Brief Communication

Quinidine Delays $I_K$ Activation in Guinea Pig Ventricular Myocytes

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A major action of the antiarrhythmic agent quinidine is prolongation of cardiac repolarization. In these experiments, the time-dependent effects of quinidine on the delayed rectifier potassium current, $I_K$, a current contributing to cardiac repolarization, were investigated in acutely disaggregated guinea pig ventricular myocytes using the whole-cell recording configuration of the patch-clamp method. The effect of quinidine on $I_K$ was dependent on the duration of depolarization. After long (2,000 msec) pulses, $I_K$ was reduced by 30 ± 27% (SD; n = 8, paired) by 10 μM quinidine; in contrast, after short (100 msec) pulses, the drug decreased $I_K$ 65 ± 35% (p<0.05). This effect was found both in paired experiments as well as when quinidine-pretreated cells were compared to non-pretreated cells. Quinidine significantly delayed $I_K$ activation (9 ± 20 msec at baseline vs. 44 ± 25 msec in drug, p<0.05), but did not alter the subsequent time course of activation (time constant 659 ± 118 msec). These findings are consistent with the hypothesis that quinidine promotes occupancy of a channel state from which opening does not occur. (Circulation Research 1988;62:1055-1058)

The widely used antiarrhythmic agent quinidine prolongs cardiac repolarization both in patients and in vitro experiments. Colatsky attributed the increase in action potential duration by quinidine in rabbit Purkinje fibers to block of the outward delayed rectifier current. The experiments reported here indicate a mechanism whereby quinidine may exert this effect. Recent findings suggest that a single-open, single-closed-state model for this current (which McDonald and Trautwein referred to as "$I_K$") is inadequate in some tissues. Bennett et al found that $I_K$ deactivation kinetics in calf Purkinje fibers were biexponential; moreover, both components had reversal potentials near $E_K$ and $P_m/P_K$ was ≤ 0.02. Gintant et al similarly reported biexponential deactivation kinetics of this current in canine Purkinje cells. Instead of a single-open, single-closed-state model, both groups postulated a channel with one open (O) state and two closed states ($C_1, C_2$), one of which would not contribute directly to opening:

$$C_2 \leftrightarrow C_1 \leftrightarrow O$$

We now report that quinidine decreases $I_K$ in single guinea pig ventricular myocytes; moreover, our findings suggest that quinidine promotes occupancy of a state of the channel from which opening does not occur.

Materials and Methods

Isolated ventricular myocytes were obtained from 200–300-g guinea pigs by collagenase dissociation. Hearts were rapidly removed, mounted on a Langendorff apparatus, and perfused with calcium-free, Joklik-modified minimum essential medium (GIBCO Laboratories, Chagrin Falls, Ohio). The medium was supplemented with 25 mM NaHCO$_3$ and was equilibrated with 95% O$_2$-5% CO$_2$. The pH was adjusted to 7.4 at 37°C with NaOH. Following a 1-minute perfusion with calcium-free solution, hearts were perfused for 10–20 minutes with solution containing 0.15% collagenase (120–153 units/mg; Worthington Type II) and 50 μM CaCl$_2$. Following the collagenase perfusion, hearts were removed from the Langendorff apparatus, minced, and gently agitated in a shaking bath. Cells were washed and stored for subsequent use at 37°C in enzyme-free Joklik solution containing 1 mg/ml bovine serum albumin and 0.5 mM CaCl$_2$.

A small aliquot of a suspension of dissociated cells was placed in a 0.5-ml chamber mounted on the stage of an inverted microscope. After a brief period during which the cells adhered to the coverslip at the bottom of the chamber, the cells were superfused at 1 ml/min with bath solution containing (mM) NaCl 145, KCl 4.5, MgCl$_2$ 2.0, CaCl$_2$ 1.0, CoCl$_2$ 3.0, HEPES 10, glucose 10, pH 7.4. The bath temperature was measured by a small thermistor and temperature was maintained at 37 ± 1°C by a Peltier-effect device.

All recordings were made in the whole-cell, voltage-clamp configuration of the "patch clamp" technique using heat-polished glass electrodes with tip resistances of 1–3 MΩ when filled with (mM) KCl 120, MgCl$_2$ 2, CaCl$_2$ 1, HEPES 10, EGTA 11, K$_2$-ATP 5, Na$_2$-creatine phosphate 10 (pH 7.2). Voltage-clamp
command pulses were generated by a 12-bit digital-to-analog converter. Membrane current was filtered at 50 Hz by a four-pole Bessel filter and digitized at 100 Hz for subsequent analysis. In these experiments, the holding potential was $-70 \text{ mV}$, and pulses of variable duration to $+20 \text{ mV}$ were applied to activate $I_K$. $I_K$ was measured as the peak magnitude of the time-dependent current during a repolarizing step to $-30 \text{ mV}$.

Only cells that remained rod-shaped with clear cross-striations during superfusion with 1 mM $\text{CaCl}_2$ and had resting potentials more negative than $-80 \text{ mV}$ were studied. Cell lengths ranged from 90 to 130 $\mu\text{m}$ and widths ranged from 20 to 40 $\mu\text{m}$ (estimated surface area 5,600–16,000 $\mu\text{m}^2$). Following assessments in the absence of drug, the perfusate was changed to one containing 10 $\mu\text{M}$ quinidine (Aldrich, Milwaukee, Wisconsin; >99% purity) and data were collected again after 10–30 minutes. As described below, additional experiments were performed in cells that had been perfused with quinidine for 30–60 minutes prior to application of an electrode.

A least-squares Marquardt search was used to fit deactivating $I_K$ and tail current amplitudes to monoexponential and biexponential functions of time. Fits were compared using a generalized $F$ test, as previously described. Analysis of variance was used to compare quinidine-induced changes in $I_K$ after depolarizing pulses of varying durations. If the null hypothesis could be rejected at the $p < 0.05$ level, Duncan’s test was used for pairwise comparisons. Baseline and quinidine data were compared by Student’s $t$ test. All data are presented as mean ± SD except in the figures, where standard error bars are used.

**Results**

Figure 1 shows reduction of $I_K$ by quinidine. $I_K$ was activated by a 500-msec step to $+20 \text{ mV}$, and $I_K$ amplitude was reported as the magnitude of the time-dependent current elicited with deactivation at $-30 \text{ mV}$. During prepulse control, $I_K$ was slightly greater than 300 pA. Following 10 $\mu\text{M}$ quinidine, $I_K$ fell to approximately 40 pA within 10 minutes. During wash-out, $I_K$ was partly restored to 150 pA within 20 minutes. In general, the preparations were stable for 20–30 minutes under these experimental conditions.

The time course of $I_K$ activation was studied by generating envelopes of current tails. An example of control $I_K$ tails following depolarizing pulses of variable duration to $+20 \text{ mV}$ is shown in Figure 2 (top traces) while the same envelope of current tails experiment after the preparation was exposed to 10 $\mu\text{M}$ quinidine is also shown in Figure 2 (bottom traces). Quinidine exerted two effects: the magnitude of $I_K$ activated after long pulses was reduced and $I_K$ activation was delayed. In the example shown in Figure 2, the magnitude of the current tail after a 2,000-msec pulse was 303 pA prior to drug and 126 pA in quinidine, a 58% reduction. In contrast, after the 100-msec pulses (the shortest ones shown in Figure 2), a 46-pA current tail was recorded prior to drug administration but $I_K$ was absent in quinidine. Changes in $I_K$ in eight paired experiments are shown in Figure 3: the reduction after short pulses (100 msec) was significantly greater than after longer pulses ($p < 0.05$; analysis of variance). After 2,000-msec pulses, $I_K$ was reduced 30 ± 27% by quinidine, whereas after 100-msec pulses, the drug decreased $I_K$ 65 ± 35%. Thus, block of $I_K$ by quinidine was partially relieved by increasing the duration of depolarization.

Quinidine did not significantly change holding current at $-30 \text{ mV}$ or $-70 \text{ mV}$. At $-30 \text{ mV}$, holding current was 10 ± 50 pA predrug and 56 ± 40 pA in quinidine and, at $-70 \text{ mV}$, 125 ± 115 pA predrug and 46 ± 103 pA in quinidine.

As noted above, the preparation we used was usually stable for 20–30 minutes after electrode contact with the cell. We therefore also compared, in unpaired experiments, the time course of $I_K$ activation in cells superfused with quinidine for 30–60 minutes prior to study to those not superfused with quinidine. As can be seen in Figure 4, the time course of $I_K$ activation was quite different in the two groups: quinidine-treated cells again showed no $I_K$ activation (mean tail current $=0\pm 16 \text{ pA}$, $n = 6$) after 100-msec pulses, compared with a mean $I_K$ of 65 ± 34 pA prior to drug administration in the eight paired experiments described above and 59 ± 47 pA in a further nine cells not exposed to quinidine. This difference between control and quinidine-exposed cells is statistically significant ($p < 0.05$).

When the time course of the current during depolarization is used to assess $I_K$, we (manuscript in preparation) and others have observed multieponential or sigmoid activation kinetics. Gintant et al found two exponentials were required to fit current...
activated during long depolarizations in canine Purkinje cells (34–36°C; 20-second pulses to −15 mV). In one example, the time constants were 0.67 and 3.3 seconds, and "creep" toward the end of the pulse was noted. Since, as discussed further below, other currents may have been present during depolarizing pulses in

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Envelopes of current tails predrug (top) and after 10 minutes exposure to quinidine 10 μM (bottom). Preparations were held at −70 mV and pulsed every 5–8 seconds to +20 mV for variable periods (100; 200; 400; 800; 1,200; and 2,000 msec), followed by deactivation at −30 mV for 3–5 seconds. All six traces during the activating and deactivating sequences are shown superimposed. The calibration bars at the bottom intersect at current = 0 and time = start of the depolarizing pulse. All traces are on the same time and current scale; the upper 1-second time bar is positioned at current = 0 for the baseline traces.

our experiments, we confined our analysis to the tail currents. We adopted the following approach to quantifying the delay in activation. The amplitudes of tail currents elicited by depolarizations of variable durations (≥100 msec) were fit with a monoexponential function of time. Under these conditions, the time intercept of the monoexponential fit was used as an index of the delay in \( I_k \) activation. These intercepts differed significantly (9 ± 20 vs. 44 ± 25 msec; \( p < 0.05 \)), again indicating a delay in activation of \( I_k \) in the presence of quinidine. The time constants of the monoexponential fits were similar: 663 ± 173 msec predrug and 659 ± 118 msec in quinidine (n = 8; paired; \( p = \text{NS} \)).

\( I_k \) deactivation at −30 mV following 2-second pulses to −30 mV each of these experiments was better fit with a biexponential rather than a monoexponential function. This finding agrees with those of Bennett et al10 and Gintant et al.7 However, Hume12 reported a monoexponential deactivating time course for \( I_k \) in non-drug-treated guinea pig myocytes; different experimental conditions (25°C; deactivation at −50 mV) may explain this difference. The time constants for the biexponential fits were similar predrug and during drug: the shorter time constant was 143 ± 20 msec predrug and 153 ± 56 msec in quinidine, while the longer one was 575 ± 113 msec predrug and 499 ± 174 msec in quinidine (n = 8; paired; \( p = \text{NS} \)).

**Figure 3.** \( I_k \) tail amplitudes (quinidine/baseline as a %) as a function of activating pulse duration. The reduction after 100- and 200-msec pulses was significantly greater than that after longer pulses (paired; n = 8). **\( p < 0.05 \)** compared with all other values; *\( p < 0.05 \)** compared with 800-, 1,200- and 2,000-msec values (analysis of variance).

**Discussion**

Although previous in vitro studies have used in Purkinje fiber preparations,10,14 a major effect of quinidine in man is prolongation of repolarization in ventricular muscle. Thus, our findings are consistent with the notion that blockade of outward current(s)10,14 by quinidine contributes to its effect on repolarization in ventricular muscle. Other workers have recently reported that quinidine depressed \( I_k \) in guinea pig15...
ventricular myocytes; however, no possible mechanism(s) of the effect of quinidine on \( I_\text{K} \) has been proposed. In these studies, we have found that quinidine delays \( I_\text{K} \) activation. Moreover, this effect was easily detected with depolarizing pulses whose duration (100–200 msec) and potential (+20 mV) corresponded to those of normal ventricular muscle action potentials. These experiments were not designed to study the effect of quinidine on \( I_\text{K} \). Under our experimental conditions, quinidine tended to reduce holding current at -70 mV, although the difference was not significant. Reduction of \( I_\text{K} \) by quinidine, which has been reported by others, may also contribute to action potential prolongation.

We measured \( I_\text{K} \) as the magnitude of the current tail following a depolarizing pulse to +20 mV. A potential of -30 mV was chosen for deactivation since the tails are smaller at more negative potentials closer to \( E_\text{K} \) as well as more positive ones, where incomplete deactivation is seen. Furthermore, tail current amplitude was used as a measure of \( I_\text{K} \) to avoid potential contamination by other currents during depolarizing pulses. These may include the sodium and calcium currents and currents associated with electrogenic pumping. The sodium current was likely present during depolarization but its kinetics at 37°C are sufficiently fast that it could not be expected to contribute to our tail current measurements. It is unlikely that the calcium current played a major role in these experiments since 3 mM CoCl\(_2\) was present in the perfusate. A role for electrogenic pump currents is more difficult to exclude. However, we have observed that quinidine produces a delay in \( I_\text{K} \) tail current development similar to that reported here in voltage-clamped guinea pig ventricular myocytes superfused with extracellular solution modified to minimize electrogenic pump currents (Tris 150 mM, Na 0 mM, MgCl\(_2\) 2.0 mM, KCl 4.5 mM, glucose 10 mM, CaCl\(_2\) 0.1 mM, CdCl\(_2\) 0.1 mM, 30°C; unpublished observations).

Bennett et al\(^6\) and Gintant et al\(^7\) suggested a multiple closed-state model for this potassium channel. Such a formulation can account for the bieponential deactivation curves reported in their Purkinje experiments. In these experiments, two closed states, a full model for quinidine’s effects on \( I_\text{K} \) is not yet apparent. Our working hypothesis is that quinidine’s affinity for this channel is a function of channel state, in analogy to the modulated receptor hypothesis of Hille\(^8\) and Hondeghem and Katzung. Further studies are required to fully characterize the effects of quinidine on this repolarizing current.

**Note added in proof:** Another explanation for the delay we observed is that quinidine may block a contaminating current component in the \( I_\text{K} \) tails.\(^17\)

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**Key Words** • quinidine • electrophysiology • delayed rectifier • repolarization
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