-type inactivation.

A subsequent study84 of cloned Shaker K⁺ channels has suggested the existence of two sites for drug binding on the
intracellular face of the channel. One of these sites, which was proposed to be deeper and accessible to TEA\(^+\), results in competition with C-type inactivation. In Shaker K\(^+\) channels, this effect is observable only when permeant (K\(^+\)) ions are removed.\(^{39}\) In other channels, a competitive interaction between drug binding and C-type inactivation has been observed for drugs that act relatively deeply within the pore (eg, quinidine, flecainide, and propafenone)\(^{38}\) but in the presence of physiological levels of [K\(^+\)]. Not only does this suggest that a conformational change occurs in deep pore residues that are accessible from the intracellular side of the membrane, it also suggests that this region may be a more energetically important target for drug-channel interaction in channels other than in Shaker, where permeation-block effects appear to dominate.\(^{84}\)

A second site of lipophilic drug binding that is proposed to be closer to the intracellular surface does not involve allosteric inhibition of C-type inactivation.\(^{38}\) Thus, the interaction between drugs and C-type inactivation, which may have important implications for use-dependent properties, is already very complicated. For Shaker K\(^+\) channels alone, there is one external site of interaction and at least two separate internal sites for interaction.\(^{84}\) This situation is likely to become more complex in the future. This is foreshadowed in the study of Baukrowitz and Yellen\(^{84}\). They note that there is also an unexplained positive allosteric effect of several compounds that is not accounted for in either of their two intracellular modulation paradigms. Since this noninhibitory drug binding site is more superficially located than the competitive site, it is interesting to speculate that this region may mediate acceleration of C-type inactivation with N-terminal inactivation or binding of large compounds that may not penetrate far into the intracellular vestibule. Perhaps not coincidentally, relatively large methanesulfonanilide compounds\(^{36,77}\) are dependent on C-type inactivation for high-affinity binding to HERG. Since these compounds are among the most selective nonpeptide K\(^+\) channel blockers known to date, it is possible that the C-type inactivated conformation may provide a substrate for development of highly specific class III antiarrhythmic drugs.

This section has focused on C-type inactivation and its modulation by compounds that act directly on the channel. However, it is important to note that evidence indicating that C-type inactivation can be strongly dependent on intracellular regulatory factors is accumulating.\(^{91-96}\) Modulation of C-type inactivation rates and recovery rates by phosphorylation has been demonstrated.\(^{92,94-96}\) It seems likely that phosphorylation of intracellular residues modulates C-type inactivation through an allosteric as opposed to permeation-type mechanism. Thus, the additional conformational changes that occur during and that influence C-type inactivation may play an important role in the kinetic modification of channels by second messengers.

**Conclusion**

Inactivation is a basic conformational change intrinsic to the majority of voltage-activated K\(^+\) channels. Great strides in our understanding of the molecular and biophysical basis of inactivation have been made in recent years. However, much work remains to be done in understanding the diversity of interactions between N-type and C-type inactivation, channel block, and the determinants of voltage-dependent development and recovery from inactivation. There is extreme diversity in the kinetic and potentially molecular properties of inactivation, particularly of C-type inactivation. This diversity is so substantial that it may be questioned whether there is a single C-type mechanism or whether there are enough distinguishing characteristics to justify multiple-pore-closure inactivation types (eg, P-type inactivation).\(^{85}\) Irrespective of the terminology used to classify inactivation, drug-channel interactions may show a strong dependence on such differences in inactivation mechanism and kinetics. A thorough understanding of the molecular diversity of inactivation and of drug dependence on inactivation properties has the potential to provide a powerful tool for development of antiarrhythmic drugs with greater specificity and desirable use-dependent properties.

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