Ketanserin Inhibits Depolarization-Activated Outward Potassium Current in Rat Ventricular Myocytes

Zhi-Hao Zhang, Mohamed Boutjdir, Nabil El-Sherif

Abstract Ketanserin (KT), an antihypertensive agent, has been shown to prolong action potential duration (APD) and QT interval and to induce torsade de pointes in some patients. We previously suggested that the prolongation of APD could be due to KT inhibition of the fast component of the delayed rectifier current (I_{kr}) in guinea-pig myocytes. However, in other tissue such as human atrium, Purkinje fibers, epicardial cells, and rat ventricular myocytes, the transient outward potassium current (I_{to}) is one of the major repolarizing currents. We investigated the possibility that KT could also increase APD by blocking I_{to}. Action potentials and membrane currents were recorded from rat ventricular myocytes known to have a large I_{to} by using whole-cell patch-clamp techniques. We found that KT (50 μmol/L) significantly prolonged APD at 50% repolarization by 218% (P<.05) and APD at 90% repolarization by 256% (P<.05) with no significant effect on other action potential parameters. Time-dependent I_{to} and sustained current (I_{to,s}) were measured in the presence of 400 nmol/L nisoldipine during depolarizing pulses to 40 mV from a holding potential of −100 mV every 10 seconds. KT resulted in a concentration- and time-dependent inhibition of charge area of I_{to} evaluated by integration with an EC_{50} of 8.3 μmol/L. The inhibitory effect of KT (10 μmol/L) was seen at voltages from 0 to 80 mV without any shift of the current-voltage relation in peak I_{to}. KT did not significantly change activation, inactivation, and reactivating curves of I_{to}. Kinetic analysis of I_{to} showed a biexponential fit of inactivation in 80.5% of total tracings studied at voltages between −30 and 80 mV (n=149, R=.99±.01). The inhibitory effect of KT was more prominent on charge areas of the slow component (Q_{s}) than the fast component (Q_{f}) of I_{to}. KT exerted an open-channel block of I_{to}, and an apparent acceleration of the I_{to} inactivation as a result of its potent block of Q_{s}, and KT prolongation of APD is not only mediated exclusively through I_{to}, block but also through inhibition of I_{to} and I_{to,s}, especially in cells where these currents are prominent. KT block of potassium outward currents in the heart could explain the QT prolongation reported in patients treated with KT. (Circ Res. 1994;75:711-721.)

Key Words • K+ current • myocytes • patch clamp • class III antiarrhythmic agent

Ketanserin (KT), both serotonin and weak α1-receptor antagonist, has been evaluated for the treatment of hypertension and peripheral vascular disease.1,2 In the heart, KT has been found to markedly prolong the action potential duration (APD) in rabbit ventricular myocardium,3 canine ventricular muscle, and Purkinje fibers.4 In addition, prolongation of the QT interval has been reported in KT-treated patients.5-7 The antiarrhythmic action of KT in ischemic/reperfused rat heart has been ascribed to a prolongation of APD.8

Recently, we reported that KT prolongs APD by exerting an open channel block of the fast component of the delayed rectifier potassium current (I_{kr}) in guinea pig ventricular myocytes, with little or no effect on the inward rectifier potassium current (I_{k1}).10 It has been shown that transient potassium outward current (I_{to}) is the major outward repolarizing current in the cardiac action potential of several mammalian tissues, including human atrium10,11 and rabbit, dog, and rat ventricular myocytes.12-16 In these preparations, it is possible that KT could alter APD by interacting with I_{to}. To test this hypothesis, we used rat ventricular myocytes known to have a large I_{to} to study the electrophysiological and pharmacological effect of KT on I_{to} recorded by whole-cell patch-clamp techniques.

Materials and Methods

Isolation of Cardiac Myocytes

Cardiac myocytes were obtained from hearts of Wistar rats (200 to 250 g) by enzymatic dissociation according to the method of Wittenberg et al.17 with some modifications.18 The heart was perfused with a HEPES-buffered solution containing (mmol/L) NaCl 117, KCl 5.7, NaHCO3 4.4, KH2PO4 1.5, MgCl2 1.7, HEPES 20, glucose 11, creatine 10, and taurine 20, along with 21 mU/mL insulin, and the pH was adjusted to 7.4 with NaOH. The heart was then perfused with fresh buffer mixed with 1.5 mg/mL collagenase type A or B (Boehringer Mannheim Corp) and 20 μmol/L calcium for 50 to 60 minutes. The ventricles were then cut off and stirred to obtain cells. Cells were suspended in Petri dishes containing HEPES buffer with 1 mmol/L CaCl2 and 0.5% bovine serum albumin (pH 7.4). All solutions used for perfusion were gassed with 100% O2 and warmed to 37°C. After incubation for 30 minutes, a small aliquot of the medium containing single cells was trans-
Compositions of Solutions

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AP indicates action potential; CP, creatine phosphate.

ferred to a 500-μL chamber mounted on the stage of an inverted microscope (Nikon Inc). Rod-shaped noncontracting cells with clear striations were used for the whole-cell voltage-clamp studies. All experiments were carried out at room temperature (20°C to 22°C).

**Solutions and Drugs**

The compositions of external and internal solutions for recording action potential, potassium, and calcium currents are given in the Table. For potassium current recordings, NaCl was replaced by choline chloride in the external solution to eliminate the fast inward sodium current (Iₙa). Since only L-type calcium current (Iₙa) was found (authors’ unpublished data) and reported in adult rat ventricular cells by others, the calcium channel blocker nisoldipine (400 nmol/L) was added to the external solution. For Iₙa recording, 50 μmol/L tetrodotoxin and 5 mmol/L 4-aminopyridine were added to the external solution to block Iₙa and Iₙa, respectively, as previously described. The pH values of external and internal solutions were adjusted to 7.4 and 7.1, respectively. KT was obtained from Janssen Research Foundation and dissolved in distilled water as a stock solution of 10 mmol/L. All drugs are from Sigma Chemical Co unless otherwise indicated.

**Voltage-Clamp Recording**

Whole-cell current- and voltage-clamp recording techniques were used to record action potentials and membrane ionic currents, respectively. A programmable horizontal puller (model P-87, Sutter Instrument Co) was used to pull the electrodes. The resistance of the capillary glass electrodes (Drummond Scientific Co) used was 1 to 3 MΩ when filled with internal solution (electrode tip, 1 to 2 μm). The volume of the recording chamber was 0.5 mL, and the rate of superfusion was 3 mL/min. The junction potential was always compensated and was <5 mV. Membrane currents were recorded with a Dagan model 3900A patch-clamp amplifier (Dagan Corp). Capacitive currents were elicited by 10-mV depolarizing pulse from −80 mV and then compensated. Later on, the capacitive tracings were fitted by a single exponential equation, and membrane capacitance (Cₘ) was calculated according to the Δequation Cₘ=τ₋1ΔEm, where τ is the time constant for cell membrane charge. Iₙa is maximum capacitative current, and ΔEm is clamp voltage. The average Cₘ was 145.3±10.3 pF (n=28). Under current-clamp conditions, the action potential was elicited by passing a current pulse through the recording patch electrode (5- to 10-millisecond duration, two times threshold). Under voltage-clamp conditions, the cells were stimulated at 0.1 Hz to ensure that Iₙa and Iₙa were recovered completely between two voltage steps. Action potentials were also recorded at 0.1 Hz under the current-clamp mode. Membrane currents were generated by using appropriate protocols as indicated in “Results.”

Because extracellular choline chloride (sodium ion substitute) has been found to shift the voltage-dependent inactivation curve of Iₙa to the left (negative potentials), a holding potential of −100 mV was used to record the fully activated Iₙa. To ensure that nisoldipine block of Iₙa at a holding potential of −100 mV was complete, we first recorded current tracings (Iₙa) in control external solution usually used to record Iₙa in the absence of nisoldipine by an 800-millisecond depolarizing pulse from a holding potential of −40 mV to a test pulse of 0 mV, where Iₙa is maximally activated. The current tracing obtained by this protocol corresponded to Iₙa and is shown in Fig 1A, tracing 1. Second, we recorded a mixture current of Iₙa and Iₙa by changing only the holding potential from −40 to −100 mV. Nisoldipine (400 nmol/L) was then added to the superfusion solution, and the current tracings were recorded until a steady-state effect was achieved. Control current recorded at a holding potential of −100 mV was subtracted from the current obtained in the presence of
Fig. 1. Nisoldipine block of calcium current (I_Ca). I_Ca was recorded in control external solution (same as for transient outward potassium current [I_K] recording, see "Materials and Methods") after 5 minutes of membrane rupture with an 800-millisecond depolarizing pulse from -40 to 0 mV. The current tracing obtained is shown in panel A, tracing 1. A mixture current was also recorded in control solution and in the presence of nisoldipine (400 nmol/L) by using the above-mentioned voltage protocol except that the holding potential was changed to -100 mV. The nisoldipine-sensitive current (nisoldipine-Sens) was obtained by subtracting the two currents. The nisoldipine-sensitive current is shown in panel A, tracing 2. Panel B illustrates I_K, recorded by using a depolarizing pulse from a holding potential of -100 mV to a test pulse of 40 mV in the presence of nisoldipine (400 nmol/L) and cobalt (5 mmol/L).

nisoldipine. The nisoldipine-sensitive current is shown in Fig 1A, tracing 2. Comparison of the nisoldipine-sensitive current with I_K showed that both currents were almost identical. Similar results have been found in four other experiments. To further investigate this issue, we studied a commonly used inorganic calcium channel blocker, cobalt (5 mmol/L), to record I_K at a holding potential of -100 mV and compared it with I_K, in the presence of nisoldipine (400 nmol/L, n = 5). The results are illustrated in Fig 1B and show that I_K recorded with nisoldipine and cobalt are identical, indicating a similar degree of I_K inhibition. Therefore, we concluded that under our experimental conditions nisoldipine (400 nmol/L) completely blocks I_Ca.

The peak of time-dependent current during 400- or 800-millisecond depolarization was measured as the amplitude of I_K. In a few experiments, the peak of I_K overlapped with the capacitive current (noncompensated) in baseline or drug-treated cells; thus, the current at 8- to 10-millisecond depolarization was measured as the peak I_K. A sustained time-independent steady-state current (I_ss) followed the initial I_K in all experiments. This I_ss has been reported to be distinct from I_K and to be tetraethylammonium (TEA) sensitive in rat ventricular myocytes and was therefore labeled I_s by Apkon and Nerbonne.25 However, in rabbit atrial cells, I_ss was TEA and barium insensitive but sensitive to chloride channel blockers and was thus labeled as chloride current.26 Because of the complex ionic nature of I_K, we report here on the effect of KT on the time-dependent component of the outward current I_K and only on the quantitative effect of KT on the I_K. The characterization of KT effect on I_K was achieved by subtracting the current that remained at the end of the depolarizing pulse from the zero current. The subtraction procedure does not completely exclude the possibility that a residual current from both I_K and I_ss may overlap.

I_K was recorded at hyperpolarized voltages from -40 to -140 mV. The absolute value at the end of hyperpolarization was measured as the amplitude of I_K. I_Ca was also recorded in some experiments. Details of I_Ca recording are described elsewhere.26 Briefly, I_Ca was recorded by depolarization to 0 mV from a holding potential of -80 mV every 10 seconds, and the amplitude of I_Ca was measured between peak I_Ca and current at the end of the 200-millisecond pulse.

### Data Analysis

Membrane currents were digitally recorded and analyzed by using pCLAMP software (version 5.5.1, AXON Instruments Inc). CLAMP and CLAMPFIT were used to measure amplitude and inactivation time constants of ionic currents, respectively. One- and two-exponential equations were used to fit I_to inactivation. SIGMAPLOT fitting programs (nonlinear curve fitting) were used to fit activation and inactivation of I_K by the Boltzmann equation: 1/[1 + exp((V - V_1/2)/k)], where V is the conditioning voltage, V_1/2 is the voltage at which half-maximal effect is obtained, and k is the slope. Dose-response curves were fit by the Hill equation: B_max/[1 + (EC_50/D^n)], where B_max is the maximum block of charge area of I_K, EC_50 is the concentration of KT for half-maximum block, D is the concentration of KT, and n is the Hill coefficient. The charge, Q, was calculated as the integral under the time-dependent inactivated membrane current (see Fig 9). Computer-simulated current tracings were generated by Equations 2 and 3 shown in "Discussion." Data are presented as mean±SEM. Student's t test for paired data was used to compare control conditions with drug interventions. A value of P<.05 was considered to be statistically significant.

### Results

#### Prolongation of APD by KT in Rat Ventricular Myocytes

A typical action potential recorded from a cell during superfusion with control solution and KT (50 μmol/L)–containing solution is shown in Fig 2. KT superfusion of the cell resulted in 218% and 256% prolongation of APD at 50% and 90% repolarization (APD_{50} and APD_{90}), respectively, without any significant change in resting membrane potential (from -78.0±2.0 to -77.5±3.5 mV) or action potential amplitude (from 98.0±4.0 to 97.5±4.5 mV, n = 4). Similar results were found in four other cells, and the average APD_{50} and APD_{90} values were 38.5±7.7 and 130.4±8.9 milliseconds during the control condition and 80.5±7.3 and 330.5±2.4 milliseconds after KT treatment (P<.05), respectively.

#### Reversible and Time-Dependent Inhibition of I_K by KT

Outward currents were evoked when adult rat ventricular myocytes were superfused with control external sodium-free solution by depolarizing pulses to 40 mV for 400 milliseconds from a holding potential of -100 mV every 10 seconds. Because the time-independent component, I_ss, seemed to be pharmacologically and
Fig 2. Effect of ketanserin (KT) on action potential duration (APD) in a rat ventricular myocyte. Action potentials were recorded during the control condition (Con) and after 3 minutes of 50 μmol/L KT superfusion. The cell was stimulated every 10 seconds. KT resulted in 218% prolongation for APD at 50% repolarization and 256% for APD at 90% repolarization, with no significant effect on resting membrane potential and action potential amplitude.

Fig 3. Effect of ketanserin (KT) on transient outward potassium current (Iₒ). The subtracted time-dependent outward currents shown were elicited every 10 seconds by a single 400-millisecond depolarizing pulse to 40 mV from a holding potential of −100 mV. Twenty-four superimposed tracings of Iₒ were recorded during the control condition (CON) and after 4 minutes of KT application. The three selected tracings indicated by the arrows (during CON and at 1 and 1.5 minutes after KT [50 μmol/L] superfusion) illustrate the blocking effect of KT on Iₒ amplitude and the acceleration of inactivation.

The three effects illustrated in Fig 3 comprised a rapidly activating and time-dependent inactivating component of Iₒ. The activation of Iₒ was fast and complete within 8 milliseconds at 40 mV, and the decay of Iₒ at 40 mV was rapid over the first 100 milliseconds of the pulse and slower thereafter.

External superfusion of the cell with solution containing 50 μmol/L KT resulted in both a decrease in Iₒ amplitude and an apparent acceleration of Iₒ decay. Fig 3 shows 24 superimposed tracings of Iₒ, starting with the control condition (first tracing to the right) and during application of KT (tracings 2 through 24). The effect of KT on Iₒ started within 20 seconds and reached a steady-state level at stimulation No. 22 (≈3.3 minutes of KT superfusion). KT reduced peak Iₒ from 879.2 to 700.0 pA during the first 60 seconds and to 441.7 pA at 90 seconds of superfusion.

The effects of KT on peak Iₒ were reversible on washout. Fig 4 illustrates the time course of KT block and the reversibility of Iₒ in drug-free solution. In Fig 4A, individual current tracings (a, b, and c) recorded at the times shown in panel B represent steady-state peak Iₒ during the control condition, after 50 μmol/L KT superfusion, and at washout, respectively. KT markedly reduced peak Iₒ by 77.7% (from 879.2 to 195.8 pA) and accelerated the Iₒ inactivation. Almost 79.6% of this inhibition was reversed after KT removal (from 195.8 to 700.0 pA). However, the apparent KT acceleration of Iₒ inactivation was only partially reversed. Fig 4B illustrates the amplitude of peak Iₒ plotted against time during 2 minutes of the control condition, at 4 minutes after application of KT, and after 6 minutes of washout.

Fig 4. Reversible inhibition of transient outward potassium current (Iₒ) by ketanserin (KT). A, Tracings show a typical effect of KT (50 μmol/L) on whole-cell Iₒ. Iₒ was induced by the voltage protocol shown in the inset. The three selected tracings represent currents during the control condition (tracing a), steady-state effect of 50 μmol/L KT (tracing b), and washout (tracing c). These tracings were taken from panel B at the times indicated. B, Graph illustrates the time course of KT effect on Iₒ from a cell that was initially superfused with control external solution (CON) for 2 minutes, followed by superfusion with a solution containing KT and washout during the time indicated. KT resulted in a marked inhibition of Iₒ. This effect was reversible for Iₒ amplitude and partially reversible for Iₒ inactivation.

Concentration-Dependent Block of Iₒ Charge Area by KT

To better determine the concentration of KT necessary for half-maximal effect, the reduction in total charge movement (integral of the current throughout the depolarizing pulse; see Equation 1) instead of peak current was used, and six concentrations of KT (0.1, 1, 10, 20, 50, and 100 μmol/L) were studied. The percentage block of Iₒ was defined as (Q•/Q•) (Q•/Q•) and plotted as a function of logarithm [KT] in Fig 5. The dose-response curve was
Fig 5. Graph shows concentration-dependent block of transient outward potassium current (I_o) charge area by ketanserin (KT). The percent block of I_o charge area by KT was defined as (Q_con−Q_con)/Q_con, and plotted as a function of the logarithm of KT concentration ranging from 0.1 to 100 μmol/L. A smooth line through the points was obtained by fitting the data using the Hill equation: y=B_max/[1+(EC50/D)n], where B_max is the maximum block of charge area of I_o, EC50 is the concentration of KT for half-maximum block, D is the concentration of KT, and n is the Hill coefficient, as described in “Results.” The concentration of KT that resulted in half inhibition of I_o charge area (EC50) is 8.3 μmol/L, with a Hill coefficient of 0.8. The number next to the data points indicates the number of experiments. The voltage steps used to elicit I_o are shown in the inset.

generated in a total of 24 cells, and the number of cells for each concentration of KT is indicated between parentheses. KT reduced the charge area of I_o by 1.2±0.8% at 0.1 μmol/L (n=5), 15.7±3.0% at 1 μmol/L (n=5), 50.0±4.1% at 10 μmol/L (n=8), 62.3±7.7% at 20 μmol/L (n=5), 75.1±8.5% at 50 μmol/L (n=5), and 82.1±3.5% at 100 μmol/L (n=6), respectively. The following Hill equation was used to fit the data points: B=B_max/[1+(EC50/D)^n]. The fitting parameters were as follows: B_max=93.6%, EC50=8.3 μmol/L, and n=0.8.

Effect of KT on Current-Voltage Relations and Steady-State Activation and Inactivation of I_o

Fig 6A illustrates a typical effect of 10 μmol/L KT on I_o at three selected potentials: 0, 40, and 80 mV. Fig 6B shows current-voltage curves of I_o generated by applying 12 depolarizing pulses from a holding potential of −100 to −30 mV for 800 milliseconds with a 10-mV increment (inset). The amplitude of I_o was normalized by cell capacitance at each voltage, and the average data from six experiments were plotted as a function of the depolarizing voltages. I_o started to activate at −20 mV and continued to increase with depolarization. In the presence of KT (10 μmol/L), the amplitude of I_o was significantly (P<0.05) reduced starting at potential 0 through 80 mV. The values for percent block of I_o by KT at 0, 30, 60, and 80 mV were 49.9±11% (from 1.6±0.3 to 0.6±0.1 pA/pF, P=0.009), 53.0±5.5% (from 5.3±0.9 to 2.2±0.3 pA/pF, P<0.003), 50.2±7.9% (from 9.5±1.4 to 4.6±0.6 pA/pF, P=0.02), and 50.8±4% (from 11.4±1.7 to 5.6±0.7 pA/pF, P<0.05), respectively. Therefore, the KT block of peak I_o is not voltage dependent.

The steady-state activation and inactivation values of I_o were obtained by the double-pulse protocol described below and were fitted by the Boltzmann function as follows: I(V)=I/(1+exp(V−V_1/2)/k), where

I(V) is the normalized current as a function of voltage. For the steady-state activation, the membrane was held at −100 mV and depolarized from −30 mV with a 10-mV increment to 60 mV for 15 milliseconds and depolarized to a test potential of −40 mV for 100 milliseconds every 10 seconds. Fig 7 illustrates the normalized tail peak of I_o at −40 mV from five experiments expressed as function of conditioning voltages that varied from −30 to 60 mV. During control conditions, average V_1/2 was 6.3±1.6 mV and k was 15.2±1.5 mV. KT at 10 μmol/L did not significantly change the activation parameters; V_1/2 was 3.9±0.6 mV and k was 16.4±0.5 mV.

The steady-state inactivation was generated by holding the membrane at different potentials ranging from −120 to −10 mV for 1000 milliseconds before applying the test potential at a fixed voltage of 60 mV. Fig 7 illustrates normalized peak I_o from six experiments expressed as a function of conditioning voltages that varied from −120 to −10 mV. During control conditions, average V_1/2 was −53.3±3.4 mV and k was −14.2±1.4 mV. After the addition of 10 μmol/L KT, the inactivation curve was slightly shifted to more negative potentials; V_1/2 was −60.3±2.3 mV and k was −14.1±2.3 mV.

Effect of KT on Kinetic Properties of I_o

We analyzed the reactivation of I_o evoked by a 600-millisecond depolarizing pulse at 60 mV from a holding potential of −100 mV, followed by the same depolarizing pulse at variable resting intervals ranging from 10 to 650 milliseconds. Current tracings obtained from such a protocol are illustrated in Fig 8A in the
and R = 0.99 ± 0.01. The activation curve was generated by a 10-second series of 15-millisecond pre-pulses from a holding potential of −100 to −30 mV throughout 60 mV with 10-mV increments, followed by a repolarizing test pulse to −40 mV for 100 milliseconds. The inactivation curve was elicited by a series of 1000-millisecond pre-pulses from −120 to −20 mV, followed by a depolarizing pulse to 60 mV. The peak tail I\textsubscript{to} and the fully activated I\textsubscript{to} after the pre-pulses were normalized by their maximum tail I\textsubscript{to} and peak I\textsubscript{to} and plotted as functions of the prepulse voltage for activation and inactivation, respectively. Averaged data from five experiments for the activation and another six experiments for the inactivation were fitted by the Boltzmann equation: \( V/\text{V}_1/2 = 1/(1 + \exp[(V - \text{V}_1/2)/k]) \), where \( V \) is the conditioning voltage, \( \text{V}_1/2 \) is the voltage at which half-maximal effect is obtained, and \( k \) is the slope, as described in "Results." The \( \text{V}_1/2 \) and \( k \) for the activation were 8.3 ± 1.6 and 15.2 ± 1.5 mV for the control condition and 3.9 ± 0.5 and 18.3 ± 0.5 mV for the effects of KT. The \( \text{V}_1/2 \) and \( k \) for the inactivation were −53.3 ± 3.4 and −14.2 ± 1.4 mV for the control condition and −60.3 ± 2.2 and −14.1 ± 2.3 mV for the effects of KT. There was no significant effect of KT on either \( \text{V}_1/2 \) or \( k \) for both activation and inactivation.

Averaged data from five experiments also showed that KT did not significantly change the \( \tau_{\text{r}} \) (from 42.2 ± 5.9 to 37.5 ± 4.2 milliseconds). The time course of I\textsubscript{to} decay at voltages from −30 to 80 mV before and after the addition of KT was fitted by monoeponential and biexponential equations (fast and slow components).

We found that 80.5% of the total control tracings of I\textsubscript{to} decay were characterized by two components (\( n = 149, R = 0.99 ± 0.01 \)) and the remaining 19.5% were characterized by only one component. Among 19.5% of the tracings that were fitted with one exponential, 10 \( \mu \)mol/L KT changed 62.1% of these tracings into two exponentials. In the absence of KT, the average time constant of the fast (\( \tau_f \)) and slow (\( \tau_s \)) inactivation of I\textsubscript{to} was voltage insensitive and was equal to 50.6 ± 8.5 and 413.0 ± 22.6 milliseconds at 40 mV, respectively. KT did not significantly change \( \tau_f \) (51.2 ± 12.4 milliseconds, \( n = 5 \)) and \( \tau_s \) (353.6 ± 44.1 milliseconds, \( n = 5 \)).

Although the above results showed that KT did not significantly affect the time constants of I\textsubscript{to} inactivation, there was an obvious apparent acceleration of I\textsubscript{to} inactivation as shown in Fig 3. Since the total outward charge movement of I\textsubscript{to} is determined not only by the kinetics of \( \tau_f \) and \( \tau_s \) but also by the amplitude of the two components, we analyzed the area of I\textsubscript{to} inactivation by integration. The area under the I\textsubscript{to} inactivation versus depolarizing time represents the total charge movement. The total charge area (\( Q_{\text{Total}} \)) of both fast and slow components is defined by the following equation:

\[
Q_{\text{Total}} = \int_0^{800} A_e^f e^{-t/\tau_f} dt + \int_0^{800} A_e^s e^{-t/\tau_s} dt
\]

where \( A_f \) and \( A_s \) are the peak currents of fast and slow components, respectively, \( \tau \) is the time constant of decay (\( \tau_f \) and \( \tau_s \) are the fast and slow components), and \( t \) is the depolarization time from 0 to 800 milliseconds. Fig 9A shows the effect of 10 \( \mu \)mol/L KT on the area of reconstructed fast and slow inactivation components of I\textsubscript{to} at 40 mV. The inactivation of I\textsubscript{to} was well described by a biexponential function with \( A_1, A_2, \tau_1, \) and \( \tau_2 \) of 512 pA, 539 pA, 47 milliseconds, and 388 milliseconds, respectively, in the control condition and 278 pA, 147 pA, 50 milliseconds, and 321 milliseconds, respectively, in the presence of KT. The integration of the two components revealed that KT reduced both \( Q_f \) and \( Q_s \) from 23.8 and 204.1 to 13.8 and 43.2 ± 5 pA, respectively.
The percent block of Qf and Qs by KT was 42.9% and 78.8%. The voltage dependency of fast and slow components before and after the drug was investigated at voltages from 30 to 60 mV and is shown in Fig 9B. KT significantly (P<0.05) depressed both fast and slow component charge areas of Ito at 30, 40, 50, and 60 mV with a more potent block on the slow component. These results suggest that the apparent KT acceleration of Ito inactivation could result from its more potent reduction of Qs.

The question of whether KT exerts an open channel block of Ito, as reported for the delayed rectifier Ito in guinea pig myocytes,9 has been investigated. Fig 10A illustrates the rate of KT block on Ito during channel activation at 40 mV. Within 50 milliseconds of the channel opening, 330 points were sampled in the presence and absence of three concentrations of KT (1, 10, and 50 µmol/L). The percentage block of Ito was defined by (Ito - Ito) / Ito, and plotted as a function of depolarizing time. The points measured for KT concentrations of 1, 10, 20, and 50 µmol/L were fitted by single-exponential equation. The fitting resulted in a smooth line (Fig 10A) with a time constant of block onset (τ0) of 17.0, 5.3, and 2.3 milliseconds for 1, 10, and 50 µmol/L KT, respectively. Averaged τ0 data for KT concentrations of 1, 10, 20, and 50 µmol/L were 17.9±1.0 (n=3), 5.3±0.5 (n=7), 4.1±0.4 (n=4), and 1.94±0.2 milliseconds (n=6), respectively.

To calculate the apparent Kf and the rate of block, 1/τ0 was plotted as a function of KT concentrations (Fig 10B), and the relation was fitted by a linear equation: 1/τ0=k[D]+l, where D is the concentration of KT, and k and l are the association and dissociation rate constants, respectively. Rate constants k and l were calculated and found equal to 9.0±0.9×10^5 M^-1 s^-1 and 86.6±0.3 s^-1, respectively. The apparent Kf for KT was calculated from l/k and was equal to 9.6 µmol/L at 40 mV.

**Effect of KT on Isub**

Although the present study focused on the effects of KT on Ito, it was also noted that KT markedly inhibited Isub. Fig 11, upper panel, illustrates total (nonsub-
Fig 11. Effect of ketanserin (KT) on the sustained outward current (I_{sus}). The upper panel shows tracings of total outward currents composed of a time-dependent inactivating current (I_o) and I_{sus}, elicited every 10 seconds by a single 400-millisecond depolarizing pulse to 40 mV from a holding potential of −100 mV. Con indicates the control condition. The amplitude of I_{sus} was measured at the end of the depolarizing pulse and the zero current. The upper panel shows that KT markedly blocked I_{sus} in a time-dependent manner. The time course of KT effect on the amplitude of I_{sus} and the reversibility upon washout are shown in the lower panel.

extracted) outward currents recorded in response to a depolarizing pulse from −100-mV holding potential to 40 mV during the control condition and after the beginning of KT (50 μmol/L) superfusion. KT markedly inhibited the current measured at the end of the depolarization pulse (I_{sus}) by 83.3% (from 800 to 133.3 pA).

In the lower panel, the time course of KT inhibition of I_{sus} and the washout are shown. To determine the EC_{50} of KT effect on I_{sus}, the dose-response curves for KT concentrations of 0.1, 1, 10, 20, 50, and 100 μmol/L were studied. The percentage block of I_{sus} was defined as (I_{con−KT})/I_{con} and plotted as a function of logarithm [KT] and is shown in Fig 12. The dose-response curve was generated by the protocol shown in the inset from a total of 19 cells, and the number of cells for each concentration of KT is indicated between parentheses. KT reduced the amplitude of I_{sus} by 2.1±2.0% at 0.1 μmol/L (n=4), 5.6±2.0% at 1 μmol/L (n=3), 44.1±5.6% at 10 μmol/L (n=7), 60.0±5.0% at 20 μmol/L (n=5), 81.0±6.0% at 50 μmol/L (n=5), and 85.1±4.2% at 100 μmol/L (n=5). The Hill equation was used to fit the data points. The fitting parameters were B_{max} of 93.4% and EC_{50} of 11.2 μmol/L.

**Effect of KT on I_{K_1} and I_{Ca} in Rat Ventricular Myocytes**

Other membrane conductance such as I_{K_1} and I_{Ca} could participate in the action potential prolongation by KT. We investigated the effects of KT on I_{K_1} and I_{Ca}. Hyperpolarization to −120 mV from −40 mV produced a time-independent inward current, I_{K_1}, which was blocked by CsCl.23 The inset of Fig 13A shows I_{K_1} tracings recorded at −100 mV during 2 minutes after rupture of the membrane and 5 to 8 minutes after addition of 50 μmol/L KT. Superfusion of cells (n=4) for up to 8 minutes did not have any significant effect on

Fig 12. Graph shows concentration-dependent block of sustained current (I_{sus}) by ketanserin (KT). The percent block of I_{sus} by KT was defined as (control I_{sus}−KT I_{sus})/control I_{sus} and plotted as a function of the logarithm of KT concentration ranging from 0.1 to 100 μmol/L. A smooth line through the points was obtained by fitting the data using the Hill equation: y=B_{max}/[1+(EC_{50}/D)^{n}], where B_{max} is the maximum block of I_{sus}, EC_{50} is the concentration of KT for half-maximum block, D is the concentration of KT, and n is the Hill coefficient, as described in "Results." The concentration of KT that resulted in half inhibition of I_{sus} (EC_{50}) is 11.2 μmol/L with a Hill coefficient of 1. The number next to the data points indicates the number of experiments. The voltage step used to elicit I_{sus} is shown in the inset.

Fig 13. Effect of ketanserin (KT) on inward rectifier potassium current (I_{K_1}) and L-type calcium current (I_{Ca}). I_{K_1} was induced by 10 hyperpolarizing pulses from a holding potential of −40 mV with a 10-mV step increment. The cell was superfused with KT (50 μmol/L) for 5 minutes. A. Graph shows the effect of KT on the current (I_{K_1})-voltage (Vc) relation. Con indicates the control condition. A typical example of KT effect on I_{K_1} at a voltage of −100 mV is shown in the inset of panel A. B. Tracings show that KT did not have any significant effect on I_{Ca}.
I_{Ks} (normalized I_{Ks} values were $-4.35 \pm 0.24$ pA/pF for control values and $-4.03 \pm 0.15$ pA/pF for KT values). Similar results were found at voltages from $-40$ to $-140$ mV. The amplitude of $I_{Ks}$ recorded at the end of hyperpolarization was normalized by cell capacitance before and after the addition of KT and plotted as a function of clamp voltages in Fig 13A. KT failed to induce any significant changes at all voltages.

In Fig 13B, a depolarizing pulse to 0 mV from $-80$ mV was used to elicit $I_{Ca}$ as previously described. KT (50 $\mu$mol/L) was applied to the cells for up to 10 minutes and did not show any significant effect on $I_{Ca}$ (Fig 13B, control $I_{Ca} = -815.5 \pm 25$ and KT $I_{Ca} = -791.2 \pm 42$ pA, n=3).

**Discussion**

The results of the present study show that KT exerts an inhibitory effect on $I_{Na}$ and $I_{Nax}$ in a dose-dependent and reversible manner. The inhibitory effect on $I_{Na}$ was mediated through both a decrease in $I_{Na}$ amplitude and an apparent acceleration of $I_{Na}$ decay.

KT has been shown to prolong APD in rat heart, rabbit ventricular myocardium, and canine Purkinje and ventricular muscle. This effect was suggested to be due mainly to KT block of a delayed rectifier, which represents the major repolarizing current in those tissues. However, in most other mammalian tissues including human atrium, epicardial cells, and rat ventricle, a depolarization-activated potassium current, $I_{Nax}$, plays a major role in action potential repolarization. Although a calcium-dependent component of this outward current has been described in some preparations, only a calcium-independent component was reported in rat ventricular myocytes. The time course of this $I_{Na}$ comprises a fast inactivating and a sustained component. Although the sensitivity of the fast inactivating component to 4-aminopyridine has been commonly reported, the exact ionic nature of the sustained component has not been completely elucidated. Apkon and Nerbonne reported a substantial attenuation of the sustained component by TEA and have referred to this TEA-sensitive current as the delayed rectifier ($I_{D}$). Castle and Slawsky reported the persistence of a time-independent outward rectifying component of the sustained current even in the presence of 135 mmol/L TEA in rat ventricular myocytes. On the other hand, Duan et al. found that $I_{Nax}$ in rabbit atrial myocytes, was insensitive to TEA and barium but was reduced by the chloride transport blockers, suggesting the involvement of a nonactivating chloride current. In the present study, KT reduced both components of the outward current (Fig 11, upper panel). Our data could be interpreted to suggest that macroscopic $I_{Na}$ and $I_{Nax}$ may reflect the activation of two distinct types of voltage-gated potassium channels in adult rat ventricular myocytes. Recently, molecular cloning of rat heart cDNA, called RHK1, has been reported. The expression of RHK1 in Xenopus oocytes and subsequent voltage-clamp studies showed a rapidly activating and inactivating 4-aminopyridine-sensitive transient outward current exhibiting time- and voltage-dependent characteristics very similar to the time-dependent component $I_{Nax}$ shown in our experiments. So far, molecular studies have not yet revealed the presence of an additional potassium channel clone distinct from RHK1 that encodes for channel properties similar to the $I_{Nax}$ in rat adult ventricular myocytes. Further electrophysiological support for the hypothesis that different channel proteins underlie $I_{Na}$ and $I_{Nax}$ would be provided by the demonstration of two distinct potassium channel types in single-channel recordings. Our results, however, do not exclude the possibility that other outward potassium currents are present and participate in action potential repolarization in adult rat myocytes. Because of the complex nature of the sustained current, we are reporting only on the effect of KT on the fast-inactivating time-dependent current $I_{D}$ and only quantitatively on $I_{Nax}$.

The block of $I_{D}$ by KT was characterized by a decrease in peak current and an apparent increase in the rate of inactivation. KT induced a small hyperpolarization shift in the steady-state inactivation and showed no significant effect on the time course of recovery from inactivation. These characteristics contrast with the time-independent inhibition of $I_{Ca}$ seen with the commonly used blocker 4-aminopyridine, which has been reported to bind strongly to closed channels with the inactivation gate open.

The time-dependent block of $I_{D}$ by KT and the absence of significant change of $I_{D}$ inactivation parameters by KT could be attributed to preferential binding of KT to the open state of the channel or “open channel block.” Furthermore, the lack of instantaneous inhibition of $I_{Na}$ by KT upon depolarization argues against a possible binding of the drug to the resting state of the channel. On the other hand, the possibility that the open blocked channel may inactivate cannot be excluded and is indicated with dashed lines in the scheme below. The kinetic interaction of KT with the channel can be summarized as follows:

![Diagram]

where R is the resting state of the channel, O is the open conducting state, OD is the drug-bound open state, and I is an absorbing inactivated state.

In rat ventricular myocytes, the time course of $I_{D}$ decay has been shown to be best described by a biexponential process on one hand and by a monoeponential process on the other, depending on the experimental settings. Dukes and Morad reported that subtraction of $I_{Ca}$ from the total current, or nifedipine pretreatment, eliminated the slowly inactivating component of $I_{D}$. They attributed the slow component of $I_{D}$ inactivation to $I_{Ca}$ contamination of total current. Under our control experimental conditions, which contained 400 mmol/L nisoldipine that completely blocked $I_{Ca}$, the time course of $I_{D}$ decay was still best described by a two-exponential process in 80.5% of the cells; the remaining 19.5% showed a monoeponential decay. Thus, the discrepancy in $I_{Na}$ decay kinetics could not be explained only by differences in experimental protocols but may be also related to such factors as the duration of the fitted current and/or to the relative overlap of the sustained current with the transient current. Further-
more, Apkon and Nerbonne\(^ {25} \) and Castle and Slawsky\(^ {29} \) reported that the contribution of the transient versus sustained currents to the total outward current was variable from cell to cell. These cell-to-cell variations could also be due to the regional variable distribution and expression of ionic currents documented within the ventricular wall in several mammalian species, including rat heart.\(^ {33} \)

In the present study, although KT reduced both the fast and slow components of the integrated area of \( I_{o} \), resulting in the apparent acceleration of \( I_{o} \) inactivation, it did not significantly change the time constants of \( I_{o} \) inactivation. This could be due to the difficulties associated with the accurate measurements of the fast phase of current decay at higher concentrations. Therefore, we used the data from Fig 10 to reconstruct the current tracings shown in Fig 14. The normalized current tracings for control (Con) and KT-treated cells were simulated by the following equations:

\[
(2) \quad I_{\text{Con}} = (1 - e^{-\tau_{f}}) \cdot [(A_{f} \cdot e^{-t/\tau_{f}}) + (A_{s} \cdot e^{-t/\tau_{s}})]
\]

\[
(3) \quad I_{\text{KT}} = I_{\text{Con}} \cdot (1 - B_{\text{Max}} \cdot e^{-t/\tau_{s}})
\]

The parameters \( \tau_{f} \), \( \tau_{t} \), and \( \tau_{s} \) are the activation time constant and the fast inactivation and slow inactivation time constants of \( I_{o} \), respectively. \( A_{f} \) and \( A_{s} \) are the normalized amplitudes of the respective fast and slow components of inactivation of \( I_{o} \). \( B \) and \( \tau_{s} \) represent the maximum block and the time constant of block onset, respectively. The resulting current tracings from the computer simulation are plotted in Fig 14. The time to peak current declined with increasing KT concentration. On the other hand, the simulated tracings were best fit by two exponentials, and KT did not have any major effect on the slow component of the \( I_{o} \) inactivation. However, KT decreased \( \tau_{s} \) mostly at high concentrations (from 47 milliseconds for the control condition to 38.9, 29.3, and 21.7 milliseconds for KT concentrations of 1, 10, and 50 \( \mu \)mol/L, respectively). This discrepancy with the experimental data may have resulted from the fact that the simulation takes into account only the time-dependent block of open channels and a different degree of \( I_{\text{so}} \) contamination. Nevertheless, this model confirmed the experimental finding that KT caused an apparent acceleration of the inactivation due to an open channel block.

KT did not have any significant effect on the rate of recovery from inactivation, indicating that the rate of recovery from block upon repolarization is the same as the rate of recovery from inactivation.

Our study suggests that the KT inhibition of both \( I_{o} \) and \( I_{\text{so}} \), together with its inhibition of the fast component of the delayed rectifier, \( I_{kr} \), may account for the reported prolongation of the APD in vitro and the QTc interval in vivo by KT. Since other conductance could also be involved in the repolarization of the action potential, we have investigated the effects of KT on the inward rectifier and calcium current. Concentrations of KT up to 50 \( \mu \)mol/L had no significant effect on either \( I_{kr} \) or \( I_{ca} \). We have not attempted to characterize the existence of the background potassium current, \( I_{k} \), in rat ventricular myocytes, which is similar to the recently described \( I_{k} \) in guinea pig ventricular myocytes.\(^ {34, 35} \) Therefore, an effect of KT on such a current cannot be excluded.

KT also has a serotonin \( S_{2} \)-receptor antagonist and weak \( \alpha_{1} \)-adrenergic antagonist effects.\(^ {36} \) The present reported KT effects are unlikely to be mediated through these receptors because KT was tested in the absence of any \( S_{2} \) or \( \alpha_{1} \)-receptor agonist in isolated myocytes.

The finding that KT can inhibit \( I_{kr} \), \( I_{o} \), and \( I_{\text{so}} \) can have obvious pharmacotherapeutic implications. KT is used as an effective antihypertensive agent in patients with essential hypertension. However, a potential cardiotoxic effect associated with the marked prolongation of the QT interval and the induction of the malignant ventricular arrhythmia, torsade de pointes, especially in settings of electrolyte imbalance, cannot be excluded.

Acknowledgments

This study was supported by Veterans Administration Medical research funds. We would like to thank Dr Mark Restivo and Edward B. Caref for their continuous scientific discussions, technical advice, and constructive comments on the manuscript. We also thank Kathleen Stergiopolous for the excellent technical assistance in some experiments.

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_Circ Res._ 1994;75:711-721
doi: 10.1161/01.RES.75.4.711

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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