Quinidine Interactions With Human Atrial Potassium Channels

Developmental Aspects

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Abstract—Clinical studies have suggested that quinidine is less effective when used for the treatment of atrial arrhythmias in pediatric patients compared with its clinical effectiveness in the adult patient population. Age-related changes in the cardiac actions of quinidine on action potential duration and interaction with potassium channels in several mammalian species also have been reported. We investigated the effects of postnatal development on quinidine's interaction with major repolarizing currents (I_{to}, I_{Kur}, I_{ns}, and I_{K1}) in human atrial myocytes, using the whole-cell configuration of the voltage-clamp technique. Our results indicate that there are age-related changes in both the IC_{50} for quinidine blockade of I_{to}, as well as the mechanism of quinidine unblocking. In contrast, quinidine was found to inhibit both adult and pediatric I_{K1} and I_{Kur} in an age-independent manner, whereas the nonselective cation current (I_{ns}), which contributes to the sustained outward current (I_{sus}), was insensitive to quinidine. The results from this study help to clarify the electrophysiological mechanism by which quinidine elicits its antiarrhythmic effect in the pediatric and adult human population. **(*Circ Res*. 1998;83:1224-1231.)**

Key Words: human ■ atrial myocyte ■ development ■ quinidine ■ K^+ channel

Quinidine is one of the most commonly used drugs for treatment of both atrial and ventricular rhythm disturbances.[1] Quinidine's efficacy as an antiarrhythmic agent is believed to result from its effects on conduction velocity and repolarization of the cardiac action potential.[1,2] Quinidine's ability to prolong the duration of the cardiac action potential has been attributed to its ability to inhibit several different types of potassium ion channels expressed in mammalian cardiac tissue, including the transient outward K current (I_{to}), the inwardly rectifying K current (I_{K1}), the rapidly activating delayed rectifier (I_{Kr}), and the ultrarapid delayed rectifier (I_{Kur}).[3] However, the mechanisms by which quinidine exerts its inhibitory effects on these potassium currents are not understood fully.

Previous studies in dog, rabbit, and rat cardiac tissue have documented that the ability of quinidine and other antiarrhythmic agents to alter conduction and repolarization change significantly during postnatal development.[4–8] Quinidine has been shown to prolong both the QT interval[9] and repolarization[4] of the Purkinje fiber action potential to a significantly greater extent in young canines compared with adult animals. Recent evidence suggests that developmental changes in the effects of antiarrhythmic agents on cardiac tissue may be attributed to age-related changes in drug-channel interactions.[6–8] For example, quinidine block of rabbit ventricular I_{to} and I_{K1} has been shown to be significantly different in neonatal versus adult myocytes, with cells from neonates being more sensitive to the action of quinidine than adults.[8]

It seems likely that age-related differences in quinidine interaction with cardiac ion channels may also exist in man. Recent clinical studies have documented that quinidine and flecainide exhibit a markedly reduced clinical efficacy against atrial tachyarrhythmias in pediatric patients[10] compared with their relatively high clinical efficacy in adults.[11] Because changes in drug-channel interaction have been indicated as a potential mechanism underlying postnatal changes in the actions of antiarrhythmic agents, we initiated a study to compare the effects of quinidine on major repolarization currents (I_{to}, I_{K1}, I_{Kur}, and the nonselective cation current, I_{ns}) expressed in pediatric and adult human atrial myocytes.

Materials and Methods

Human Atrial Specimens and Isolation of Cardiac Myocytes

Human myocytes used in the experiments investigating the effects of quinidine on human atrial potassium channels were isolated from specimens of right atrial appendages obtained during surgery from the hearts of 23 adult patients (35 to 75 years of age) and 17 pediatric patients (4 days to 2 years of age) undergoing cardiopulmonary bypass surgery. Atrial tissue obtained from a separate group of patients was used to study the effects of age on I_{to} density (Figure 1).

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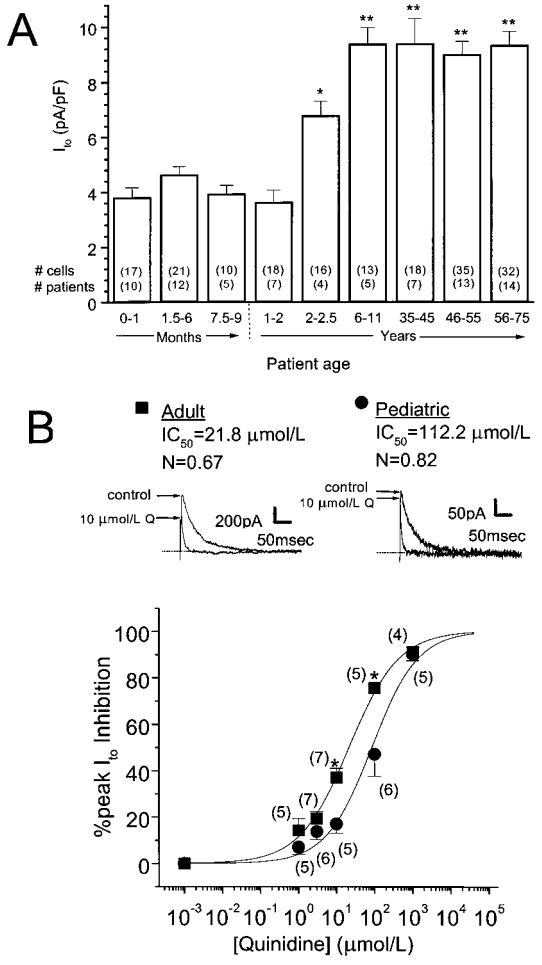

Figure 1. Effect of age on I_{to} density and sensitivity to quinidine. A, Effect of age on I_{to} density. Patients were divided into 9 age groups as shown. I_{to} was elicited by an 800-ms pulse to 60 mV from a holding potential of −40 mV. I_{to} was corrected for the sustained current. A significant increase in I_{to} density occurred after the second year of life. I_{to} underwent a significant maturational increase (from 4.2±0.4 pA/pF in patients 1 to 2 years old to 6.97±0.5 pA/pF in patients 2 to 2.5 years old) during the second year of life. At 11 years, human atrial I_{to} density (9.4±0.7 pA/pF) did not appear to be different from the density of the current recorded from patients >11 years old (9.01±0.5 pA/pF). B, Effect of age and quinidine on I_{to}. Currents were elicited by an 800-ms pulse to 60 mV from a holding potential of −70 mV. Inserts show typical examples of the effect of 10 μmol/L quinidine on the time course and peak amplitude of I_{to} in a pediatric and adult atrial myocyte. Graph shows the relationship between quinidine concentration and inhibition of peak I_{to}. Values indicate mean±SE. Number of cells is given by each mean value. Average data were fit by the equation: $1/(1+(IC_{50}/X)^N)$. Estimated values for adult I_{to} were IC_{50}=21.8 μmol/L, N=0.67. For pediatric I_{to}, the values were IC_{50}=112.2 μmol/L, N=0.82. There was a significant developmental difference in the percentage of quinidine-induced inhibition by 10 and 100 μmol/L of the drug, with pediatric I_{to} inhibited to a smaller extent (adult: 37±4% at 10 μmol/L, 75±1.7% at 100 μmol/L; pediatric: 17±4% at 10 μmol/L, 47±9% at 100 μmol/L). IC_{50} values for adult and pediatric populations were significantly different (P<0.0001). *P<0.05 vs all groups <2 years old; **P<0.05 vs all groups <6 years old.

Atrial tissue was obtained in accordance with Tulane University School of Medicine institutional guidelines. Specimens of atrial tissue were considered free from pathology if all of the following criteria were met: (1) the specimen appeared grossly normal on removal; (2) there was no evidence of right atrial enlargement (eg, P wave amplitudes >2.5 mm); and (3) there was no evidence for significant right atrial pressure overload (right atrial pressure >7 mm Hg). Human atrial myocytes were isolated enzymatically from the obtained specimens. The isolation procedure was identical to that previously described by Crumb et al[12] and typically produced an initial yield of 40% to 60% rod-shaped, calcium-tolerant cells. Cells were used within 8 hours after isolation. Currents were recorded from myocytes with characteristically normal morphology (rod-shaped, clear striations, no surface blebs).

Solutions and Drugs

Recordings of potassium currents were made from myocytes bathed in an "external" solution having the following chemical composition (in mmol/L): NaCl 137, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 11, and HEPES 10 (adjusted to pH 7.4 with NaOH). All recordings were made in the presence of 0.2 mmol/L $CdCl_2$ to block the L-type calcium current (I_{Ca}).[13] In most of the experiments, cells were held at −40 mV to inactivate the fast inward sodium current (I_{Na}). In some cells, I_{to} was elicited with depolarizing steps from a holding potential of −80 mV. As demonstrated by Li and Keung,[14] no sodium current was elicited under these conditions, suggesting complete inactivation of Na channels. Glass pipettes were filled with "internal" solution containing (in mmol/L): K-aspartate 120, KCl 20, Na_2-ATP 4, EGTA 5, and HEPES 5 (adjusted to pH 7.2 with KOH). When the nonselective cation current was recorded, a potassium-free internal solution of the following composition was used (in mmol/L): CsCl 140, Na_2-ATP 4, Mg_2Cl 1, EGTA 5, and HEPES 5 (adjusted to pH 7.2 with CsOH). Quinidine hydrochloride was obtained from Sigma Chemical Co and dissolved in ethanol to form stock solutions of 8-, 40-, and 125-mmol/L concentrations. Pilot experiments indicated that the level of ethanol present in the quinidine stock solution was without effect on ionic currents when added to the bath alone. Steady-state drug effects were typically reached 8 to 10 minutes after onset with quinidine-containing solution. All experiments were performed at room temperature (22°C to 24°C).

Voltage-Clamp Technique and Data Acquisition

The whole-cell patch-clamp technique was used to record ionic currents under voltage-clamp.[15] An Axopatch amplifier (Axon Instruments) interfaced via a 333-kHz Digidata 1200 acquisition board to a personal computer running pClamp (Version 6.0+) software was used to voltage-clamp the isolated myocytes. Borosilicate glass pipettes were made using a horizontal pipette puller, and pipette tips were heat-polished. Pipette tip resistance was ≈1 to 2.5 MΩ when the electrodes were filled with the internal solution.

Capacitative transients were evoked by a 10-mV step (from −40 to −50 mV) and well described by a single exponential function. Before compensation, the mean τ_{rec} of transient decay was estimated to be 0.32±0.02 ms in adult (n=96) and 0.11±0.01 ms in pediatric cells (n=57). Cell capacitance (C_m) was obtained by integration of the area under the capacitative transient. Mean values of C_m obtained by this method were 90.2±3.14 pF and 35.7±1.07 pF for adult and pediatric myocytes, respectively. The series resistance (R_s) for the pathway between the pipette and cell membrane was estimated from the equation $R_s=\tau/C_m$. The uncompensated R_s was 3.53±0.12 MΩ for adult and 3.1±1.01 MΩ for pediatric cells. To minimize the duration of the capacitative surge and voltage drop across the pipette, R_s was compensated electronically (typically by 30% to 60%). The amplitude of I_{to} always was measured as the difference between the peak current and the steady-state level of current remaining at the end of the pulse (typically of 800-ms duration). To determine the effects of quinidine on the inwardly rectifying potassium current (I_{K1}) and the sustained current (I_{sus}), a p/4 post hoc leak subtraction procedure was used.

Statistics and Data Analysis

Data are presented as mean±SEM. Data analysis and curve fitting were performed using the software packages Clampfit (pClamp software, Axon Instruments), Origin (Microcal Software), and

GraphPad Prism (GraphPad Software, Inc). Differences between group means were evaluated for statistical significance using Student *t* test. An unpaired *t* test was used to compare statistically best-fit values of the drug concentration producing 50% inhibition (IC_{50}) of adult and pediatric I_{to}, I_{sus}, and I_{K1} (GraphPad Prism).

Results

Effect of Postnatal Development on I_{to} Amplitude

In a previous study, it was reported that there is an age-related difference in human atrial I_{to} density, with pediatric current having half the density of the adult I_{to}.[12] Because the time course over which I_{to} undergoes its developmental change was not well defined, we compared values of I_{to} amplitude obtained for 9 different age groups ranging in age between 0 to 1 month and 56 to 75 years. As demonstrated in Figure 1A, the amplitude of human atrial I_{to} did not change significantly during the first 2 years of life, after which it doubled in amplitude between the ages of 2 and 11 years and then remained relatively constant until at least 75 years of age.

Affinity of Adult and Pediatric I_{to} for Quinidine

To define the age-dependent change in quinidine's effect on human I_{to}, cells from the 2 age groups were exposed to 5 different concentrations of quinidine (1, 3, 10, 100, and 1000 μmol/L), and dose-response curves for quinidine's effect on the peak I_{to} were constructed (Figure 1B). Because of the spontaneous time-induced shift in I_{to} voltage dependence of inactivation, individual cells were exposed to a single concentration of quinidine. For the same reason, peak currents before and at the time of exposure to quinidine were measured during a depolarizing step to 60 mV from a holding potential of −80 mV. Mean values obtained at different quinidine concentrations were fit with the equation $1/(1+(IC_{50}/[D])^N)$, where [D] is the concentration of quinidine, IC_{50} is the quinidine concentration producing a 50% inhibition of I_{to}, and N is the Hill coefficient. The least squares best-fit parameters for pediatric inhibition of peak I_{to} were IC_{50}=112.2 μmol/L and N=0.82. For adult cells, the values were IC_{50}=21.8 μmol/L and N=0.67. Comparison of the IC_{50} values obtained for the 2 age groups using a *t* test (GraphPad Prism) indicated that pediatric I_{to} was significantly less sensitive to quinidine than were adult cells ($P<0.0001$).

In addition to decreasing I_{to} density, quinidine also accelerated the rate of I_{to} decay in both adult and pediatric cells (Figure 1B), a behavior suggesting that channel block develops after channel opening.[16] In both the absence and presence of quinidine, the decaying phase of I_{to} was well fit by a single exponential function for the 2 age groups. The effect of quinidine on current decay kinetics was concentration-dependent but age-independent. At 60 mV, the τ_{rec} of I_{to} decay in adult cells was decreased from 77.7±5 ms (n=12 to 14) under control conditions to 41.3±1.4 ms (n=5 to 7), 22.4±3.1 ms (n=12 to 14), and 14.6±2 ms (n=5 to 8) in the presence of 1, 3, and 10 μmol/L quinidine, respectively. Quinidine produced a similar reduction in the τ_{rec} of I_{to} decay in pediatric cells. Under control conditions, the τ_{rec} for decay at 60 mV was 64.5±5.4 ms (n=15 to 18), which decreased during exposure to 1, 3, and 10 μmol/L quinidine to 39.6±5.2 ms (n=5), 18.5±1.4 ms (n=5 to 7), and 20.1±2.4 ms (n=5), respectively.

A drug-induced reduction in peak current amplitude could result from a drug-induced shift in voltage dependence of inactivation, as well as open channel block. Equilibration in the presence of 10 μmol/L quinidine for 8 to 10 minutes resulted in a hyperpolarizing shift in the midpoint for I_{to} inactivation by 7.9±1.9 mV in pediatric cells (n=6) and 9±1.9 mV in adult cells (n=11). However, the amplitude of the shift in the midpoint was not significantly different from the amplitude of the spontaneous shift observed for cells exposed to quinidine-free solutions for the same time interval (at 1 to 5 minutes, V_{mid}=−22.9±1.3 mV; at 20 to 25 minutes, V_{mid}=−32.7±1.7 mV). This suggests that quinidine itself does not produce a significant shift in the voltage dependence of inactivation and that the change in the midpoint of the inactivation curve measured during quinidine perfusion is due to perfusion (time) alone. The slope of adult and pediatric voltage dependence of I_{to} inactivation was not affected by quinidine either (adult: n=8, control k=−5.2±0.2 mV, 10 μmol/L quinidine k=−6.1±0.3 mV; pediatric: n=6, control k=−6.3±0.6, 10 μmol/L quinidine k=−7.8±1.5 mV).

Voltage Dependence of I_{to} Recovery From Quinidine Block

Recovery from quinidine block was defined using a paired-pulse protocol (Figure 2A). Channel block was initially produced using an 800-ms–long pulse, and the time course for recovery from block then was defined using a second pulse evoked after a variable recovery time, ranging from 3 to 8000 ms. Under drug-free conditions, the recovery of channels from inactivation at −40 mV could be well described by a single exponential equation having a τ_{rec} significantly smaller in adult cells (292±27 ms) compared with pediatric cells (450±49 ms; Figure 2A through 2C). This difference in τ_{rec} decreased progressively as the recovery potential was made more hyperpolarized, with τ_{rec} becoming nearly identical at −80 mV (adult, 62±5 ms; pediatric, 71±7 ms; Figure 2C). In the presence of quinidine, the recovery of I_{to} from block was a double-exponential process (Figure 2A and 2B). The fast τ_{rec} was similar to the recovery τ_{rec} observed during control conditions and was assumed to reflect the recovery of drug-free channels from inactivation. The τ_{rec} for the slow phase of I_{to} recovery observed in quinidine (τ_{quin}) was assumed to reflect the time course of quinidine unbinding from channels. Pediatric I_{to} was found to recover significantly slower from quinidine block at −40 mV (τ_{quin}=2938±454 ms) compared with adult channels (τ_{quin}=1628±187 ms; $P<0.007$; Figure 2D). We also observed a marked developmental difference in the voltage dependence of the τ_{rec} for quinidine unblocking. In adult cells, the unblocking τ_{rec} increased significantly with hyperpolarization from −40 to −80 mV, whereas in pediatric cells, the unblocking τ_{rec} underwent an apparent, although not statistically significant decrease with hyperpolarization (Figure 2D). The marked difference in voltage sensitivity suggests that the mechanisms for quinidine unblocking are different in pediatric versus adult myocytes. The voltage dependence for quinidine unblocking (Figure 2D) in both age groups was also signifi-

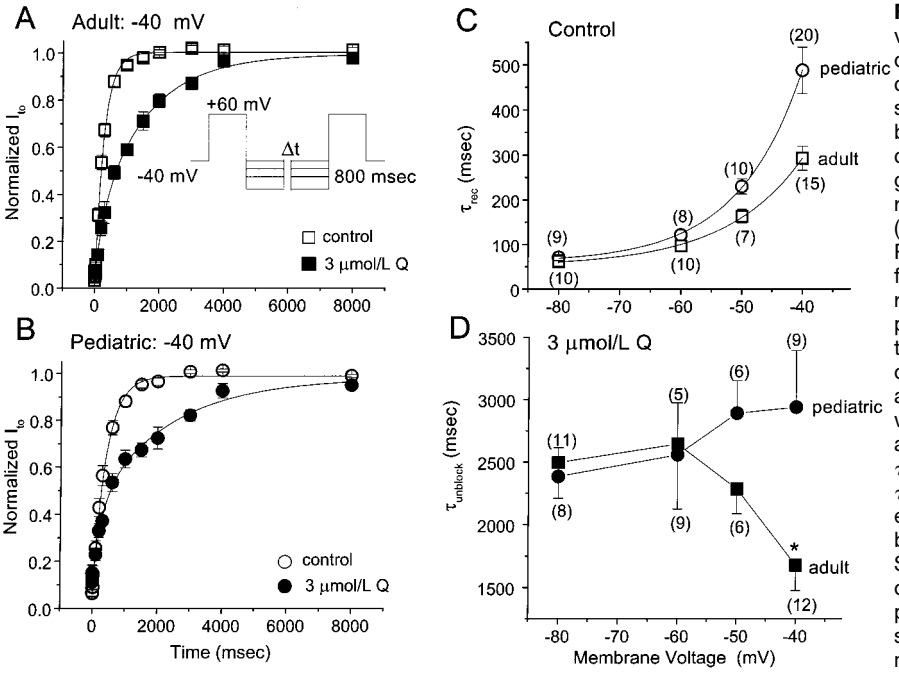

Figure 2. Comparison of the kinetics and voltage dependence of I_{to} recovery from quinidine block in pediatric and adult myocytes. As illustrated in the inset of (A), steady-state level of block was produced by an 800-ms–long pulse to 60 mV. The conditioning pulse was followed by a single test pulse to 60 mV after a variable recovery time (Δt) at 4 different potentials (−40, −50, −60, and −80 mV). A and B, Recovery of adult (A) and pediatric (B) I_{to} from block at V_h=−40 mV (n=7 and 12, respectively). Currents during the test pulse have been normalized to the amplitude of I_{to} after a long rest. In the absence of quinidine, recovery of pediatric and adult I_{to} was a monoexponential process, with pediatric I_{to} recovering almost twice as slow as the adult (pediatric τ_{rec}=449.9±48.5 ms; adult τ_{rec}=291.8±26.6 ms; P<0.05). In the presence of 3 μmol/L quinidine, recovery from block was a double-exponential process. Significant age-dependent difference was observed in the τ_{quin} at this potential, with pediatric I_{to} recovering from block more slowly than adult (adult: τ_{fast}=318.2±60.9 ms, τ_{quin}=1627.7±186.5 ms; pediatric: τ_{fast}=274.4±69.3 ms, τ_{quin}=2937.9±454.5 ms; P<0.05). C, Plot of τ_{rec} of recovery from inactivation of pediatric and adult I_{to} under control conditions. Data were fit using a monoexponential function. τ_{rec} changed e-fold for 10.6 mV change in membrane potential for pediatric I_{to} and e-fold for 12.7 mV for the adult current. D, Significant developmental difference was observed in the voltage dependence of I_{to} recovery from block. Recovery from quinidine-induced block of I_{to} got significantly faster when adult cells were depolarized (P<0.05). However, pediatric I_{to} showed flat voltage dependence. *P<0.05.

cantly different from that observed for recovery from channel inactivation under control conditions. In the absence of quinidine, the recovery τ_{rec} changed e-fold (≈2.7) for a 10.7-mV change in the membrane potential for pediatric cells and e-fold for every 12.6 mV for adult I_{to} (Figure 2C). In contrast, τ_{quin} changed by <20% over a 40-mV voltage range in pediatric cells and displayed a voltage dependence opposite of control τ_{rec} in adult cells. This suggests that the mechanism underlying the slow interpulse recovery of I_{to} in the presence of quinidine does not result from a simple slowing of recovery from inactivation.

Effects of Quinidine on I_{K1}, I_{sus}, and I_{ns}

Previous work by Wang et al[17] reported that quinidine significantly suppressed the sustained current left after inactivation of I_{to} with an estimated IC_{50} of 5 μmol/L. Initial experiments suggested that this sustained current (designated I_{Kur}) resulted from the expression of the gene Kv1.5.[18] However, it was found later that the sustained outward current (I_{sus}) is carried by a combination of at least 2 currents (≈70% I_{Kur} and ≈30% by a nonselective current, I_{ns}).[19] Figure 3A and 3B shows the effect of 100 μmol/L quinidine on the total steady-state current. The steady-state current measured at the end of an 800-ms pulse to 60 mV was suppressed by 57±6% in the presence of 100 μmol/L quinidine. The quinidine-sensitive (difference) current was an outwardly rectifying current (Figure 3B, inset), suggestive of a selective blockade of I_{Kur}.

To further test this hypothesis, we defined the effect of quinidine on the nonselective cation current (I_{ns}) using potassium-free (cesium-containing) pipette solutions, an experimental condition that rapidly eliminated other K-selective currents (eg, I_{K1}, I_{Kur}, and I_{to}), allowing us to define selectively quinidine's effect on I_{ns}. As demonstrated in Figure 3C, I_{to} was abolished within 3 minutes after rupturing the membrane when using a pipette filled with potassium-free solution. The current left represented the nonselective cation current.[19] As shown in Figure 3C and 3D, 100 μmol/L quinidine had no definable effect on I_{ns}. This supports the conclusion that quinidine's inhibitory effect on the total steady-state current (Figure 3B) is exclusively due to quinidine-induced block of I_{Kur}, whose density is known not to be age-independent.[12] Dose-response curves defining the inhibitory effect of quinidine on the total sustained outward current (I_{sus}=I_{Kur}+I_{ns}) in pediatric and adult myocytes also were defined from measurements of quinidine's effect on the sustained current at the end of an 800-ms pulse during a step to 60 mV. The IC_{50} values obtained after fitting the data to the Hill equation were not significantly different between the 2 age groups (adult IC_{50}=6.6 μmol/L; pediatric IC_{50}=5.1 μmol/L; P>0.76). Similarly, the maximal level of inhibition of the steady-state current by quinidine was ≈70% for both age groups, which is consistent with previous observations that ≈70% of the total sustained outward current is carried by I_{Kur} and that the amplitude of I_{ns} is age-independent.[19]

Previous work by Wu et al[7] in rabbit myocytes has shown that age-related changes in the sensitivity of I_{K1} to quinidine may also occur. Although no developmental change in the characteristics of I_{K1} has been reported for human cardiac tissue,[12] it was difficult to rule out the possibility that postnatal changes in I_{K1} sensitivity to quinidine sensitivity may not also exist in human tissue. To define the effects of

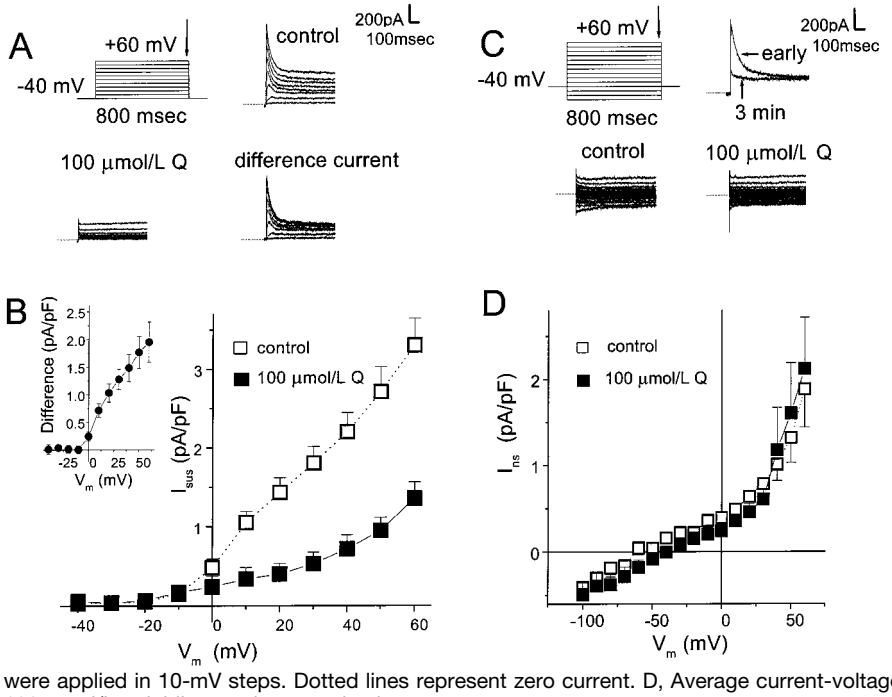

Figure 3. Effect of quinidine on sustained and nonselective currents. Diagram of the voltage protocol is shown in the top left corner of (A). Currents were produced by 800-ms–long pulses ranging between −40 and 60 mV from a holding potential of −40 mV. Arrow indicates current amplitude, measured at the end of the pulse. Representative currents recorded in the absence and presence of 100 μmol/L quinidine, as well as quinidine-sensitive (difference) current are also shown in (A). B, Mean data showing the effect of quinidine on the current-voltage relationship for the sustained current (n=6 to 9). Quinidine (100 μmol/L) reduced the current by 57±6% at 60 mV. The drug-sensitive current is shown in the inset (B). C and D, Effect of 100 μmol/L quinidine on the nonselective cation current. C, In the presence of cesium-containing internal solution, the transient outward current was abolished 3 minutes after rupturing the cell membrane (top right corner). Currents before and after quinidine exposure were elicited by the voltage protocol shown in the top left corner of (C). Cells were held at −40, and 800-ms pulses between −100 and 60 mV were applied in 10-mV steps. Dotted lines represent zero current. D, Average current-voltage relationship showing the lack of effect of 100 μmol/L quinidine on the nonselective current.

quinidine on I_{K1} in human atrial myocytes, currents were evoked by pulses ranging between −100 and −20 mV from a holding potential of −40 mV. Current amplitudes were measured at the end of an 800-ms pulse. Because the nonselective current exhibits a linear current-voltage relationship over this voltage range, a p/4 post hoc leak subtraction method was used to isolate I_{K1} from other currents (eg, I_{ns}).[19] Figure 4A shows representative current traces of I_{K1} before and after exposure to 100 μmol/L quinidine, as well as the drug-sensitive current. The current-voltage relationship for I_{K1} before and after exposure to quinidine, and the quinidine-sensitive current are shown in Figure 4B.

Exposure to 100 μmol/L quinidine inhibited I_{K1} by 47±4%. The dose-response curve for quinidine inhibition of I_{K1} in pediatric and adult cells is shown in Figure 4C. As indicated by the low N value for current blockade (0.27 for pediatric cells, 0.25 for adult cells), I_{K1} displayed a shallow concentration dependence for blockade by quinidine. Although the IC_{50} for quinidine blockade of I_{K1} was apparently lower for pediatric cells (IC_{50}=4.1 μmol/L) compared with adult cells (IC_{50}=42.5 μmol/L), these values were not significantly different, based on comparison of the best fit parameters using an unpaired *t* test ($P>0.16$).

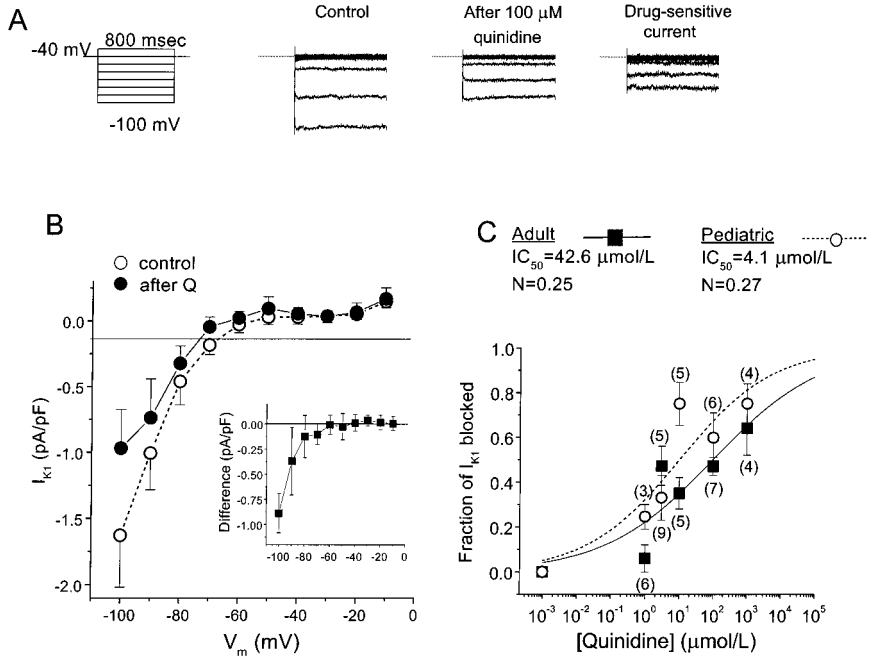

Figure 4. Effect of quinidine on I_{K1}. A, Currents were elicited by 800-ms–long pulses applied to voltages between −100 and −20 mV from a holding potential of −40 mV (top left). Examples of currents before and after exposure to 100 μmol/L quinidine, as well as digitally subtracted drug-sensitive currents are shown. B, Averaged current-voltage relationship for the currents under control conditions and in the presence of 100 μmol/L quinidine (n=6 to 9). Quinidine inhibited the current at −100 mV by 47±4%. The inset shows the mean current-voltage relationship for the quinidine-sensitive (difference) current. C, Dose-response curves for quinidine block of adult and pediatric I_{K1} measured at −100 mV. A linear leak subtraction that incorporated a p/4 pulse protocol was used. Current amplitudes were measured at the end of the 800-ms–long pulses. Measurements were made from cells superfused with a single dose of quinidine. The smooth curves indicate the best fit of the dose response curve to the data (solid curve indicates adult; dashed curve, pediatric). The IC_{50} values for quinidine block of I_{K1} were not significantly different between the 2 age groups (adult IC_{50}=42.6 μmol/L; pediatric IC_{50}=4.1 μmol/L; $P>0.2$).

Discussion

Quinidine's Interaction With Human I_{to} Is Age-Dependent

We investigated the effects of age on the interactions of quinidine with potassium channels expressed in human atrial myocytes obtained from pediatric and adult patients. Our results indicate that there are significant developmental changes in both quinidine's affinity for human atrial I_{to}, as well as its mechanism of unblocking. Pediatric I_{to} had a ≈5-fold higher IC_{50} for inhibition by quinidine compared with adult cells ($P<0.0001$; Figure 1B). In addition, adult and pediatric cells displayed a difference in their voltage dependence of recovery from quinidine blockade, with the voltage dependence of recovery from quinidine block being slowed with hyperpolarization in adult cells and relatively independent of voltage in pediatric cells (Figure 2D). The voltage dependence of quinidine unblocking in adult cells seems most consistent with an activation trapping mechanism.[20,21] According to this mechanism, the charged form of quinidine becomes trapped within the channel lumen by a closed activation gate, and recovery from block then is determined by the rate of spontaneous channel opening, which becomes progressively smaller with increasing hyperpolarization. The lack of significant voltage dependence of quinidine unblocking in pediatric cells (Figure 2D) suggests that either quinidine is not trapped by the activation gate or it is trapped equally at all voltages between −40 and −80 mV in pediatric cells. Additional experiments will be needed to distinguish between these possibilities. Possible explanations for the age-dependent difference in inactivation recovery kinetics and quinidine unblocking kinetics (Figure 2C and 2D) include age-related changes in the mixture of multimeric proteins that constitute a functional channel (eg, due to subunit switching during development, as has been reported to occur between Kv1.4 and Kv4.2/4.3 in developing rat ventricular myocytes),[22] changes in post-translational modification of the channel, or changes in the expression of an accessory subunit (eg, β) that modulates channel function. Further experiments will be required to distinguish between these possible mechanisms.

Effects of Quinidine on I_{Kur}, I_{ns}, and I_{K1}

The lack of effect of quinidine on the nonselective component (I_{ns}) of the sustained current (I_{sus}) (Figure 3D) indicates that quinidine's effect on the total steady-state current in human atrial myocytes is a result of selective inhibition of I_{Kur}. Quinidine's effect on I_{Kur} was found to be age-independent with an IC_{50} of 5 to 7 μmol/L ($P>0.76$) in pediatric and adult atrial myocytes. Our study also showed that quinidine inhibited the human atrial I_{K1} at therapeutically relevant concentrations. Although the estimated IC_{50} values for quinidine inhibition of pediatric and adult I_{K1} differed by a factor of 10 ($IC_{50}=4.1$ μmol/L in pediatric versus $IC_{50}=42.6$ μmol/L in adult), the concentration dependence of quinidine blockade was extremely shallow ($N=0.25$ and 0.27; Figure 4C), and the 95% confidence intervals for the estimated IC_{50} values for the 2 age groups overlapped (0.07 to 25 700 μmol/L in adult versus 0.0003 to 65 850 μmol/L for pediatric cells). Consequently, the estimated IC_{50} values for quinidine block of I_{K1} in pediatric and adult cells were not significantly different.

Comparison of Results With Previous Studies

Our data on the effects of age on I_{to} sensitivity to quinidine are opposite of those reported by the study on quinidine's effects on rabbit cardiac I_{to},[7] suggesting the existence of species variations in the effects of antiarrhythmic drugs on I_{to}. Interestingly, the postnatal changes in gating behavior of rabbit I_{to} are also opposite to those observed in human atrial I_{to},[12,23] indicating that significant species and/or tissue differences in the pattern of postnatal changes in ionic currents also exist.

There are conflicting reports on the effect of quinidine on the inwardly rectifying potassium current (I_{K1}) in different animal species, including results suggesting both inhibition[7,24] or lack of effect of quinidine on I_{K1}.[25–27] Quinidine's effect on I_{K1} also has been reported to be age-dependent in rabbits, with the neonatal current being more sensitive to inhibition by the quinidine when compared with the adult current.[7] However, rabbit I_{K1} has been shown to undergo significant developmental changes,[28,29] whereas no such developmental changes have been demonstrated for human I_{K1}.[12]

These results indicate that species-related differences in channel expression and the pattern of postnatal changes exist for both I_{to} and I_{K1} in mammalian cardiac tissue. In support of this hypothesis, Dixon et al[30] have suggested that the Kv4.3 channel may underlie the bulk of the I_{to} in canine and human cardiac myocytes, whereas both Kv4.2 and Kv4.3 are likely to contribute to I_{to} in rat. A recent study also indicates that significant regional diversity of K channel expression also may exist between cells present in different areas of the heart (eg, atrial versus ventricular).[31] For this reason, extrapolation of data on the pharmacology of ion channels from one species to another, or from one region of the heart to another, should be done with caution.

A study comparing human atrial action potentials from pediatric and adult patients was the first to suggest the existence of developmental difference in the contribution of I_{to} to action potential repolarization.[21] Later work[12,32] demonstrated age-related differences in both the density and inactivation recovery kinetics of human atria I_{to}, a finding confirmed by this study, as well. In contrast to the results from these 2 studies, work by Gross et al[33] suggested that the amplitude and I_{to} inactivation recovery kinetics do not change as a function of age. The reason for the differences in the findings of these groups is unclear. Although in the study by Mansourati and Le Grand,[32] I_{to} was recorded from cells obtained only from dilated atria, the specimens used in the study by Crumb et al,[12] as well as the tissue used in this study, came only from nondilated atria (see Materials and Methods). The lack of I_{to} in some pediatric cells, as well as its smaller magnitude, also could contribute to the observed age-related difference in the antiarrhythmic properties of quinidine in humans.

Clinical Relevance

Clinical studies have suggested that there are age-related differences in the effectiveness of quinidine when used for the treatment of atrial arrhythmias, with quinidine being less effective in the pediatric population compared with the adult population.[10,11] In theory, age-dependent differences in the pharmacokinetics of quinidine, as well as differences in the pharmacodynamic interaction of quinidine with heart tissue, could contribute to the clinically observed developmental differences in the antiarrhythmic effectiveness of quinidine. Previous studies have shown that quinidine is cleared by both hepatic and renal mechanisms. Approximately 60% to 85% of quinidine administered is cleared by hepatic metabolism, with hepatic clearance in pediatric patients being 2 to 3 times as rapid as the clearance in adults.[1] Renal clearance involves both glomerular filtration and active tubular secretion and accounts for 15% to 40% of total clearance.[1] When renal clearance is taken into account, the total quinidine clearance is independent of age. The plasma concentration of quinidine has been found to be significantly affected by the dose administered and by the time at which the plasma sample is obtained, but not by the age of the patients.[34] This information suggests that the difference in the antiarrhythmic effectiveness of quinidine in pediatric and adult population cannot be accounted for by an age dependence of the disposition kinetics of quinidine.

To our knowledge, this work is the first to investigate the effect of postnatal development on quinidine's interactions with human cardiac potassium channels. Potassium currents play an important role in regulating the shape of the cardiac action potential and the duration of the electrical refractory period. Our results indicate that quinidine produces significant age-related differences in its ability to inhibit the transient outward current. Developmental changes in quinidine's affinity for the transient outward current, as well as a developmental change in I_{to} density, may contribute to the reported difference in quinidine's antiarrhythmic effectiveness in pediatric and adult patients by making pediatric tissue less sensitive to quinidine-induced changes in repolarization and refractoriness.

Quinidine was observed to inhibit the sustained outward current (I_{Kur}) at relatively low concentrations (IC_{50}=5 to 7 μmol/L) and in an age-independent manner, suggesting that inhibition of this current also may contribute to its antiarrhythmic activity in both age groups. Quinidine was also observed to reduce the amplitude of I_{K1} by ≈20% to 30% at therapeutic concentrations, an effect that could contribute to drug effects on diastolic excitability. These results help to clarify the electrophysiological mechanisms by which quinidine elicits its antiarrhythmic effect in pediatric and adult patients.

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