

Inhibition of Human *Ether-A-Go-Go* Potassium Channels by Cocaine

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ABSTRACT

Cocaine is a potent cardiac stimulant and its use has been linked to life-threatening arrhythmias in humans. A prominent effect of cocaine in the heart is a suppression of the delayed-rectifier potassium current (I_K) that is important for cardiac repolarization. In this study, cocaine was found to be an inhibitor of HERG channels that underlie the rapidly activating component of I_K . HERG was expressed in tsA201 cells and the whole-cell currents were measured using the patch-clamp technique. HERG currents are inhibited in a dose-dependent fashion with an IC_{50} value of $5.6 \pm 0.4 \mu M$. The cocaine inhibition increases over the range of voltages at which the channels activate, indicating that cocaine preferentially binds to

open or inactivated channels. At more depolarized potentials, at which the channels are maximally activated, the cocaine inhibition is constant indicating that the binding of the drug is not directly influenced by voltage. Cocaine reduces both the peak tail currents and the instantaneous currents measured by applying voltage steps under conditions where channels are open. The data are consistent with the inhibition of open channels. Cocaine also accelerates the rapid decay of the current at depolarized voltages suggestive of an interaction with inactivated channels. The data indicates that cocaine inhibits the channels by preferentially binding to a combination of open and inactivated states.

Recent reports have documented the high incidence of sudden death related to cocaine abuse; the majority of these fatalities have been attributed to cardiovascular complications (Kloner et al., 1992; Bauman et al., 1994; Billman, 1995). Cocaine produces numerous effects on the heart, including coronary vasospasms, myocardial infarction, arrhythmias, and ventricular fibrillation (Billman, 1995). These actions of cocaine can be traced to two prominent effects: an increase in sympathetic stimulation to the heart and coronary vasculature and an inhibition of cardiac ion channels. In whole-animal studies, cocaine acts as a cardiac stimulant, causing increases in heart rate and blood pressure (Billman, 1990). These effects are related to the action of cocaine on autonomic nerves, where it inhibits norepinephrine uptake into the nerve terminals and enhances the sympathetic output (Billman, 1990; Isner and Chokshi, 1991). Cocaine also acts as a local anesthetic that inhibits ion channels, resulting in severe disturbances in cardiac electrophysiology (Kloner et al., 1992; Bauman et al., 1994; Schindler, 1996). The mechanisms that underlie the cardiotoxic effects of cocaine are not well understood and are probably related to a combination of both the sympathomimetic and local anesthetic properties of this drug.

One of the hallmark effects of cocaine on cardiac electro-

physiology is an increase in the QT interval, which is consistent with a general slowing of myocardial repolarization (Billman, 1990; Beckman et al., 1991; Temeszy-Armos et al., 1992; Erzouki et al., 1993). In isolated cardiac myocytes, cocaine prolongs action potentials and inhibits the delayed rectifier current (I_K) important for repolarization (Kimura et al., 1992; Clarkson et al., 1996). The data suggest that reduction of I_K may contribute to the abnormal repolarization observed in humans after the use of cocaine.

The recently cloned human ether-a-go-go channel (HERG) displays similar rectification (Sanguinetti et al., 1995; Trudeau et al., 1995) and pharmacology (Snyders and Chaudhary, 1996; Zhou et al., 1998) as the rapidly activating component of the native I_K . Reduction of HERG current by naturally occurring mutations or class III antiarrhythmic drugs prolong the cardiac action potential and produce long QT syndromes that increase the likelihood of arrhythmia and sudden cardiac arrest (Curran et al., 1995). The effects of cocaine on the QT interval closely resemble the actions of class III antiarrhythmic drugs. The repolarization abnormalities observed in vivo after the ingestion of cocaine may result, at least in part, from the inhibition of HERG channels.

In this study, the effect of cocaine on HERG channels heterologously expressed in a mammalian cell line was investigated. The tsA201 cells used in this study do not express

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ABBREVIATIONS: HERG, human *ether-a-go-go*; I-V, current-voltage.

an endogenous HERG channel and have minimal background currents. These properties eliminate the requirement for including pharmacological agents to isolate the cocaine-sensitive component from the numerous overlapping potassium currents that are expressed in native cardiac cells. This approach has enabled a more thorough characterization of the state- and voltage-dependence of cocaine binding.

Materials and Methods

Expression in Cultured Cells. The HERG clone was provided by Dr. G. Robertson. A standard calcium phosphate precipitation procedure was used to transfect tsA201 cells (O'Leary, 1998). Within 24 h of transfection, tsA201 cells express whole-cell K^+ currents of between 0.5 and 3 nA of outward current at 0 mV and >5 nA of outward tail current at -80 mV. tsA201 cells express a relatively small amount of endogenous K^+ current (≤ 100 pA). These endogenous currents activate rapidly, display little time-dependent changes in conductance, and produce negligible tail currents. In most cases, these currents do not interfere with the accurate measurement of HERG current. Cells expressing the endogenous current displaying a rapidly activating component of current that coincides with the voltage pulse transient. Cells expressing large amounts of this rapidly activating current (>100 pA) were discarded.

Electrophysiology. Whole-cell patch recordings were made using sylgard-coated (Dow Corning Corp., Midland, MI) patch electrodes fashioned from Corning 8161 glass (Wilma Glass Company, Buena, NJ). Series resistance was less than $2\text{ M}\Omega$ and was 80% compensated. The series resistance errors were <3 mV and the expected charging time constant of the cells is $<10\text{ }\mu\text{s}$. The liquid junction potentials were not corrected. Currents were recorded using an Axopatch 200A amplifier and pCLAMP software (Axon Instruments, Foster City, CA). Holding potentials were -80 mV unless otherwise stated. Internal solution consisted of 120 mM KCl, 5 mM EGTA, and 10 mM HEPES, pH 7.4. External solution consisted of 136 mM NaCl, 2 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , and 10 mM HEPES, pH 7.4. The solution surrounding the cells was exchanged using a large-bore perfusion pipette positioned immediately adjacent to the cell. The bath temperature was maintained at 20°C using a Medical Systems TC-202 temperature controller. For the action potential clamp experiments, the bath temperature was increased to 35°C . Data are reported as the mean \pm S.E. and plotted using SigmaPlot (Jandel Scientific, Chicago, IL).

Results

Properties of HERG Channels Expressed in tsA201 Cells. Figure 1 shows whole-cell K^+ currents measured from tsA201 cells transiently transfected with HERG. Long depolarizing pulses (25 s) to voltages between -60 and $+40$ mV were used to activate the channels and the tail currents measured upon repolarization to -80 mV. The small currents observed at depolarized voltages and the large tail currents are typical of HERG (Sanguinetti et al., 1995; Trudeau et al., 1995). The current-voltage relationship measured near the end of the depolarizing voltage pulses increases for voltage steps more depolarized than -50 mV, reaching a maximum at approximately -10 mV (Fig. 1B, \bullet). For voltage steps >-10 mV, the amplitude of the currents paradoxically decrease because of rapid voltage-dependent inactivation, which produces the characteristic inward rectification of these channels (Sanguinetti et al., 1995; Trudeau et al., 1995).

Another unusual feature of HERG is the large "hooked" tail current. The rising phase of the tail current results from

the rapid recovery from inactivation and reopening of HERG upon hyperpolarization (Sanguinetti et al., 1995). The slow decay of the tail current reflects the normal deactivation of the channels. The amplitude of the tail current increases with prepulse potential reaching a maximum at voltages >0 mV. Figure 1B plots the normalized tail currents versus prepulse voltage for the currents shown in Fig. 1A. The smooth curve through the tail current data is a fit to the Boltzmann equation with a midpoint of -29.5 mV and a slope of 5.3 mV. The properties of HERG currents expressed in tsA201 cells are similar to those previously reported for HERG channels expressed in other mammalian cell lines (Snyders and Chaudhary, 1996; Zhou et al., 1998).

Cocaine Inhibition of HERG. The effect of cocaine on HERG current was determined by giving test pulses to $+20$ mV during bath application of the drug. The onset of cocaine inhibition is rapid, reaching equilibrium within 30 s after the start of perfusion. Figure 2A shows HERG currents in the absence (top trace) and after application of 0.1, 1, 10, and 100 μM cocaine, respectively. Cocaine causes a dose-dependent reduction in the amplitude of HERG current measured at $+20$ mV and the tail current measured at -80 mV. To quantify the cocaine inhibition, the current measured in the presence of cocaine was normalized to control current and plotted versus concentration (Fig. 2C). The cocaine inhibition measured at $+20$ mV (\blacklozenge) is equivalent to that determined from the tail currents at -80 mV (\bullet), indicating that cocaine block is not highly sensitive to membrane voltage. The smooth curve is a fit of the tail current data to a single-site model with an IC_{50} value of $5.6 \pm 0.4\text{ }\mu\text{M}$ ($n = 11$).

Recent studies suggest that inactivation of HERG may play a critical role in the binding of antiarrhythmic drugs (Wang et al., 1997; Ficker et al., 1998). These findings conflict

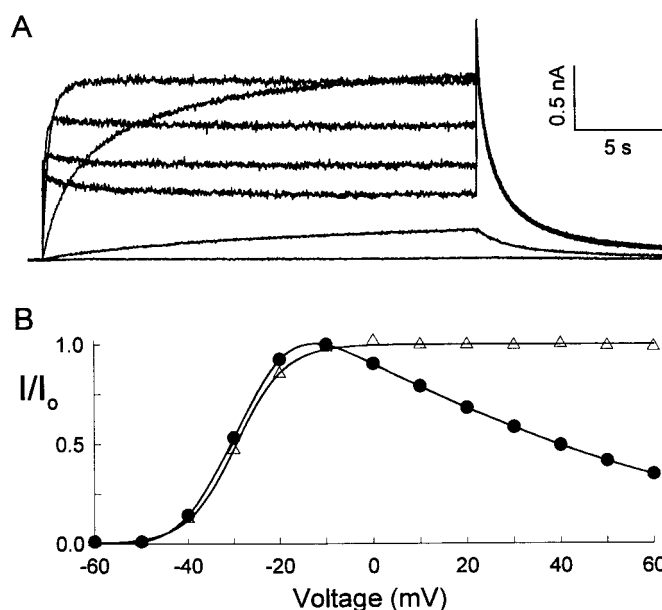


Fig. 1. Properties of HERG channels expressed in tsA201 cells. Currents were elicited by depolarizing steps between -60 and $+40$ mV. The holding potential was -80 mV. A, whole-cell HERG currents transiently expressed in tsA201 cells. B, the current-voltage relationship determined from the currents shown in A. \bullet , the normalized currents measured after 25 s of depolarization. The peak tail currents were measured and normalized to the current at $+40$ mV (\triangle). The smooth curve is a fit to the Boltzmann equation with a midpoint of -29 mV and a slope of 5.3 mV.

with other studies suggesting that inactivation may interfere with drug binding (Kiehn et al., 1996; Snyders and Chaudhary, 1996; Zou et al., 1997). The contribution of inactivation gating to cocaine binding was further examined by raising the external concentration of K^+ , which is known to reduce the inactivation of HERG (Schönherr and Heinemann, 1996; Smith et al., 1996; Wang et al., 1997). For example, raising the concentration of external K^+ from 2 to 100 mM increases the inactivation time constant measured at +20 mV from 3.6 ± 0.2 ms ($n = 13$) to 16.5 ± 0.3 ms ($n = 5$) and increases the residual noninactivating current from $14.0 \pm 1.0\%$ ($n = 13$) to $31.4 \pm 1.7\%$ ($n = 5$). Raising the external concentration to 100 mM caused the inactivation of HERG to become slow and incomplete. Figure 2B shows the effects of raising external K^+ on the cocaine inhibition of HERG. Raising $[K^+]_o$ to 100 mM does not alter the IC_{50} value for cocaine inhibition ($IC_{50} = 6.5 \pm 1.5$ μ M, $n = 17$) regardless of whether the dose-response relationship is determined from depolarizing pulses or from the peak of the tail currents

(Fig. 2C). The data indicate that despite weakening inactivation, increasing the concentration of external K^+ does not alter cocaine binding.

Cocaine Preferentially Binds to the Activated States of HERG Channels. Many anesthetics are known to selectively bind to different gating states of ion channels. The inhibition produced by these drugs generally increases with depolarization suggesting that they preferentially bind to the open or inactivated states (Hille, 1977; Hondeghem and Katzung, 1977). Usually, such state-dependence reflects increases in binding affinity or the accessibility of the drug to the binding site as channels gate. In addition, membrane voltage may also directly influence drug binding. At physiological pH, cocaine is positively charged (Nettleton and Wang, 1990) and electrostatic interactions with the membrane electric field could contribute to binding (Strichartz, 1977).

To examine the requirements for cocaine binding, the development of the inhibition as a function of voltage was studied in more detail. The fractional inhibition was determined from the ratio of currents measured before and after application of 5 μ M cocaine and the percentage inhibition plotted versus voltage (Fig. 3). Cocaine inhibition increases at the hyperpolarized voltages at which the channels begin to activate, reaching a maximum of 51% at 0 mV. With depolarizations > 0 mV, the cocaine inhibition is relatively constant, indicating that the binding is no longer dependent on membrane voltage.

Also plotted is the voltage-dependent activation of the channels determined from the peak tail currents (Fig. 1B) measured before and after application of cocaine (Fig. 3). At concentrations that inhibit currents by $\approx 50\%$ (5 μ M), cocaine produces a hyperpolarizing shift in activation. The smooth curves are Boltzmann fits to the data with midpoints of -31.9 ± 0.15 mV ($n = 4$) and -34.9 ± 0.13 mV ($n = 4$) for control and after addition of cocaine, respectively. Paired T-tests show that cocaine significantly shifts the midpoint of activation by -4.0 mV ($p < 0.005$). The slope factors of these fits are not significantly different, indicating that cocaine does not alter the voltage sensitivity.

Cocaine inhibition develops over the range of voltages (-40

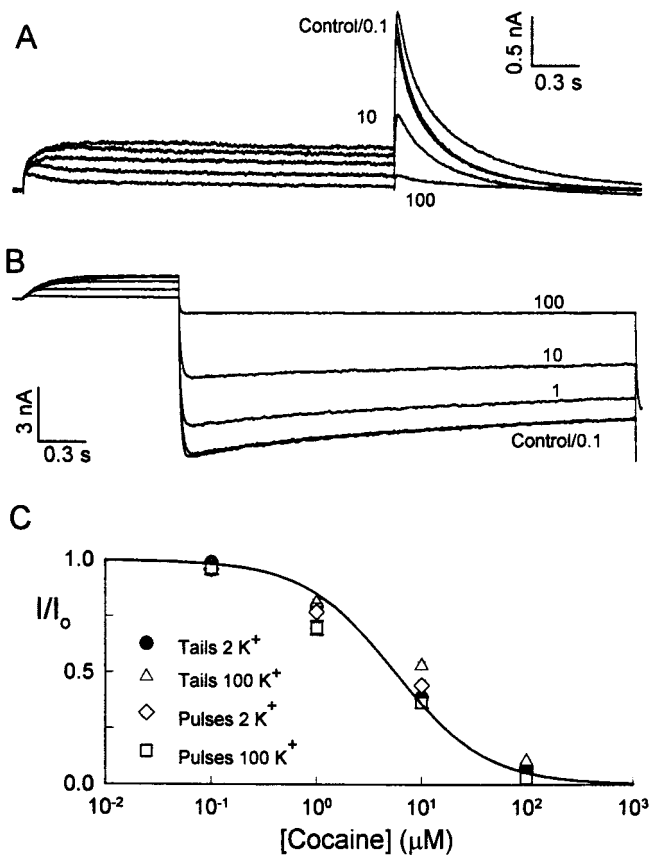


Fig. 2. Cocaine inhibition of HERG. A, currents were activated by depolarizing pulses to +20 mV (pulses) and tail currents measured after returning the voltage to -80 mV (tails). The currents are shown before (top trace) and after the bath application of 0.1, 1, 10, and 100 μ M cocaine. B, cocaine inhibition of HERG currents after raising external $[K^+]_o$ to 100 mM measured using the same pulse protocol as in A. C, dose-response relationship for cocaine inhibition. The cocaine-modified currents were normalized to their respective control currents (i.e., drug-free) and plotted versus concentration. The normalized amplitude of the currents measured after 3 s of depolarization to +20 mV (\diamond , $n = 10$) or at the peak of the tail currents (\bullet , $n = 11$) with 2 mM $[K^+]_o$. The dose-response relationship determined with 100 mM $[K^+]_o$ from pulses (\square , $n = 8$) or from tail currents (\triangle , $n = 8$). The smooth curve is a fit of the tail current data obtained with 2 mM $[K^+]_o$ to a single-site model with an IC_{50} value of 5.6 ± 0.4 μ M.

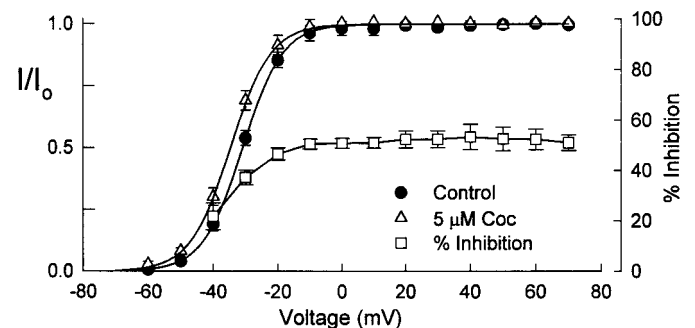


Fig. 3. Effect of cocaine on activation. Currents were activated by pulsing to voltages between -60 and $+80$ mV, and the tail currents measured -80 mV (Fig. 1A). The peak tail currents were normalized to the values at $+40$ mV and plotted versus voltage. The relative amplitudes of the tail currents are shown before (\bullet) and after (\triangle) application of 5 μ M cocaine ($n = 4$). The smooth curves are Boltzmann fits to the data with midpoints and slope factors of -31.0 ± 0.2 mV and 6.4 ± 0.1 mV for controls and -34.9 ± 0.1 mV and 6.2 ± 0.1 mV after application of 5 μ M cocaine. The percentage inhibition (\square) was calculated from the ratio of currents $[100 \times (1 - I_{Coc}/I_{Con})]$ measured near the end of the 25-s depolarizing pulses before and after application of cocaine ($n = 4$).

to +20 mV) at which HERG channels activate. The data indicate that cocaine binds preferentially to the open or inactivated state of the channel. Such selective binding would tend to stabilize the channels in the open or inactivated state, which may explain the observed hyperpolarizing shift in activation. At voltages more depolarized than +20 mV, where the channels are fully activated, the fractional inhibition is relatively constant to voltages up to +80 mV. This finding has two important implications: 1) outside of its coupling with activation, cocaine binding has little intrinsic voltage dependence, suggesting that the drug does not bind within the membrane electric field and 2) any voltage-dependent gating that occur over this more depolarized range of voltages does not promote additional cocaine binding. Rapid inactivation is well known to underlie the strong rectification of HERG at voltages more depolarized than 0 mV (Fig. 1B, ●). The progressive increase the fraction of channels occupying the inactivated state induced by strong depolarization does not further alter cocaine binding.

Effect of Cocaine on the Instantaneous I-V Relationship. The voltage sensitivity of cocaine inhibition was further investigated by examining the instantaneous current-voltage relationship. Instantaneous currents were obtained using a triple-pulse protocol (Fig. 5A). Currents were activated by depolarizing to +20 mV before stepping to -80 mV to promote recovery from inactivation. Test pulses were then used to inactivate the fully primed channels. Examples of the currents elicited by this protocol are shown in Fig. 5B. The instantaneous currents were determined by extrapolating the exponential inactivation time course of the test currents back to the beginning of the test pulse. These currents provide an estimate of the current amplitudes of open channels before the onset of inactivation (Smith et al., 1996). In the absence of drug, the instantaneous I-V relationship is nearly linear between -60 and +40 mV, illustrating the important role of channel gating in the rectification of these channels (Fig. 4A). After application of 5 μ M cocaine, the amplitudes of the currents are reduced compared with control currents. Importantly, the voltage-sensitivity of the instantaneous I-V relationship is not altered by cocaine. This is clearly seen when the instantaneous currents measured in the presence of cocaine are normalized to their paired controls (dashed line). This is inconsistent with a voltage-dependent blocking mechanism that predicts some deviation between the control and cocaine-modified currents at the more depolarized voltages. The downward shift of the instantaneous currents is consistent with a simple reduction in the fraction of open channels. These findings, along with the steady-state measurements of cocaine inhibition (Fig. 3) and the insensitivity of the IC_{50} value to test pulse voltage (Fig. 2C), further supports the conclusion that cocaine binding is not directly influenced by membrane voltage.

One potential concern with these estimates of cocaine inhibition of open channels is that drug bound during depolarizing prepulses, where the channels are inactivated, may not dissociate before the measurement of the instantaneous current. This would cause a reduction in the amplitude of the tail current that is not related to the inhibition of the open channels. However, unlike many drugs that inhibit HERG, cocaine accelerates rather than slows the recovery from inactivation (Fig. 6). The data indicate that cocaine rapidly dissociates as the channels recover from inactivation. It is

unlikely that persistent cocaine binding to inactivated channels contributes to the reduction in the instantaneous currents observed in these experiments.

The fractional inhibition ($1 - I_{Coc}/I_{Ctrl}$) was calculated from the instantaneous currents measured before and after application of cocaine. When the influence of channel gating on cocaine block is minimized, the fractional inhibition remains constant between -60 and +40 mV, further supporting the idea that cocaine binding to open channels is not voltage-dependent (Fig. 4B). Also plotted is the steady-state inhibition measured after 4 s of depolarization (see Fig. 3). The progressive increase in the steady-state inhibition for voltages between -30 and 0 mV cannot be attributed to a direct electrostatic interaction between the positively charged cocaine and the membrane electric field. Rather, the increase in cocaine inhibition coincides with the range of voltages at which the channels activate. At voltages >0 mV, where the channels are fully activated, the inhibition of instantaneous currents where channels are open is similar to the steady-state inhibition measured under conditions at which the majority of the channels ($\approx 85\%$) are inactivated.

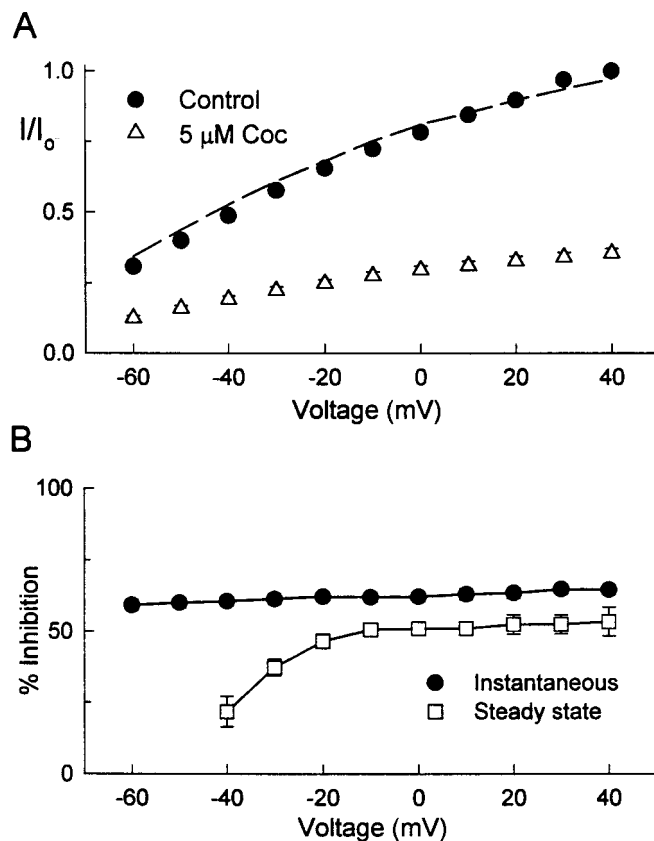


Fig. 4. Cocaine inhibition of open channels. The instantaneous currents were measured using a triple-pulse protocol consisting of a depolarizing conditioning pulse (+60 mV/400 ms), a hyperpolarizing pulse (-80 mV/30 ms) and a family of test pulses to voltages between -60 and +40 mV (Fig. 5A). The instantaneous currents were determined by extrapolating the decay of the currents measured during test pulses back to the beginning of the depolarizing step (Fig. 5B). A, instantaneous current-voltage relationship measured before (●) and after (△) bath application of 5 μ M cocaine ($n = 8$). The dashed line is the relative amplitude of the cocaine-modified currents normalized to paired control currents at +20 mV. B, the percentage inhibition of the instantaneous currents [$(1 - I_{Coc}/I_{Ctrl}) \times 100$] is plotted versus voltage (●, $n = 8$). Also plotted is the steady-state cocaine inhibition determined after 3 s of depolarization (data from Fig. 3).

Cocaine (5 μ M) inhibits a similar fraction of the HERG current regardless of whether the channels are open or inactivated. Activation of HERG channels clearly enhances the inhibition, suggesting that channel opening may play an important role in cocaine binding. However, the data also suggest that the conformational changes associated with inactivation do not promote additional cocaine binding over that already observed for open channels. One possibility is that open and inactivated channels may have similar affinity for cocaine.

Cocaine Accelerates the Inactivation of HERG. Because of the atypical gating of this channel, the rapid rate of C-type inactivation plays an unusually important role in determining the amplitudes of HERG currents. In addition to blocking the channels, cocaine also alters the kinetics of inactivation; suggesting that cocaine may modulate currents through changes in gating. The time course of inactivation was measured using a triple pulse protocol. A depolarizing conditioning pulse was applied to activate and inactivate the channels. This was followed by a short hyperpolarization to allow channels to recover from inactivation and a series of depolarizing test pulses of variable amplitude to inactivate the fully primed channels (Fig. 5A). Using this protocol, the decay of the currents measured during test pulses accurately

reflects the time course of inactivation (Smith et al., 1996). Inactivation is slow and incomplete at hyperpolarized voltages and the rate progressively increases with depolarization (Fig. 5B). The current decay was fitted with a single exponential and the inactivation time constant plotted versus voltage (Fig. 7A, \bullet). The time constants decrease with voltage consistent with an increasing rate of inactivation. The shape of the currents and the voltage dependence of inactivation were retained after addition of 5 μ M cocaine (Fig. 5C). Over the range of voltages tested, cocaine caused HERG channels to inactivate rapidly compared with control currents (Fig. 7A).

Cocaine Accelerates the Recovery from Inactivation. Cocaine also alters the time course of recovery from inactivation. Depolarizing conditioning pulses were used to inactivate the currents and test pulses between -140 and 0 mV applied to stimulate recovery (Fig. 6A). At voltages more hyperpolarized than -70 mV, the inward tail currents display a prominent hook caused by the rapid recovery and slow deactivation of HERG channels at these voltages (Spector et al., 1996a). These currents were fitted with the sum of two exponential values with the rapid component reflecting the recovery from inactivation. At voltages more depolarized than -60 mV, the time course is best described by a single exponential value that predominately reflects the recovery from inactivation. The recovery time constants were measured and plotted versus voltage (Fig. 7A). The time constants increase with depolarization, reaching a peak at -40 mV, consistent with a voltage-dependent process that slows with depolarization. At voltages more depolarized than -40 mV, the time constants decrease because of an increasing contribution of inactivation to the current kinetics at these voltages. Cocaine accelerates the recovery, but does not otherwise alter the shape of the currents (Fig. 6C). After application of cocaine, the time constants are reduced over the entire voltage range, which is suggestive of a more rapid recovery from inactivation (Fig. 7A).

Modeling of Inactivation Gating. To further investigate the effects of cocaine on gating, the inactivation and recovery time constants were fitted with a first-order kinetic model (Fig. 7A). The model provides a reasonable description of the data before and after application of 5 μ M cocaine. Consistent with the direct measurements, cocaine increases the inactivation rate constant at 0 mV from 0.147 ms^{-1} in control experiments to 0.220 ms^{-1} after addition of cocaine. In contrast, the recovery rate constants are similar before (0.014 ms^{-1}) and after (0.012 ms^{-1}) addition of cocaine. Figure 7B plots the theoretical inactivation (β) and recovery (α) rate constants predicted by the model. With the exception of the most depolarized voltages, cocaine uniformly increases the inactivation rate constants compared with control currents.

Cocaine Inhibition during the Cardiac Action Potential. Because of the complex gating of HERG, it is difficult to predict the effects of cocaine on the currents during a cardiac action potential, where the membrane voltage is continually changing with time. To gain a better understanding of the effects of cocaine on repolarization, the action potential clamp technique was employed. An action potential wave form recorded from a guinea pig myocyte was used as the command voltage to activate HERG currents (Fig. 8A). In control experiments, HERG currents activate during the rapid upstroke and plateau phase of the action potential (Fig.

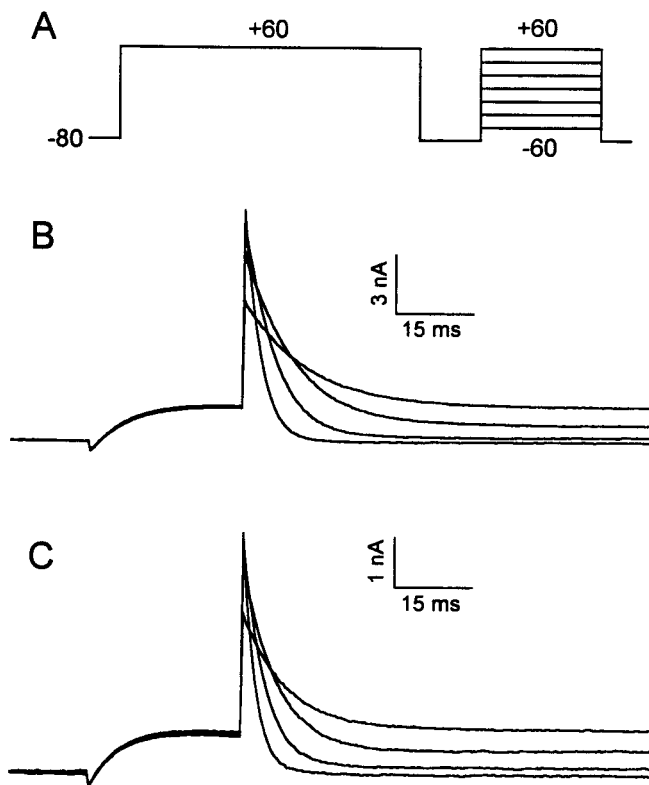


Fig. 5. Cocaine accelerates the inactivation of HERG. A, currents were activated by a conditioning pulse to $+60$ mV for 400 ms. The voltage was returned to -80 mV for 30 ms to allow the channels to recover before applying test pulses to voltages between -40 and $+60$ mV. Only the final 10 ms of the $+60$ mV conditioning pulses are shown. The decay of the current during the test pulses is well fitted by a single exponential. B, representative currents showing the development of inactivation with time constants of 11.8 (-60 mV), 12.3 (-40), 9.4 (-20), and 5.7 (0) ms, respectively. C, same cell as in B after treatment with 5 μ M cocaine with time constants of 8.0 (-60 mV), 8.4 (-40), 6.6 (-20), and 4.2 (0) ms. The inactivation time constants were measured for control ($n = 13$) and cocaine-modified ($n = 12$) currents and plotted versus voltage (Fig. 7A).

8B). This is consistent with HERG currents elicited by step depolarization and reflects a combination of slow activation and rapid inactivation. During repolarization, the currents paradoxically increase because of rapid recovery and reopening of the channels. Although HERG conducts outward K^+ current throughout the action potential, it contributes most significantly to cardiac repolarization during the terminal phases of the action potential as the channels reopen. Cocaine ($5 \mu\text{M}$) broadly inhibits the HERG current throughout the action potential wave form. The inhibition progressively increases during the course of the action potential, reaching approximately 55% at voltages between -10 and -80 mV. This pattern of cocaine inhibition tends to selectively reduce the delayed rectifier current during the late phases of the cardiac action potential, thus weakening the repolarization of myocardial cells that express HERG channels.

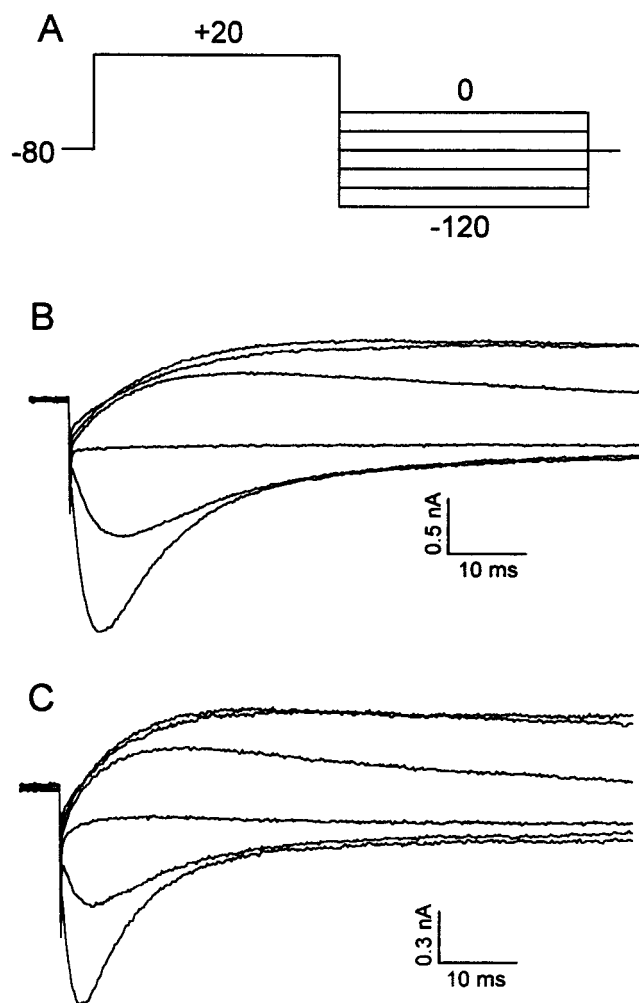


Fig. 6. Effect of cocaine on recovery from inactivation. A, conditioning pulses ($+20$ mV/200 ms) were used to inactivate the channels before applying hyperpolarizing test pulses to voltages between -140 and 0 mV. With test pulses more negative than -70 mV, the currents are biexponential, with a rapid phase reflecting recovery from inactivation followed by slower deactivation (see text). At voltages more depolarized than -50 mV, the currents are fit with a single exponential. B, representative currents with recovery time constants of 2.0 (-140 mV), 3.7 (-120), 7.8 (-80), 11.1 (-60), and 13.2 (-40) ms. C, same cell as in B after the bath application of $5 \mu\text{M}$ cocaine with time constants of 1.4 (-140 mV), 2.4 (-120), 4.8 (-80), 7.2 (-60), and 7.9 (-40) ms. The recovery time constants were determined for control ($n = 11$) and cocaine-modified ($n = 13$) currents and plotted versus voltage (Fig. 7A).

A prominent effect of cocaine on cardiac electrophysiology is an increase in the QT interval, which is often attributed to abnormal repolarization (Beckman et al., 1991; Temesy-Armos et al., 1992; Erzouki et al., 1993). The delayed rectifying potassium current plays an essential role in cardiac repolarization and is an important determinant of action potential duration. In this study, cocaine was found to be a potent inhibitor of HERG channels that underlie the rapidly activating component of the delayed rectifier current. The dose-response relationship for cocaine inhibition of HERG is consistent with a single high-affinity binding site with an IC_{50} value of $5.6 \mu\text{M}$. HERG channels are inhibited by cocaine within the range of concentrations (0.4 – $70 \mu\text{M}$) detected in post mortem plasma samples of people who died after the use of cocaine (Mittleman and Wetli, 1984) and is likely to contribute to the cardiotoxic effects of this drug in vivo.

Cocaine has several prominent effects on HERG currents: 1) a reduction in the current amplitude, 2) a hyperpolarizing shift in activation, and 3) an increase in the rate of inactivation. This wide spectrum of effects suggests that the cocaine inhibition of HERG is complex and that the drug may act by several different mechanisms. At depolarized voltages, cocaine reduces the amplitudes of the currents but does not

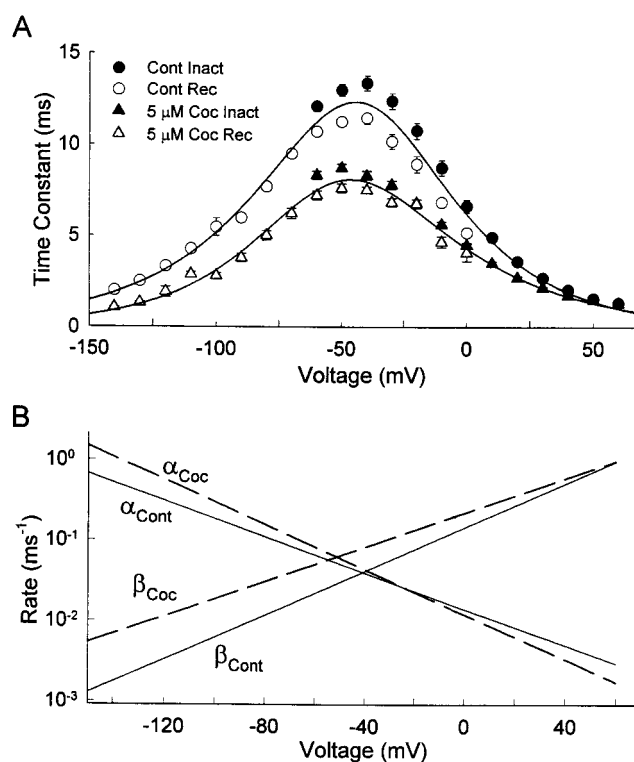


Fig. 7. Modeling the effects of cocaine on inactivation gating. The inactivation and recovery time constants were determined from data similar to that shown in Figs. 5 and 6. A, Plot of the mean \pm S.E.M. of the inactivation (\bullet , $n = 13$) and recovery (\circ , $n = 11$) time constants of control experiments. Similar plot of the inactivation (\blacktriangle , $n = 12$) and recovery (\triangle , $n = 13$) time constants after bath application of $5 \mu\text{M}$ cocaine. The smooth curves are fits to a first order model [$\tau = (\alpha + \beta)^{-1}$], where $\alpha(V) = \alpha(0)\exp(V/k_\alpha)$ and $\beta(V) = \beta(0)\exp(-V/k_\beta)$. Parameters for control data: $\alpha(0) = 0.145 \text{ ms}^{-1}$, $k_\alpha = 31.8 \text{ mV}$, $\beta(0) = 0.014 \text{ ms}^{-1}$, and $k_\beta = 38.7 \text{ mV}$. After treatment with $5 \mu\text{M}$ cocaine: $\alpha(0) = 0.220 \text{ ms}^{-1}$, $k_\alpha = 40.5 \text{ mV}$, $\beta(0) = 0.012 \text{ ms}^{-1}$, and $k_\beta = 31.1 \text{ mV}$. B, plot of the theoretical inactivation (β) and recovery (α) rate constants predicted by the model before (Cont) and after (Coc) application of $5 \mu\text{M}$ cocaine.

significantly alter their time course. Either cocaine inhibits the channels under resting conditions or they are rapidly inhibited as the channels open and inactivate. These mechanisms are difficult to distinguish in HERG because the unusually rapid inactivation precludes an accurate determination of events occurring during the rising phase of the current. However, two findings support an activated state inhibition as the mechanism of cocaine action: 1) cocaine inhibition increases over the range of voltages where the channels activate, and 2) cocaine causes a hyperpolarizing shift in the current-voltage relationship. These data are inconsistent with models in which cocaine binds preferentially to closed channels.

At depolarized voltages, open and inactivated states are in rapid equilibrium; distinguishing between cocaine binding to these states has proved difficult. To further investigate these mechanisms, the effect of cocaine on the instantaneous currents was examined (Fig. 4). The instantaneous currents are measured under conditions in which channels have fully recovered, but before the onset of inactivation, and provide a reasonable estimate of the current flow through open channels. Cocaine causes a reduction in the instantaneous currents with no change in voltage sensitivity. Over a wide range of voltages, cocaine causes a downward shift in the instantaneous I-V relationship consistent with a simple decrease in the number of channels conducting current. In addition, cocaine also inhibits HERG tail currents (Fig. 2). The peak tail currents measured at hyperpolarized voltages reflect the activity of channels that have recovered from inactivation but have not yet closed. The reduction in the amplitudes of both the instantaneous and tail currents are consistent with the cocaine inhibition of open channels.

The mechanism of cocaine inhibition of the open channels is not known. Cocaine could act by binding within the pore

and block the channel, as has been recently demonstrated for the antiarrhythmic drug MK-499 (Mitcheson et al., 2000). Alternatively, cocaine may bind to a site outside of the pore and cause a conformational change that inhibits permeation. A definitive test of the blocking mechanism has been hampered by the usually rapid inactivation of these channels, which precludes a detailed study of the effects of cocaine at the single channel level. Further studies of the cocaine inhibition of open HERG channels are necessary to identify the mechanism of action.

The role of inactivation in cocaine inhibition is unclear. Raising the concentration of external K^+ disrupts inactivation but does not alter the IC_{50} value for cocaine inhibition (Fig. 2). In addition, the fractional cocaine inhibition remains constant (Fig. 3) over the range of voltages at which the HERG channels rectify because of an increase in inactivation (Fig. 1). Finally the cocaine inhibition measured at depolarized voltages, at which channels are predominately inactivated ($\approx 85\%$), is nearly equivalent to the inhibition of the instantaneous currents determined under conditions where the channels are open (Fig. 4). If inactivation were critical for cocaine binding, then some decrease in the IC_{50} value or increase in the inhibition would be expected as the channels inactivate. These findings are more consistent with the conclusion that cocaine binding is minimally altered as the channels shift between open and inactivated states. Overall, the data indicate that cocaine can readily distinguish between closed and open channels but does not discriminate between open and inactivated channels.

Because of the unusual gating of HERG, inactivation has been shown to be an important determinant of current amplitude. At depolarized voltages, HERG currents are typically small because most of the channels rapidly inactivate at these voltages. Manipulations that slow C-type inactivation, such as raising external $[K^+]$ (Wang et al., 1996) or pore mutations (Schönherr and Heinemann, 1996; Smith et al., 1996), increase the amplitude of HERG current. By contrast, cocaine accelerates HERG inactivation, a result that is expected to further reduce the currents at depolarized voltages. The mechanism underlying this faster inactivation is not known. One possibility is that cocaine binding to open or inactivated channels may accelerate the relaxation of HERG current by shifting the equilibrium away from the open state. Alternatively, cocaine binding may promote faster inactivation by an allosteric mechanism. Further studies will be necessary to elucidate these potential mechanisms.

Recent studies indicate that the permanently charged quaternary derivative of cocaine preferentially acts from the internal side of the HERG channel (Zang et al., 2000). In this study, cocaine binding was found to be insensitive to membrane voltage, indicating that the binding site is not located within the membrane electric field and is not altered by inactivation or changes in the concentration of external K^+ . Taken together, these data suggest that cocaine inhibits HERG by binding to a site located superficially on the internal side of the channel. Drugs that bind to such internal sites may not sense the conformational change associated with C-type inactivation, which is believed to occur near the external mouth of the channel, compete for K^+ binding sites located deep within the permeation pathway and although positively charged at physiological pH, may not directly interact with the membrane electric field. Overall, the data

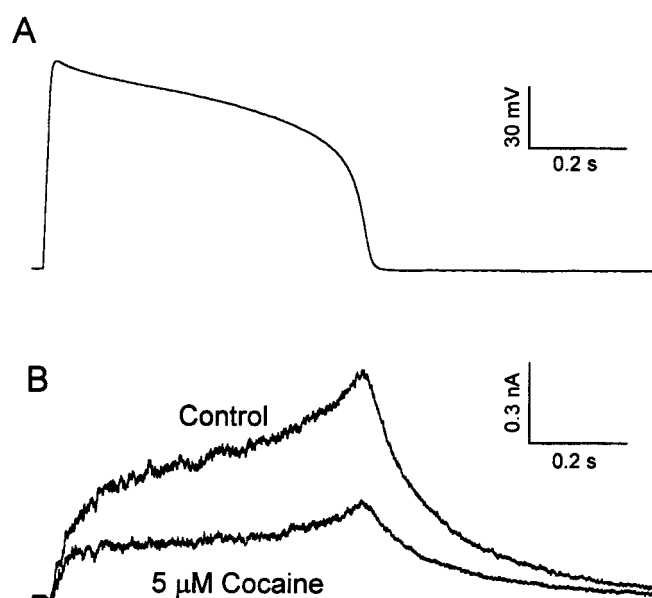


Fig. 8. Effect of cocaine on HERG currents evoked by an action potential wave form. A, the action potential of a guinea pig ventricular myocyte was used as the command voltage to stimulate HERG current. B, representative HERG currents elicited by the wave form shown in A before (top trace) and after (bottom trace) bath application of $5 \mu M$ cocaine. Peak currents are inhibited 55% by cocaine. Similar effects were observed in three additional experiments. The temperature in this experiment was $35^\circ C$.

suggest that the narrow part of the pore and the external mouth of the channel involved in C-type inactivation are remarkably well insulated from the cocaine binding site.

HERG channels are inhibited by a variety of class III antiarrhythmic drugs. The data suggest that these drugs inhibit HERG by preferentially binding to the open (Jurkiewicz and Sanguinetti, 1993; Trudeau et al., 1995; Yang et al., 1995; Snyders and Chaudhary, 1996; Spector et al., 1996b; Busch et al., 1998) or inactivated (Ficker et al., 1998; Suessbrich et al., 1999) states of the channel. In this study, cocaine was found to inhibit HERG currents at both depolarized and hyperpolarized voltages, cause a hyperpolarizing shift in activation, and accelerate inactivation. These effects can be explained by assuming that cocaine binds to a combination of open and inactivated states of the channels.

Physiological Relevance. Although the cardiotoxic effects of cocaine on the heart are well documented, the mechanism by which this drug promotes arrhythmias remains controversial. In part, this stems from the pharmacological properties of cocaine, which acts as both a local anesthetic and a sympathomimetic agent (Billman, 1990, 1995; Kloner et al., 1992; Bauman et al., 1994). Enhanced sympathetic stimulation is known to increase heart rate and myocardial contractility and to mediate vasoconstriction in peripheral and coronary blood vessels (Billman, 1990). One proposed mechanism is that cocaine-induced vasoconstriction and localized ischemia may promote myocardial infarctions and ventricular arrhythmias (Billman, 1990, 1995; Kloner et al., 1992). However, recent evidence suggests that cocaine may induce lethal arrhythmias in the absence of acute myocardial damage (Mittleman and Wetli, 1984; Tazelaar et al., 1987; Virmani et al., 1988; Dressler et al., 1990). The mechanism by which cocaine induces cardiac arrhythmias remains poorly understood.

Naturally occurring mutations and class III antiarrhythmic drugs reduce HERG currents in vivo, resulting in long QT syndromes that predispose otherwise healthy persons to ventricular arrhythmias and sudden death (Roden et al., 1996). These lethal events occur in the absence of other complications such as preexisting heart disease (Roden et al., 1996) or ischemia (Priori and Corr, 1990). Because of its highly specific block of HERG, cocaine may produce an acquired form of long QT syndrome. In addition, one of the suspected triggers for sudden death in patients with long QT syndromes is emotional or physical stress, which is believed to act via an increase in sympathetic stimulation (Roden et al., 1996). Thus, cocaine presents two important predisposing factors identified as contributing to cardiac arrhythmias, namely an increase in the QT interval and enhanced sympathetic stimulation. This condition is likely to be further aggravated by the inhibition of cardiac sodium channels (Crumb and Clarkson, 1992) with the associated slowing of conduction velocity. This combination of slowed conduction, delayed repolarization, and enhanced sympathetic stimulation may be sufficient to trigger lethal arrhythmias in humans.

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