On the Mechanism by which 4-Aminopyridine Occludes Quinidine Block of the Cardiac $K^+$ Channel, hKv1.5

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**Abstract**

4-Aminopyridine (4-AP) binds to potassium channels at a site or sites in the inner mouth of the pore and is thought to prevent channel opening. The return of hKv1.5 off-gating charge upon repolarization is accelerated by 4-AP and it has been suggested that 4-AP blocks slow conformational rearrangements during late closed states that are necessary for channel opening. On the other hand, quinidine, an open channel blocker, slows the return or immobilizes off-gating charge only at opening potentials ($>-25$ mV). The aim of this study was to use quinidine as a probe of open channels to test the kinetic state of 4-AP-blocked channels. In the presence of 0.2–1 mM 4-AP, quinidine slowed charge return and caused partial charge immobilization, corresponding to an increase in the $K_c$ of $\sim$20-fold. Peak off-gating currents were reduced and decay was slowed $\sim$2– to 2.5-fold at potentials negative to the threshold of channel activation and during depolarizations shorter than normally required for channel activation. This demonstrated access of quinidine to 4-AP-blocked channels, a lack of competition between the two drugs, and implied allosteric modulation of the quinidine binding site by 4-AP resident within the channel. Single channel recordings also showed that quinidine could modulate the 4-AP-induced closure of the channels, with the result that frequent channel reopenings were observed when both drugs were present. We propose that 4-AP-blocked channels exist in a partially open, nonconducting state that allows access to quinidine, even at more negative potentials and during shorter depolarizations than those required for channel activation.

**Key words:** channel block • gating currents • potassium channel • heart

**Introduction**

4-Aminopyridine (4-AP)\(^1\) blocks many different voltage-gated $K^+$ channels in a variety of tissues including neurons (Ulbricht and Wagner, 1976; Yeh et al., 1976), lymphocytes (Choquet and Korn, 1992), skeletal muscle (Gillespie and Hutter, 1975), and heart muscle (Kenyon and Gibbons, 1979; Simurda et al., 1989; Castle and Slawsky, 1993). Block by 4-AP is mediated by its cationic form from the intracellular face of the channel (Kirsch and Narahashi, 1983; Choquet and Korn, 1992; Kirsch et al., 1993; Bouchard and Fedida, 1995). 4-AP can bind to either open (Wagoner and Oxford, 1990; Choquet and Korn, 1992; Kirsch and Drewe, 1993) or closed (Yeh et al., 1976; Thompson, 1982; Kehl, 1990) channels and can also be trapped within deactivating channels at hyperpolarized potentials (Choquet and Korn, 1992; Kirsch and Drewe, 1993; McCormack et al., 1994; Bouchard and Fedida, 1995; Rasmusson et al., 1995). Trapping of 4-AP may occur by protection of the drug binding site by the activation gate (Kirsch and Drewe, 1993) so that it cannot be washed out with a drug-free solution when the channel is closed. Binding of 4-AP is also thought to be mutually exclusive to both fast (Campbell et al., 1993; Castle and Slawsky, 1993; Stephens et al., 1994) and slow inactivation (Castle et al., 1994; Fedida et al., 1996). It has also been recently shown to prevent slow gating charge immobilization associated with C-type inactivation (Fedida et al., 1996).

The study of $K^+$ channel gating currents can give important additional information on the state dependence of drug action on the channels. $K^+$ channel closure is characterized by a slowed return of gating charge after channel opening (Taglialatela and Stefani, 1993; Stefani et al., 1994) manifested as a reduced peak and slowing of the decay of the gating current waveform upon repolarization (Porozo et al., 1993; Stefani et al., 1994). To explain the slowing, models of gating include either additional slow transitions that carry little charge, before the open state that delays charge return after opening (Taglialatela and Stefani, 1993; Bezanilla et al., 1994; Zagotta et al., 1994) or a more concerted rearrangement of subunits as a final step to channel opening, reversal of which slows charge return on repolarization (Bezanilla et al., 1994; McCormack et al., 1994; Stefani et al., 1994). 4-AP accelerates the time course of Shaker $K^+$ channel off-gating currents (McCormack et al., 1994; Bouchard and Fedida, 1995), and this has been interpreted as a prevention of the late slow steps in channel activation gating that lead to...
opening (McCormack et al., 1994; Bouchard and Fedida, 1995). This could then reduce or prevent predominantly open state-dependent inactivation (Campbell et al., 1993; Castle et al., 1994; Fedida et al., 1996).

Quinidine is an antiarrhythmic drug that blocks cardiac Na⁺ channels to alter action potential duration and slow conduction (Colatsky, 1982; Clarkson and Hondeghem, 1985; Slawsky and Castle, 1994) and blocks repolarizing cardiac K⁺ channels to prolong action potential duration (Colatsky, 1982; Inaizumi and Giles, 1987; Slawsky and Castle, 1994; Wang et al., 1995). These antiarrhythmic actions correspond to its class Ia and class III properties. When expressed in heterologous systems, RHK1 (Yatani et al., 1993), Kv1.2 (Tseng et al., 1996) in Xenopus oocytes, and hKv1.5 channels expressed in mouse L cells (Snyders et al., 1992; Yeola et al., 1996) or in HEK cells (Fedida, 1997) are all blocked by quinidine. Quinidine is suggested to bind only to open channels and either has extremely low affinity or is unable to bind to closed channels (Snyders et al., 1992; Slawsky and Castle, 1994; Clark et al., 1995). Evidence for open channel block has been acceleration of K⁺ current inactivation without effects on activation or on the steady state voltage dependence of inactivation (Kehl, 1991; Slawsky and Castle, 1994; Clark et al., 1995), and the slowing of deactivation tail currents, which suggests that quinidine has to dissociate before channels can close (Yatani et al., 1993; Yeola et al., 1996; Fedida, 1997). Detailed studies of quinidine-induced block of hKv1.5 show that block is most likely mediated at an intracellular site within the internal mouth of the channel through a combination of a charge-based block and hydrophobic interactions (Snyders et al., 1992; Snyders and Yeola, 1995; Yeola et al., 1996). Gating currents indicate that quinidine rapidly blocks only open hKv1.5 channels with a marked slowing of off-gating charge or gating charge immobilization, with little evidence for closed channel block even at high concentrations (Fedida, 1997).

Here we attempt to use quinidine, a known open channel blocker, to probe the state dependence of 4-AP binding in hKv1.5. Our investigation is centered on gating current measurements and the clear difference in action between 4-AP and quinidine. The data indicate that the 4-AP-blocked channels undergo slowing of gating charge return by quinidine, and this effect occurs even at potentials negative to the threshold of channel opening. Additional data from single channels strongly suggest that quinidine has access to channels that have 4-AP bound or trapped in them. We propose that 4-AP holds the channel in a conformation that is nonconductive but allows quinidine access even at potentials at which it is normally excluded. A preliminary report of this work has been published previously (Chen and Fedida, 1996).

**Materials and Methods**

**Cells and Solutions**

HEK-293 cells were transiently transfected with hKv1.5 cDNA in pRC/CMV, using LipofectACE reagent (Canadian Life Technologies, Bramalea, Ontario, Canada) in a 1:10 (wt/vol) ratio. Transfectants were detected using the phOx system (Invitrogen Corp., San Diego, CA) as described previously (Fedida, 1997). To record gating currents, patch pipettes contained 140 mM N-methyl-D-glucamine (NMDG), 1 mM MgCl₂, 10 mM HEPES, 10 mM EGTA, adjusted to pH 7.2 with HCl. The bath solution contained 140 mM NMDG, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, adjusted to pH 7.4 with HCl. In cell-attached patches, to record ionic currents, pipettes contained 135 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2.8 mM sodium acetate, 10 mM HEPES, adjusted to pH 7.4 with NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH. 4-Aminopyridine was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using HCl. Quinidine (sulfate salt) was dissolved in distilled water at a stock concentration of 500 mM and pH-adjusted to 7.4 using NaOH. The bath solution contained 135 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM dextrose, pH adjusted to 7.4 with KOH.

**Electrophysiology**

Current recording and data analysis were done using an Axopatch 200A amplifier and pClamp 6 software (Axon Instruments, Foster City, CA). Patch electrodes were fabricated using thin-walled borosilicate glass (World Precision Instruments, Inc., Sarasota, FL). After silgarding and fire polishing, pipettes used to measure current had resistances of 1.0–2.5 MΩ. Capacitance compensation was routinely used, but series compensation was observed. Gating currents were subtracted on-line using a P/6 protocol (Zagotta et al., 1994b). The absence of ionic current at negative membrane potentials in HEK cells allowed faithful leak subtraction of data. The passive membrane characteristics have been described in detail when we reported a mean capacity transient decay time constant of 55.0 μs (Chen et al., 1997). Capacity compensation and leak subtraction were routinely used, but series resistance compensation was only rarely used. No difference between results with and without R compensation was observed. Gating current data were sampled at 100–330 kHz and low pass filtered at 10 kHz. Single channel data were sampled at 5 kHz and low pass filtered at 1 kHz. All experiments were performed at 22°C and cells were superfused continually at a flow rate of 1–2 ml/min. All Q̅off measurements were obtained by integrating the off-gating currents until current waveforms decayed to the baseline. Significant differences between groups of data were tested using Student’s t test or ANOVA where appropriate, and a value of P < 0.05 was deemed statistically significant.

**Results**

Does 4-AP Occlude the Effects of Quinidine on Gating Currents?

In K⁺ channels, it is well established that 4-AP speeds Q̅off movement (McCormack et al., 1994; Bouchard and Fedida, 1995) and quinidine slows the return of Q̅off (Fedida, 1997). It is thought that 4-AP speeds Q̅off and
blocks K+ channels by preventing a final conformational rearrangement before channel opening (McCormack et al., 1994). On the other hand, quinidine is an open channel blocker that only binds to channels at potentials positive to the threshold potential of activation (Snyders et al., 1992; Clark et al., 1995; Fedida, 1997). This has the opposite effect to 4-AP in that the return of gating charge is slowed more as quinidine impedes closing and channels tend to reblock (Fedida, 1997). The obvious difference in the effects on Q_{off} of these two drugs allowed us to use quinidine as a probe to test whether 4-AP prevented channel opening and therefore could occlude the action of quinidine. To obtain data shown in Fig. 1, cells were held at −100 mV in the presence of 1 mM 4-AP and given a 12-ms depolarizing pulse to +40 mV to activate the channels and move all gating charge. These gating currents are identical to those observed previously in the presence of 4-AP in both Shaker K+ channels (McCormack et al., 1994) and in hKv1.5 (Bouchard and Fedida, 1995). I_{gon} represents the movement of gating charge as channels progress towards the open state, whereas, upon repolarization, I_{goff} represents the return of gating charge as the channels deactivate. In the presence of 1 mM 4-AP, I_{goff} reaches a peak rapidly and decays rapidly. These gating currents are unlike those seen without 4-AP, which have a slow component of gating charge return that results in a reduction in peak I_{goff} and slowing of the decay, on repolarization from potentials positive to the threshold of channel activation (Stühmer et al., 1991; Perozo et al., 1992; Stefani et al., 1994; Zagotta et al., 1994a). Channel opening and the associated slow transitions are thought to be prevented by 4-AP (McCormack et al., 1994; Bouchard and Fedida, 1995), which accounts for the rapid rise in I_{goff} to peak and subsequent rapid decay to baseline.

After washing on different doses of quinidine while pulsing at a rate of 0.5 Hz, a steady state was eventually reached, usually after 1–2 min. In Fig. 1, the steady state trace in 4-AP plus quinidine is superimposed over the trace in 4-AP alone in all panels. 4-AP speeding of gating charge movement caused a rapid rise and subsequent decay of I_{goff} while conserving the amount of charge moved (McCormack et al., 1994; Bouchard and Fedida, 1995). Fig. 1A shows little effect after addition of 10 μM quinidine, with the labeled arrow indicating the dose of quinidine and the peak I_{goff}. With the addition of 50 and 100 μM quinidine (Fig. 1, B and C), an obvious dose-dependent decrease in the peak I_{goff} was evident. At higher doses of quinidine up to 1 mM (Fig. 1, D–F), a slight increase in the rate of decay of the I_{gon} became apparent, and peak I_{goff} was further reduced, but the most striking action of quinidine was to further slow the time course of I_{goff} decay. At higher doses, there was also the appearance of a positive current “notch” at the beginning of the I_{goff} that reflects the slow charge return induced by quinidine. It appeared that in 4-AP plus quinidine, I_{goff} waveforms were slowed in a manner similar to that of control without any drugs (Chen et al., 1997; Fedida, 1997), but not to the extent seen in quinidine alone (Fedida, 1997). For example, 100 μM quinidine alone reduced peak I_{goff} almost to

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**Figure 1.** On- and off-gating currents (I_{gon} and I_{goff}) from hKv1.5 during 12-ms depolarizations to +40 mV from a holding potential of −100 mV in the presence of 1 mM 4-aminopyridine (4AP). Superimposed are traces from the same cells after addition of different doses of quinidine until achievement of a steady state response. (A–F) Doses of quinidine (10, 50, 100, 200, and 500 μM, and 1 mM) are labeled with arrows pointing to the peak of I_{goff}. In D–F, arrows pointing to I_{gon} indicate a dose-dependent acceleration of the decay to the baseline.
zero (Fedida, 1997). So it appeared that in the presence of 4-AP, when the channels were depolarized to potentials at which they should be fully open, the effects of quinidine on $I_{\text{goff}}$ were still present, but somewhat attenuated.

This partial protection of channels from the full action of quinidine by 4-AP was not restricted to high 4-AP concentrations. In Fig. 2, the effect of changing the 4-AP concentration on the effect of 200 µM quinidine is shown. Preexposure of cells to 4-AP at concentrations from 1 mM down to 50 µM (Fig. 2, A–D) had quite similar effects. As the 4-AP concentration was lowered, there was some minor slowing of the return of off-gating current in 4-AP alone, but the cells were still largely protected from the charge-immobilizing actions of 200 µM quinidine when it was added. The effect of quinidine was to slow the return of off-gating charge such that time to peak $I_{\text{goff}}$ was slowed and its amplitude was reduced ~50%. Not only could relatively low concentrations of 4-AP protect the channels from the full effects of quinidine, but 4-AP could apparently reverse the actions of quinidine added first. This effect is shown in Fig. 2 E. Here the cell was exposed to 10 µM quinidine alone, which reduced $I_{\text{goff}}$ markedly, as shown by Fedida (1997). Subsequent addition of 100 µM 4-AP reversed this reduction in $Q_{\text{off}}$ and produced a rapid $I_{\text{goff}}$ waveform.

When compared with control data with only 4-AP present (Fig. 3 A, ▼), experiments with 50 µM (●), 200 µM (■), and 1 mM 4-AP (▼) plus quinidine showed that apart from slowing $I_{\text{goff}}$, doses greater than 10 µM quinidine caused a small dose-dependent reduction in the returning gating charge; that is, a degree of $Q_{\text{off}}$ immobilization. The normalized fractional block of $Q_{\text{off}}$, $f = (1 - Q_{\text{off}}/Q_{\text{on}})$ was fit with the function:

$$f = \max / [1 + (K_d/D)^{-\eta_H}],$$

where max is the fitted maximum level of block (~0.2 in each case), $K_d$ is the concentration at which there is a half-maximal effect, $D$ is the quinidine concentration, and $\eta_H$ is the Hill coefficient. The $K_d$ for quinidine in the presence of 1 mM 4-AP was calculated to be 144 µM and $\eta_H$ was 1.01. At 200 µM 4-AP, $K_d$ for quinidine was 141 µM, and at 50 µM 4-AP, the $K_d$ was 67 µM. In comparison, with quinidine alone, the $K_d$ was 7.2 ± 1.6 µM and the $\eta_H$ was 0.84 ± 0.21 at a depolarization of +60 mV (Fedida, 1997). The $K_d$ for quinidine in the presence of 200 µM 4-AP is ~20× greater than with quinidine alone, while the $\eta_H$ remains relatively unchanged (~1). The slopes of the dose–response relations in quinidine and 4-AP suggests that a single binding site exists for quinidine and that cooperativity is not an important factor in the binding of quinidine to the channel, either in the absence or presence of 4-AP. At 50 µM 4-AP the $K_d$ was much lower, but still it should be noted that at all concentrations of 4-AP studied the maximum immobilization of charge was ~20%. The ratio of the maximum rate of charge returned, compared with the rate of on-gating charge movement (peak $I_{\text{goff}}/I_{\text{gon}}$) in 1 mM 4-AP alone is shown in Fig. 3 B (▼) to be ~2, but with increasing doses of quinidine (▼), the ratio decreased from 2.1 at 10 µM ($n = 1$) to 0.72 ± 0.02 at 1 mM quinidine ($n = 4$). In 50 µM 4-AP alone, the off-gating charge movement was not so accelerated
Quinidine has been shown to affect hKv1.5 ionic currents between −30 and 0 mV (during channel opening), suggesting an affinity for the open state (Snyders et al., 1992). In confirmation of this, quinidine has also been shown to slow Ig_{off} only at potentials positive to −25 mV (Fedida, 1997). If 4-AP prevented channel opening, we would expect quinidine’s effects to be fully prevented—which was not seen (Figs. 1 and 2). At least two possibilities exist that could explain these observations: (a) significant numbers of channels can transiently escape 4-AP block, open, and are then blocked by quinidine; or (b) channels with 4-AP bound exist in a conformationally “open” but blocked state that does not allow ion conduction, but that allows quinidine access to its binding site. We have tested the two hypotheses by examining gating current waveforms at different potentials, with a particular emphasis at potentials where the channel should not be open; that is, negative to the activation threshold at ∼−25 mV. Fig. 4A shows

**Figure 3.** (A) Fractional reduction (1 − Q_{off}/Q_{on}) of gating charge (mean ± SEM) in paired cells exposed to 1 mM (○), 200 μM (■), and 50 μM (●) 4-AP before (open symbols) and after (filled symbols) addition of 10–1,000 μM quinidine (n = 4–7 in each case). Gating charge was measured from the integration of Ig as in Fig. 1 until complete decay to baseline up to 12 ms for Q_{on} and 18 ms for Q_{off}. Solid lines for the quinidine data are fits from a Hill equation (Eq. 1) giving K_{d}'s of 144 μM (1 mM 4-AP, n_{H} = 1.01, max = 0.22), 141 μM (200 μM 4-AP, n_{H} = 1.21, max = 0.19), and 67 μM (50 μM 4-AP, n_{H} = 0.92, max = 0.21). (B) Ig_{off}/Ig_{on} for the same paired cells exposed to 1 mM (○) or 50 μM (●) 4-AP, before (open symbols) and after (filled symbols) addition of quinidine as in A. (C) Mean time constant of off-gating current decay (τ_{off}) for the same cells in 1 mM (○) or 50 μM (●) 4-AP, before (open symbols) and after (filled symbols) addition of quinidine. *Significant difference between corresponding 4-AP and quinidine data points (P < 0.05, Student’s unpaired t-test).

as in 1 mM 4-AP, so that the Ig_{off}/Ig_{on} ratio was ∼1.6 (○). At this concentration of 4-AP, there was a more marked reduction of the ratio in the presence of quinidine that was apparent at concentrations as low as 10 μM (●). Fig. 3C shows the dose dependence of the time constant of decay of Ig_{off} (τ_{off}). In the paired controls with 50 μM or 1 mM 4-AP (○), the τ_{off} was ∼0.4 ms and significantly increased with the addition of quinidine (●, ▼) in doses above 10 μM, reaching 1.23 ± 0.08 ms in 1 mM quinidine. This increase in τ_{off} reflects the dose-dependent slowing in the decay of Ig_{off} to baseline (Figs. 1 and 2).

At potentials at which channels were fully activated, quinidine exposure in the presence of a range of 4-AP concentrations resulted in a significant slowing in the return of gating charge on repolarization associated with a reduction in peak Ig_{off}/Ig_{on}, and 15–20% immobilization of returning charge. These effects of quinidine are significant but, in the presence of 4-AP, channels appear protected from the larger effects of quinidine alone (Fedida, 1997). At 50 μM, near the K_{d} for 4-AP alone, somewhat more Q_{off} immobilization in the presence of low concentrations of quinidine was noted (Fig. 3A). Apart from this, little difference in the action of quinidine was seen across a 20-fold concentration range of 4-AP. This finding suggested that quinidine and 4-AP were not competing for the same binding sites on hKv1.5. Rather, the data suggested that at potentials at which the channels should be fully activated, both 4-AP and quinidine could access binding sites on the channel, and that the presence of 4-AP allosterically modulated the quinidine site such that its effectiveness at charge immobilization was reduced.

**The Voltage Dependence of Quinidine Action in 1 mM 4-AP Is Changed**
gating current data collected during 9-ms depolarizing pulses to between −100 and +60 mV. Transient Ig on in the presence of 4-AP can be seen during depolarizations positive to −70 mV that peak rapidly and decay more quickly during larger depolarizations, similar to those seen in Shaker K+ channels expressed in Xenopus oocytes (Perozo et al., 1992; Stefani et al., 1994). Ig off in the presence of 4-AP were expressed in a uniform fast after pulses across the entire potential range and decayed rapidly at −70 mV. This seems to negate the first hypothesis as Ig off data in the presence of 4-AP, which represent the behavior of a large population of channels, do not show any Ig off slowing after pulses to positive potentials (which would be evidence for channels having opened) compared with pulses negative to the opening threshold. When 100 μM quinidine was added (Fig. 4 B), the same voltage protocol showed that Ig on remained relatively unchanged, but that peak Ig off decreased and decayed more slowly, as seen in the single pulse experiments (Figs. 1 and 2). Superimposed in Fig. 4 B (dotted line) is the gating current during the pulse to +60 mV, with 1 mM 4-AP alone for comparison (Fig. 4 A).

The voltage dependence of the rate of decay of Ig on (τ on) in 4-AP before (V) and after (V) addition of quinidine is shown in Fig. 4 C. The τ on could only be measured at potentials ≥−70 mV after the appearance of Ig. In 4-AP, the τ on at −70 mV was 1.77 ± 0.05 ms and increased to a maximum of 3.14 ± 0.13 ms at −10 mV, followed by a voltage-dependent decrease to 0.67 ± 0.07 ms at +70 mV as Ig on decayed faster. The curve with quinidine was similar in shape and had few significantly different points. The differences appeared to correspond with a small leftward shift of ~10–15 mV.

If slowing of the decay of Ig off is an important indicator of the action of quinidine, the voltage dependence of this effect can tell us the potentials at which the drug can access 4-AP-blocked channels. The slowing of the decay time constant of Ig off (τ off) at five different voltage precipulse (−60, −40, −20, 0, and +40 mV) is shown on an expanded time scale, normalized and superimposed in 1 mM 4-AP before and after the addition of 100 μM quinidine (Fig. 5, A–E). The data are noisy at −60 mV, but at all potentials that occur negative to (−60, −40 mV), at (−20 mV), and more positive (0 and +40 mV) to the threshold for channel activation, the currents with quinidine decayed much more slowly than in 4-AP alone. The voltage dependence of τ off (Fig. 5 F) showed a striking difference in 4-AP before (V) and after (V) quinidine over the entire voltage range. In 4-AP alone, the τ off showed a very slight increase from 0.48 ± 0.03 ms at a prepulse of −70 mV to 0.53 ± 0.02 ms at +70 mV. However, after addition of quinidine, the τ off was dramatically slowed to 2–2.5-fold with a larger voltage-dependent increase from 0.90 ± 0.05 ms at −70 mV to 1.17 ± 0.03 ms at +70 mV. The fact that significant slowing occurred throughout the
voltage range suggests that, in the presence of 4-AP, quinidine has access to the channels even at potentials where the channels would normally be closed.

The effects of quinidine with 4-AP on gating charge were examined by integrating both I_{g_{on}} and I_{g_{off}} of the data traces from Fig. 4, A and B. These integrals are shown in Fig. 6, A and B as Q_{on} movement for depolarizations from −100 mV to between −80 and +60 mV in 20-mV steps, and as Q_{off} for return of gating charge upon repolarization to −100 mV. In Fig. 6 A, in 1 mM 4-AP, Q_{on} and Q_{off} both increased more rapidly and eventually saturated at ~1.4 pC with larger depolarizations. The Q_{off} integrals shown here are similar in size and shape to those previously recorded in the presence of permeant K^+ and Cs^+ (Chen et al., 1997). When 100 μM quinidine was added to the same cell (Fig. 6 B), Q_{on} behaved similarly in its rise and saturation at 1.4 pC. However, Q_{off} did not have the same properties in quinidine, compared with 4-AP alone. Instead, the rise time was slower and the amount of charge saturated at ~1.26 pC. The +60-mV trace from the 4-AP data (Fig. 6 A) is included for comparison (Fig. 6 B, dotted line).
Quinidine Modulates 4-Aminopyridine Block of hKv1.5

Compared with 4-AP alone (Fig. 6 C, ▽), mean normalized Qoff data from depolarizations up to +70 mV in 10-mV steps show a leftward shift in the voltage dependence of charge movement in the presence of quinidine plus 4-AP (▼). The Qoff in quinidine plus 4-AP was normalized to Qoff in 4-AP alone (■) and the resulting Qoff–V Boltzmann curve was shifted left ~13 mV, showing earlier saturation to ~90% of the level in 4-AP alone (Fig. 6 C, solid lines). The valence (z) decreased slightly from 2.06 ± 0.03 to 1.89 ± 0.04 e0 after addition of quinidine (n = 4). Fig. 6 D shows the voltage dependence of the Qoff/Qon ratios from −70 to +70 mV. Since the amounts of charge moved at more negative potentials were very small, the ratios varied more as evidenced by larger error bars. There was a trend for a reduction in Qoff/Qon over the entire potential range, but this was only significant positive to −20 mV, near the threshold of channel activation. Depolarizations positive to −20 mV showed that, at most, ~10% of Qoff is inhibited, and this amount of fractional charge block for 1 mM 4-AP with 100 μM quinidine is in agreement with data obtained from the single-pulse dose–response curve in Fig. 3 A. Although the charge block did not seem to be complete until more positive potentials, the leftward shift of the Qoff–V curve in Fig. 6 C, along with measurements of τoff (Fig. 4), all suggest that quinidine

![Image of Figure 6](https://example.com/figure6.png)

**Figure 6.** Voltage dependence of Qon and Qoff in 1 mM 4-AP in the absence and presence of 100 μM quinidine. (A) Q was measured by integrating the Ig from Fig. 4 A for 9 ms for Qon and 15 ms for Qoff. (B) Steady state Q movement from Fig. 4 B after addition of 100 μM quinidine. Data from the +60-mV trace in A is included for comparison (dotted line). (C) Mean normalized Qoff at −100 mV after depolarizations to between −100 and +70 mV. Data (±SEM) are from paired cells (n = 4) in 1 mM 4-AP before (▽) and after (▼) addition of quinidine. Quinidine plus 4-AP data have also been normalized to the maximum peak Qoff in 4-AP alone (■). Boltzmann fits gave a V0.5 of −18.2 ± 1.6 mV and k of 12.4 ± 0.2 mV or valence (z) of 2.06 ± 0.03 e0 (▽) and a V0.5 of −31.9 ± 0.9 mV and k of 13.5 ± 0.3 mV or z of 1.89 ± 0.04 e0 (▼, ■). (D) Mean Qoff/Qon for the same depolarizations and from the same paired cells as in C in 1 mM 4-AP before (▽) and after (▼) addition of quinidine. In C and D, an asterisk adjacent to each point in the presence of quinidine plus 4-AP indicates a significant difference from the corresponding data point in 4-AP alone (P < 0.05, Student’s unpaired t test).
has an effect on gating charge movement throughout the voltage range in the presence of 1 mM 4-AP.

The Time-dependence of Charge Return Is Slowed in Quinidine

In Shaker K⁺ channels, Igoff has a slowed decay and decreased peak with longer depolarizing prepulses up to 8 ms (Bezanilla et al., 1994; Zagotta et al., 1994a). This time-dependent slowing is prevented by the addition of 4-AP and return of Qoff remains fast for depolarizations up to ~350 ms (McCormack et al., 1994; Fedida et al., 1996). Igon and Igoff are shown for a depolarization to +60 from −100 mV for varying lengths of time in the presence of 1 mM 4-AP (Fig. 7 A). The first pulse was 0.45 ms long, and subsequent pulses were incremented by 0.60 ms. Superimposition of the data traces showed consistent Igon and reflected the full recovery of gating charge between the pulses at a frequency of 0.5 Hz. Peak Igoff increased at short depolarizations, saturating after ~2 ms and decay of Igoff remained fast for all pulses. After addition of 100 μM quinidine to the same cell (Fig. 7 B), Igon was similar, but Igoff showed a large difference in both the peak and rate of decay. Off-gating currents showed a blunted peak and slowed decay even for the shortest depolarizations, and after ~2 ms, a notch appeared at the moment of repolarization, which was absent in 4-AP alone. Mean data for peak Igoff normalized to maximum peak Igon showed that before addition of quinidine (Fig. 7 C, ▼), the ratio increased after the first prepulse of 0.45 ms and saturated after 2 ms at a value of ~2.0. When quinidine was present (Fig. 7 C, ▼), normalized Igoff was always less than in 4-AP alone, and saturated at ~0.8 after 2 ms. This suggested that quinidine was able to affect (reduce the peak) Igoff very rapidly, and this effect was maintained for depolarizations up to 8 ms duration. The decay rate of Igoff was significantly slowed at all depolarization durations tested in the presence of quinidine. In Fig. 7 D, single exponential fits of Igoff showed that in 4-AP alone (▼), τoff was ~0.4 ms. In contrast, addition of quinidine (▼) significantly slowed τoff by ~2–2.5-fold to ~1.0 ms. Similar to the voltage-dependent effects of quinidine in the presence of 4-AP, the time-dependent effects consisted of a relative reduction in the peak Igoff and slowing of τoff by ~2–2.5-fold. These changes develop during the briefest depolarizations and saturated after a depolarizing prepulse to +60 mV of ~2 ms in length.

By contrast, inhibition of charge movement (Qoff) in the presence of quinidine plus 4-AP takes time to become established. Fig. 8, A and B, show superimposed Qon and Qoff for the data in Fig. 7, A and B. In 1 mM 4-AP alone, the Qon and Qoff rose rapidly and saturated after a +60-mV prepulse of ~3 ms in length. After addition of 100 μM quinidine, similar Qon and Qoff are evi-
dent, although the rise of $Q_{\text{off}}$ was slower, and saturation also occurred after a prepulse of $\sim 3$ ms duration. Mean data for $Q_{\text{off}}$ normalized to maximum $Q_{\text{on}}$ (Fig. 8 C) show that in quinidine plus 4-AP ($\nabla$), $Q_{\text{off}}$ saturated after a prepulse of 2.35 ms duration.

Measurements of $Q_{\text{off}}/Q_{\text{on}}$ (Fig. 8 D) show that charge was conserved in 4-AP throughout ($\nabla$), but there was a significant time-dependent loss of $Q_{\text{off}}$ in quinidine plus 4-AP ($\nabla$) after a prepulse $> 1.05$ ms. The maximum loss of charge reached $\sim 10\%$ at a prepulse of 7.05 ms, which agrees with data obtained from single-pulse dose–response experiments (Fig. 3 A), and with depolarizations $>- 20$ mV (Fig. 6 D). Quinidine seemed to exert an immobilizing effect on $Q_{\text{off}}$ very rapidly ($\sim 1$–2 ms) in the presence of 4-AP that did not occur in 4-AP alone. However, quinidine had immediate effects ($<1$ ms) in reducing peak Ig$_{\text{off}}$ (Fig. 7 C) and slowing $\tau_{\text{off}}$ (Fig. 7 D).

**Quinidine Has Access to a Single Channel Blocked by 4-AP**

The data so far suggests that 4-AP-blocked channels are accessible to quinidine at all potentials and times studied, even those at which the channel is normally closed. From this we hypothesized that, at the single channel level quinidine might be able to convert the essentially silent behavior known to occur in 4-AP (McCormack et al., 1994) to a flickering type of block seen with quinidine (Yatani et al., 1993; Tseng et al., 1996). hKv1.5 single channels have a high $P_o$ (Fedida et al., 1993; Philipson et al., 1993), so it was relatively simple to identify single channel patches from the appearance of a single opening level. Control data from such a patch are shown in Fig. 9 A. Cells were depolarized in high (135 mM) bath [K$^+$] and pipettes contained 5 mM [K$^+$].

Patches were held at $-80$ mV and given a depolarizing pulse of +60 mV for 800 ms at 0.5 Hz. Control traces in Fig. 9 A are shown after subtraction of blank traces to eliminate capacity transients. Single channel openings occurred in bursts of varying duration and within bursts, the channel was mostly open with frequent, brief closings. An ensemble average of 74 sweeps from the same cell is shown in Fig. 9 B with a dotted line indicating the baseline and the voltage protocol below to indicate the timing of the pulse. During addition of 1 mM 4-AP to the bath (Fig. 9 C), the channel displayed decreased openings, eventually becoming completely silent in the steady state. When averaged, 75 sweeps show that the average current in 4-AP could not be distinguished from the baseline. After addition of 100 $\mu$M quinidine to the same cell, representative traces in the steady state show that the single channel openings reappeared (Fig. 9 E). The channel open time was greatly reduced within bursts and closing events were prolonged. Sweeps with no openings were rare compared with 4-AP alone. An ensemble average of 80 sweeps is shown in Fig. 9 F. The average current was $\sim 50\%$ of the control average cur-

![Figure 8](https://jgp.rupress.org/figure/8)

**Figure 8.** Time dependence of $Q_{\text{on}}$ and $Q_{\text{off}}$ in 1 mM 4-AP in the absence and presence of 100 $\mu$M quinidine. (A) $Q$ was measured by integrating the Ig from Fig. 7 A and are superimposed to show homogeneity during depolarizations from $-100$ to +60 mV in 1 mM 4-AP. (B) Steady state $Q$ movement from Fig. 7 B after addition of 100 $\mu$M quinidine. (C) Mean ($\pm$SEM) $Q_{\text{on}}$ normalized to maximum $Q_{\text{on}}$ at $-100$ mV after depolarization to +60 mV for 4.5 to 7.05 ms in 1 mM 4-AP before ($\nabla$, $n = 3$) and after ($\nabla$, $n = 3$) addition of quinidine. (D) Mean $Q_{\text{off}}/Q_{\text{on}}$ for the same depolarization duration and from the same cells as in C in 1 mM 4-AP before ($\nabla$) and after ($\nabla$) addition of quinidine. In C and D, an asterisk adjacent to each point in the presence of quinidine plus 4-AP indicates a significant difference from the corresponding data point in 4-AP alone ($P < 0.05$, Student’s unpaired $t$ test).
rent (Fig. 9 B). Similar results with 4-AP and quinidine were obtained in four other single channel patches. Clearly, the addition of quinidine, in the continued presence of 4-AP, resulted in channel reopening and the adoption of a “flickering” open channel block behavior.

Open- and closed-dwell time histograms in control and in the presence of 4-AP and quinidine are shown in Fig. 10. In control, both were fit with a single exponential function and values of 1.42 ± 0.01 and 0.40 ± 0.12 ms were obtained for mean open and closed times, respectively. In 1 mM 4-AP and 100 μM quinidine, mean open time was reduced to 0.61 ± 0.07 ms (Fig. 10 C). This may be compared with the effect of 100 μM quinidine alone where the mean open time was reduced to 0.26 ± 0.12 ms. However, the closed-time histogram was well-fit by a double exponential with two mean closed times of 0.47 ± 0.11 and 3.82 ± 0.02 ms (Fig. 10 D). The first closed time likely corresponds to the closed state observed in control, which had a similar mean closed time. The second closed time was approximately eight times longer than the control closed time. Similar effects on shortening mean open time and lengthening closed events have been recently observed with intracellularly applied quinidine on single Kv1.2 channels expressed in Xenopus oocytes (Tseng et al., 1996).

**Discussion**

**The Acceleration of Charge Return**

Until recently, only 4-AP was known to prevent the off-charge slowing that accompanies K\(^+\) channel opening (McCormack et al., 1994; Bouchard and Fedida, 1995). This action of 4-AP was strong evidence that 4-AP blocked channels by preventing a final transition before the open state. Now, at least three processes are known to accelerate charge return in K\(^+\) channels. In Shaker K\(^+\) channels, external Ba\(^{2+}\) speeds Ig\(_{\text{off}}\) and accelerates the return of gating charge upon repolarization (Hurst et al., 1996, 1997). Hurst et al. (1996, 1997) suggest that Ba\(^{2+}\) destabilized the open conformation to accelerate the return of gating charge. Recently, we have shown that permeant monovalent cations also speed Ig\(_{\text{off}}\) and return of gating charge (Chen et al., 1997). Internal 4-AP increases the decay rate of Ig\(_{\text{off}}\), thereby enhancing return of gating charge (McCormack et al., 1994; Bouchard and Fedida, 1995). McCormack et al. (1994) hypothesized that 4-AP prevented channels from opening and noted a 10–15% decrease in Q\(_{\text{off}}\) in the presence of the drug. Here, in the presence of 4-AP we have shown that quinidine, a known open-channel blocker, can still slow the decay of off-charge.

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**Figure 9.** Cell-attached patch recordings from hKv1.5 with 5 mM [K\(^+\)] solution in the pipette and 135 mM [K\(^+\)] in the bath solution to depolarize the cell. (A) 10 representative control sweeps in a one-channel patch, showing openings after an 800-ms depolarizing pulse from −80 to +60 mV. Data were sampled at 5 kHz and filtered at 1 kHz, and pulses were given at a frequency of 0.5 Hz. (B) The ensemble average of 74 sweeps showing an average current over the baseline (dotted line) and a voltage pulse protocol below. (C) 10 sweeps from the same patch as A during wash in of 1 mM 4-AP using the same protocol. (D) The ensemble average of 75 sweeps showing little deviation from the baseline (dotted line). (E) 10 sweeps from the same patch after addition of 100 μM quinidine using the same protocol showing reopening of the channel and characteristic open channel block. (F) The ensemble average of 80 sweeps showing an average current between control and 1 mM 4-AP relative to the baseline (dotted line).
Quinidine modulates 4-aminopyridine block of hKv1.5 gating currents (Figs. 1 and 2), prevent full charge return in 4-AP-blocked channels (Figs. 3 and 6 D), and cause the reappearance of currents from silent single channels (Fig. 9).

Since quinidine is known to only block open channels (Snyders et al., 1992; Clark et al., 1995; Fedida, 1997), the 4-AP-blocked channels must be able to exist in a conformationally open, nonconducting state that allows quinidine access. Quinidine binds to this state with a lower affinity ($K_d = 144 \mu M$, Fig. 3 A) compared with quinidine alone ($K_d = 7.2 \mu M$; Fedida, 1997) with a small nonsignificant change in the Hill coefficient ($n_H = 1.01$ with 4-AP present, Fig. 3 A), from $0.84 \pm 0.21$ (Fedida, 1997) in quinidine alone. These differences indicate a parallel shift in the quinidine sensitivity in the presence of 4-AP of $\approx 20$-fold, and suggest a change in the affinity of the quinidine binding site. 100 $\mu M$ quinidine also reduced peak Igoff (Figs. 4 D and 7 C), and shifted the voltage dependence of $\tau_{on}$ (Fig. 4 C). Most importantly, quinidine decreased $\tau_{off}$ $\approx 2.5$-fold (Figs. 5 F and 7 D) in the presence of 1 mM 4-AP at all potentials studied. The slowing was also observed after shorter depolarizations than in quinidine alone (Fedida, 1997). These two results suggest that 4-AP-blocked channels are vulnerable to quinidine block at more negative potentials and at earlier times than the limits set by the threshold potentials ($\approx 25$ mV) and the activation time to opening ($\approx 1$ ms) required for the action of quinidine alone (Fedida, 1997).

Quinidine slows Igoff decay at all times and potentials (Figs. 1, 2, 5, and 7), which indicates access to the channel when it should be closed. Additional evidence for an action of quinidine at potentials negative to the channel opening threshold were negative shifts in the potential dependence of charge movement (Qoff–V, Fig. 6 C) and the decay rate of Igoff ($\tau_{on}$, Fig. 4 C). The direction of these shifts and their relatively small magnitude ($< 10$ mV) suggest quinidine access to a region of the channel where it can exert charge screening effects, which are not seen in the presence of quinidine alone (Fedida, 1997). To obtain further evidence for quinidine access to 4-AP-blocked channels, we performed single channel experiments (Figs. 9 and 10). The effects of 1 mM 4-AP at +60 mV on the single channels was a change from bursts of channel opening with a high $P_o$ to a complete loss of conduction with a failure to reopen or conduct at steady state. The addition of 100 $\mu M$ quinidine, while keeping the concentration of 4-AP constant, led to characteristic flickering open-channel block with only a few blank sweeps. There was a reduction in mean open time to $< 50\%$ and the addition of a second mean closed time that was approximately eight times longer than the closed time also observed in control currents. Recovery from 4-AP block was not observed before addition of quinidine, which suggested that quinidine was also able to change the state of 4-AP-bound channels, and allow them to open. The addition of the second mean closed time could reflect the presence of a 4-AP- and quini-
dine-blocked state from which return to the open state is possible, but approximately eight times slower than in the unblocked state.

Immobilization of Charge

There are several situations where the return of gating charge is slowed or immobilized. The first example of charge immobilization observed was associated with open channel block by the inactivation domain in channels exhibiting N-type inactivation (Armstrong and Bezanilla, 1977; Bezanilla et al., 1991; Demo and Yellen, 1991). Quaternary ammonium ions such as TEA (tetraethylammonium) also cause open channel block of K⁺ channels internally and prevent the conformational closing of the channel (Armstrong, 1971). It was shown that internally applied TEA slows return of gating charge (Bezanilla et al., 1991; Stühmer et al., 1991). The action of quinidine on hKv1.5 channels results in gating charge immobilization (Fedida, 1997), similar to that seen with open channel block by internal TEA and the N-type inactivating peptide (Armstrong and Bezanilla, 1977; Bezanilla et al., 1991; Stühmer et al., 1991). In the presence of 4-AP, quinidine had a partial charge immobilizing effect (at doses >50 μM), which caused a parallel shift in the dose–response relation compared with quinidine alone (Fig. 3 A; compare with Fedida, 1997). The voltage dependence of charge return showed a leftward shift of ∼13 mV in the Qoff–V curve when normalized to values in both 4-AP and quinidine (Fig. 6 C), and significant immobilization of up to 20% of gating charge was observed positive to −20 mV (Fig. 3 A). The time dependence of charge return with quinidine plus 4-AP showed that normalized Qoff was reduced by ∼10% (Fig. 8 C) and development of significant immobilization took ∼1 ms (Fig. 8 D). In 1 mM 4-AP, the gating currents showed a reduction of peak Igoff (Figs. 3 B and 7 C) and slowing of τoff (Figs. 3 C, 5 F, and 7 D) at quinidine doses higher than 10 μM, and at all potentials and prepulse lengths studied. However, the partial charge-immobilizing effects of quinidine were both voltage (Fig. 6, C and D) and time (Fig. 8, C and D) dependent and did not reach the full effect in the presence of 4-AP (Fig. 3 A; compare with Fedida, 1997). Interestingly, the conditions for quinidine-induced charge immobilization in the presence of 4-AP coincided with the threshold (∼25 mV) and onset (∼1 ms) of channel activation. This suggests that there could be coupling between full channel opening and the ability of quinidine to immobilize charge, perhaps through improved access or stronger binding to the channel.

Molecular Determinants of 4-AP Trapping, and Binding Sites of 4-AP and Quinidine

Many studies have reported the phenomenon of 4-AP trapping in K⁺ channels. 4-AP binds to closed (Yeh et al., 1976; Thompson, 1982; Kehl, 1990) or open (Wagener and Oxford, 1990; Choquet and Korn, 1992) channels. It remains trapped in the channels at hyperpolarized potentials with relief of block after wash out occurring upon subsequent depolarization (Choquet and Korn, 1992; Kirsch and Drew, 1993; McCormack et al., 1994). Trapping of blockers in channels was first proposed for quaternary ammonium compounds (Armstrong, 1971). By transplanting regions of Kv3.1 into the less 4-AP-sensitive Kv2.1, the cytoplasmic halves of the S5 and S6 transmembrane segments were determined to be important for 4-AP binding and were also implicated in forming part of the inner mouth of the pore (Kirsch et al., 1993). The actual site of 4-AP binding was thought to be guarded by the activation gate structure, which would trap the 4-AP in the closed channel (Kirsch and Drew, 1993). The differences in the S5 segment are at positions 434, 435, and 439 (using hKv1.5 numbering) and in the S6 segment at 507, 510, 512, and 514. The equivalent residues in hKv1.5 show more sequence homology with Kv3.1 than Kv2.1 and, as expected, the IC₅₀ for hKv1.5 (50 m; Bouchard and Fedida, 1995) is similar to Kv3.1 (100 μM; Kirsch et al., 1993), and quite different from Kv2.1 (18 mM; Kirsch et al., 1993).

In a mutational study of Kv1.5, two sites were found to increase or decrease affinity for quinidine when they were changed (Yeola et al., 1996). These residues at positions 507 and 514 would occur on the same side of the α-helical S6 segment, separated by two turns. Quinidine open channel block is thought to involve both electrostatic and hydrophobic components (Snyders et al., 1992; Snyders and Yeola, 1995). As such, substitutions with greater hydrophobicity at position 507 increased affinity for quinidine. It is important to note that these two sites are also two of the four sites in the S6 segment that are implicated for differential 4-AP sensitivity in Kv2.1 and Kv3.1 (Kirsch et al., 1993). This strongly suggests that these two drugs have binding sites close to one another at the inner mouth of the K⁺ channel pore. Our data supports this idea as the parallel-shifted dose–response curve of fractional charge block in quinidine plus 4-AP (Fig. 3 A) suggests allosteric modification of the quinidine site by the presence of 4-AP; the single channel data (Figs. 9 and 10) suggest quinidine modification of 4-AP binding to the channel.

Recently, it has been shown through the use of Kv2.1 and Kv3.1 chimeras that the cytoplasmic end of the S5 segment (with the same residue differences as for 4-AP sensitivity) plays an important role in the rate of the open to first closing transition (Shieh et al., 1997). This part of S5 could be a part of the structural component of an activation gate or lid. Another recent study has discovered the ability of a point mutation in Shaker K⁺ channels at site 470 (508 in hKv1.5) in the S6 trans-
membrane segment to confer the ability to trap the organic blockers decyltriethylammonium (C₁₀) and tetraethylammonium (Holmgren et al., 1997). They determined that the region in S6 near residue 508 is normally covered by the activation lid when the channel is closed. This site (508) is within the stretch of eight amino acids that contain the sites implicated for both 4-AP (507, 510, 512, and 514) and quinidine binding (507 and 514). Therefore, on a molecular level, the mechanism for 4-AP trapping and binding of both 4-AP and quinidine may be mediated by the same region of eight amino acids that represents two full turns of the S6 α-helix. 4-AP trapping also likely involves the cytoplasmic end of S5, which may form part of the activation lid.

**Quinidine Access to 4-AP-blocked Channels**

We propose a physical model (Fig. 11) where the activation lid normally shuts the channel when it is closed at potentials negative to the threshold of channel opening (A). When the lid opens, quinidine is allowed access to the channel and can reach its binding sites (Fig. 11 B). The bound quinidine must dissociate before allowing the activation lid to close the channel. This results in delayed channel closing and characteristic crossover of deactivating tail currents (Snyders et al., 1992; Fedida, 1997). 4-AP binds to sites that are likely covered by the activation lid and exposed when the channel opens (Fig. 11 C). When the channel closes, 4-AP remains bound to the channel and is trapped within. The activation lid now cannot shut completely, because the 4-AP molecule might occupy some of the sites that the lid normally covers. Incomplete closure of the activation lid could be an explanation for our observations that quinidine has access to 4-AP-blocked channels earlier in time and at potentials negative to the threshold of channel opening (Fig. 11 C). There was an obvious slowing of the rate of decay of Ig_{off} at subthreshold potentials, and earlier than expected compared with the onset of quinidine action without 4-AP. The presence of trapped 4-AP in the channels seems to allow quinidine access and provides the basis for our model of the 4-AP-blocked channel in a quinidine-sensitive but nonconducting state, analogous to the “tense” state proposed by McCormack et al. (1994). Since quinidine binding is voltage dependent and is increased at more depolarized potentials (Snyders et al., 1992; Clark et al., 1995; Fedida, 1997), quinidine affinity would be expected to be reduced at potentials negative to the threshold of activation. In addition, access to quinidine could be restricted by the partially closed activation lid and could be enhanced at potentials where the activation lid would open fully (Fig. 11 D). These two reasons may explain why significant charge immobilization of quinidine in the presence of 4-AP did not seem to occur at potentials negative to the threshold of activation and took ~1 ms to develop. Thus, the voltage-dependent increase in affinity of quinidine or the opening of the activation lid could allow for greater association of quinidine to the 4-AP-blocked channel at positive potentials. This increased affinity or enhanced access of quinidine could then confer its charge-immobilizing action. Our gating current observations that quinidine affinity was greatly reduced in 4-AP, coupled with the single channel observations that reopenings were virtually nonexistent in 4-AP alone but were facilitated by the addition of quinidine, strongly support the idea that modification of both quinidine and 4-AP binding occurred when both drugs were present at the inner mouth of the channel (Fig. 11 D).

![Figure 11](https://i.imgur.com/123456.png)

**Figure 11.** A schematic diagram of the physical interactions between quinidine, 4-AP, and hKv1.5. (A) Normal channel closing is shown by the activation gate swinging from open (solid) to shut (dotted). (B) Open channel block by quinidine results in a quinidine-blocked state, impeding the activation lid from closing the channel and slowing channel deactivation. (C) 4-AP has access to open channels and can keep the channels in a nonconducting 4-AP-blocked state when 4-AP is trapped within the channel. 4-AP-trapped channels prevent complete closing of the activation lid (dotted) and allow quinidine access to its binding site, which is close to that of 4-AP. (D) 4-AP and quinidine can coexist at binding sites within the inner mouth of the channel. Allosteric modulation of quinidine binding by the presence of 4-AP, and vice-versa, occurs. This reduces the apparent K_d for quinidine ~20-fold and allows reopening of silent channels in the presence of high concentrations of 4-AP.
Conclusion

Using quinidine as a probe of open channels, our results suggest that 4-AP-blocked channels actually allow access to quinidine at potentials and times when the channel should be closed. The simplest explanation of this result is that 4-AP actually holds the channel in an open, but nonconducting state. However, at this point, we cannot tell whether the channel is in a conformationally open but nonconducting state or if trapping of 4-AP holds the internal vestibule of the channel partially open—enough to allow quinidine binding, but not enough to allow ion conduction past narrower, deeper parts of the K⁺ channel pore. The latter explanation is more consistent with the fast off-gating currents on repolarization (McCormack et al., 1994), supporting the idea of a slow final opening transition that carries little charge. The fact that 4-AP trapping and binding of both 4-AP and quinidine may all be mediated by the same region at the inner vestibule is also important for understanding the structure of K⁺ channels and the precise mechanism of action of ion channel blockers and therapeutic drugs.

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