Effects of Cocaine and Its Major Metabolites on the HERG-Encoded Potassium Channel

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ABSTRACT
Cocaine abuse has been reported to result in QT prolongation in humans; however, the mechanisms underlying this effect are still poorly understood. In this study we compared the direct effects of cocaine and its major metabolites in human embryonic kidney 293 cells stably transfected with human ether-a-go-go-related gene (HERG). Cocaine blocked HERG-encoded potassium channels with an IC_{50} of 4.4 ± 1.1 μM (22°C). Cocaethylene (a metabolite formed in the presence of ethanol) had a significantly lower IC_{50} of 1.2 ± 1.1 μM (P < 0.0001), and cocaine’s primary pyrolysis metabolite methylecgonidine blocked HERG with a higher IC_{50} of 171.7 ± 12 μM. In contrast, 1 mM ecgonine methylester or benzoylecgonine produced only a minimal block (21 ± 4 and 15 ± 8%, respectively).

Cocaethylene is slightly more potent than cocaine as a blocker of HERG, whereas methylecgonidine has much lower potency, and both benzoylecgonine and ecgonine methyl ester are essentially inactive at clinically relevant concentrations.

Blockade of HERG by cocaine, cocaethylene, and methylecgonidine increased significantly over the voltage range where HERG activates, but became constant at voltages where HERG activation was maximal, indicating that all three drugs block open channels, but by a mechanism that is not highly sensitive to voltage per se. Cocaine and cocaethylene also significantly slowed the time course of deactivation at −60 mV, an effect consistent with open channel block. We conclude that cocaethylene is slightly more potent than cocaine as a blocker of HERG, whereas methylecgonidine has much lower potency, and both benzoylecgonine and ecgonine methyl ester are essentially inactive at clinically relevant concentrations.

Cocaine abuse is associated with a variety of cardiovascular complications, including cardiac arrhythmias and sudden death (Isner et al., 1986; Kloner et al., 1992). Recent clinical studies indicate that cocaine abuse is associated with cardiac repolarization abnormalities in the electrocardiogram that include a prolonged QT and QTc interval, as well as enhanced QTc dispersion and appearance of abnormal U waves (Perera et al., 1997; Gamouras et al., 2000). This observation is consistent with previous tissue bath studies that documented that micromolar concentrations of cocaine (IC_{50} = 4 μM) block the rapid component of the delayed rectifier current (I_{Kr}), causing a prolongation of the action potential duration (Kirimura et al., 1992; Clarkson et al., 1996) as well as the induction of early afterdepolarizations in ventricular myocytes (Kirimura et al., 1992). A recent study has also documented that cocaine blocks the cloned HERG channel with a similar IC_{50} (5.6 μM) (O’Leary, 2001). Although a direct effect of cocaine to block I_{Kr} could account for cocaine’s ability to prolong the QT interval, it is difficult to rule out the possibility that cocaine metabolites could also contribute to such effects. For example, cocaine’s half-life is approximately 48 min (Chow et al., 1985), yet in a study on patients admitted to the hospital following the onset of symptoms related to cocaine use, the maximal QT prolongation did not occur for up to 24 h after admission (Gamouras et al., 2000). In addition, it has been reported that approximately 65% of deaths related to cocaine overdose occur within 5 h after cocaine administration, with approximately 30% occurring between 2 and 5 h (Finkle and McCloskey, 1977). The aim of the current study was to define the effects of cocaine and its major metabolites on HERG-encoded potassium channels to clarify which, if any, of its major metabolites could contribute to QT prolongation by a mechanism similar to that documented for cocaine (Clarkson et al., 1996). Cocaine is metabolized primarily in the liver and plasma, resulting in the formation of two primary metabolites, benzoylecgonine and ecgonine methylester (Jeffcoat et al., 1989). Both of these metabolites have been found to be inactive as blockers of the cardiac sodium channel when studied at a drug concentration exceeding a maximal blocking concentration for cocaine (e.g., 100 μM) (Crumb and Clarkson, 1992). However, they are not devoid of biological activity. Benzoylecgonine has been shown to be a potent vasoconstrictor of cerebral arteries, causing concentration-dependent effects at levels >10^{-10} M (Kurth et al., 1993), and ecgonine methylester has been reported to produce both mild vasodilation at 10^{-8} M, or vasoconstriction (at 10^{-8}–10^{-4} M) in different arterial preparations (Kurth et al., 1993; Schreiber et al., 1994). There is also evidence that one or both of these metabolites may be responsible for me-
diating the coronary vasconstrictor effects caused by intranasal cocaine administration in humans (Brogan et al., 1992). Because both of these metabolites have been shown to have biological activity, direct experimental confirmation is necessary to determine their activity on human potassium channels, i.e., HERG. Methylecgonidine (ecgonine methylster) is an additional cocaine derivative that is the major pyrolysis product formed during the smoking of crack cocaine (Cone et al., 1994). Methylecgonidine has been shown to block the acetylcholine-activated potassium current $I_{KACl}$ with an $IC_{50}$ (12 μM) lower than that for cocaine (25 μM) (Xiao and Morgan, 1998). In addition, methylecgonidine has been reported to be both an agonist for muscarinic m₂ receptors at concentrations of $10^{-8}$ to $10^{-4}$ M, and to produce irreversible negative inotropic effects by an additional poorly defined mechanism at micromolar concentrations (Huang et al., 1997; Woolf et al., 1997). Finally, cocaethylene is a pharmacologically active metabolite of cocaine that is formed by transesterification of cocaine in the presence of ethanol (Bailey, 1993). Cocaethylene has a longer half-life (3.5–5.5 h) (Bailey, 1993) compared with cocaine (48 min) (Chow et al., 1985); can achieve plasma concentrations equal to, or greater than that of cocaine (Bailey, 1993); and has been found to block the cardiac sodium channel with a significantly higher potency than cocaine (Xu et al., 1994). In this study we defined the effect of cocaine on HERG-encoded potassium channels, and compared its effects with those of its major metabolites and by-products, including benzoylecgonine, ecgonine methylester, methylecgonidine, and cocaethylene.

### Materials and Methods

**Transfection and Cell Culture.** Human embryonic kidney (HEK) 293 cells that were stably transfected with HERG were obtained from Dr. Craig T. January (University of Wisconsin, Madison, WI). Cells were passaged and maintained in minimum essential medium with Earle's salts supplemented with nonessential amino acids, sodium pyruvate, penicillin, streptomycin, and fetal bovine serum (Zhou et al., 1998). Single cells were isolated for electrophysiological study by a 1-min trypsinization, washed twice with minimum essential medium, and stored in the same media at room temperature until used (within 8 h).

**Drugs and Solutions.** Cocaine, benzoylecgonine, and ecgonine methylester were obtained from the National Institute on Drug Abuse. Methylecgonidine (ecgonine methylester) was purchased from Sigma/RBI (Natick, MA). Drugs were dissolved in deionized H₂O to make 30 mM stock solutions that were aliquoted and stored at −20°C. Stock solutions were thawed once and the unused portion discarded. Dilutions of stock solutions were made immediately before the experiment to create the desired concentrations. The external solution (solution bathing the cells) used for recording of HERG potassium currents had an ionic composition of 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl₂, 1.2 mM MgCl₂, 11 mM dextrose, 10 mM HEPES, adjusted to a pH of 7.4 with NaOH. The internal (pipette) solution had an ionic composition of 130 mM KCl, 1 mM MgCl₂, 10 mM NaATP, 5 mM EGTA, 5 mM HEPES, adjusted to a pH of 7.2 with KOH. In several experiments the effect of methylecgonidine was defined on the human atrial sodium current. In these experiments, the external solution had an ionic composition of 135 mM tetramethylammonium chloride, 5 mM NaCl, 5 mM CaCl₂, 1.8 mM MgCl₂, 1.2 mM MgCl₂, 20 mM HEPES, 11 mM glucose, adjusted to a pH of 7.3 with tetramethylammonium hydroxide. Glass pipettes (tip resistance <1 MΩ) were filled with an internal solution of 125 mM CsF, 20 mM CsCl, 10 mM NaF, 5 mM EGTA, 10 mM HEPES, adjusted to a pH of 7.2 with CsOH. The protocol for isolating human atrial myocytes was identical to that previously published (Crumbl et al., 1995). Experiments were performed at 22 ± 1°C.

**Data Acquisition and Analysis.** Currents were measured using the whole-cell variant of the patch-clamp method (Hamill et al., 1981). Pipette tip resistance was approximately 1.0 to 2.0 MΩ when filled with potassium internal solutions. Analog capacity compensation and 40 to 60% series resistance compensation were used to yield voltage drops across uncompensated series resistance of less than 3 mV. An Axopatch 1B amplifier (Axon Instruments, Foster City, CA) was used for whole-cell voltage clamping. Creation of voltage-clamp pulses and data acquisition were controlled by a PC running pClamp software (version 8; Axon Instruments). After rupture of the cell membrane (entering whole-cell mode), current amplitude and kinetics were allowed to stabilize (3–7 min) before experiments were begun. HERG potassium currents recorded from HEK cells stably expressing HERG were elicited by 5-s depolarizing voltage steps from a holding potential of −80 mV. HERG tail currents were measured upon repolarization to −60 mV. Drugs were applied locally to cells in the bath through a micropipette perfusion system that allowed for rapid exchange of drug concentrations surrounding a cell. Up to three drug concentrations were studied in each cell. In the first series of experiments a high concentration of E-4031 was applied at the end of the experiment to confirm that adequate perfusion of the cell under study was obtained. Drug effects on tail current amplitude were measured after a steady-state level of block had been achieved, and were defined by the change in peak versus steady-state current amplitude. The cycle length for most protocols was 20 s. Drug effects were recorded after a steady-state effect had been reached in the presence of drug, and expressed as a reduction in amplitude relative to the current amplitude recorded before drug was introduced (control). Up to three drug concentrations (from low to high in sequence) were studied for each cell. Unless stated differently, each cell served as its own control.

**Statistics and Curve Fitting.** Nonlinear regression analysis was used to fit data to a sigmoidal concentration-response relationship $Y = 1/(1 + 10^{(\log IC_{50} - X/Slope)})$, where slope was the Hill slope parameter, $IC_{50}$ is the concentration producing 50% blockade, and $X$ is the drug concentration. Student’s t-test was used to compare fits of the data with this equation, assuming a fixed or variable slope parameter. Student’s t-test was also used to compare the significance of $IC_{50}$ values for different drugs (GraphPad Prism, version 3; GraphPad Software, San Diego, CA). Tail currents were fit by nonlinear regression (Clampfit; Axon Instruments). Paired data were compared for statistical difference by paired Student’s t-test, and differences between multiple groups of data were compared by analysis of variance (ANOVA), followed by Tukey’s multiple comparison test. A $P$ value of <0.05 was accepted as statistically significant. Data are presented as mean ± S.E.M.

### Results

The structures for cocaine and its major metabolites are shown in Fig. 1. The effects of these drugs on HERG-encoded potassium channels were investigated by voltage clamping HEK293 cells stably transfected with HERG. Figure 2 shows a family of HERG-encoded potassium currents recorded upon application of 5-s step depolarizations to different voltages under control conditions, and after a steady-state effect had been achieved in the presence of 3 μM cocaine. It is apparent that both the activating and deactivating currents are suppressed by cocaine. As illustrated in Fig. 3, cocaine not only reduced the amplitude of HERG potassium current, but altered the kinetics of the currents as well. Qualitatively similar effects were also observed when cells were exposed to an appropriate concentration of the cocaine metabolites cocaethylene or methylecgonidine (Fig. 3, C and D). The slowing of
the tail current kinetics was most marked for cocaine and cocaethylene, which produced a clear “crossover” of the drug affected tail current compared with the control tail current when traces were superimposed (Fig. 3, B and C). Two additional metabolites benzoylecgonine and ecgonine methylester were also investigated for blocking activity. Ecgonine methylester produced a small but significant level of block at both 20 μM (12 ± 3%, n = 7) and 1 mM (21 ± 4%, n = 6) concentrations (P < 0.05). In contrast, benzoylecgonine produced a nonsignificant level of block at both 20 μM (6 ± 2%, n = 7) and 1 mM (15 ± 8%, n = 6) concentrations. Because the level of block produced by these drugs was small at clinically relevant concentrations (<20 μM), they were not further studied. The pattern of HERG channel-blocking potencies for these drugs is qualitatively identical to that reported for sodium channel blockade where cocaine and cocaethylene are relatively potent sodium channel blockers at micromolar concentrations, whereas both at 100 μM benzoylecgonine and ecgonine methylester produce little effect (Crumb et al., 1992; Xu et al., 1994). A similar comparison of channel-blocking potencies for methylecgonidine could not initially be made because its effect on the cardiac sodium current has not been previously reported. Therefore, we also determined the effect of 100 μM methylecgonidine on the sodium current in three human atrial myocytes. Similar to its low potency in blocking HERG channels, we found that 100 μM methylecgonidine produced very little tonic blockade (2.4 ± 1.3% reduction in peak sodium current during a pulse to −20 mV after a 1-min rest at −140 mV), and very little use-dependent reduction (1.1 ± 0.6% reduction during a train of 10 pulses of 50-ms duration at 5 Hz (20°C, V_h = −140 mV, V_test = −20 mV)).

In the absence of drug, the time course of HERG tail currents has been shown to be well fit by a double exponential function (Zhou et al., 1998). To further define the effects of cocaine, cocaethylene, and methylecgonidine on HERG tail current kinetics, we fit the time course of the tail current with a double exponential function before and after exposure to drug (Table 1). Exposure to concentrations of cocaine or cocaethylene that blocked HERG tail current by approximately one-half produced a significant increase in both of the two time constants of decay (Table 1). Methylecgonidine’s effect on the tail current kinetics was smaller, more variable, and did not achieve statistical significance. Because drug effects were always determined following a 6- to 18-min perfusion time after control measurements, we also tested the hypothesis that the slowing of tail current kinetics could have been due to an effect of perfusion time (e.g., due to time-dependent changes in gating kinetics). To test this hypothesis, we defined the effect of perfusion time on tail kinetics by using control solutions and found no significant change (Table 2). Thus, the effects of drug on tail current...
whereas for both cocaethylene and methylecgonidine, the concentration-dependent effect of cocaine could be well approximated by a binding equation having a slope factor of 1, described by a Boltzman relationship. The midpoint (V_{mid}) val-
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Decay can be attributed to the presence of the drug, and not to a time effect.

To define the concentration dependence with which cocaine and its metabolites block HERG-encoded potassium currents, we defined the effects of these drugs on the current with a significantly lower IC_{50} of 1.2 ± 1.1 μM (P < 0.05), and methylecgonidine blocked the current with a significantly higher IC_{50} of 171.7 ± 1.2 μM (P < 0.001) (ANOVA and Tukey's multiple comparison test). The concentration-dependent effect of cocaine could be well approximated by a binding equation having a slope factor of 1, whereas for both cocaethylene and methylecgonidine, the data were best fit with a slope of less than unity (0.62 for cocaethylene and 0.58 for methylecgonidine) (F-test, P < 0.0001).

Previous studies have shown that some blockers of HERG potassium channels such as amitriptyline (Jo et al., 2000) block HERG with a clear voltage dependence, whereas other channel blockers, such as ketoconazole, block without a clear voltage dependence (Dumaine et al., 1998). To determine the voltage dependence of HERG block for cocaine, cocaethylene, and methylecgonidine, we defined their effects on tail current evoked upon repolarization to −60 mV after a 5-s depolarization step to +60 mV. The fraction of current blocked is plotted against the drug concentration (μM). Nonlinear regression analysis was used to fit the data to the sigmoidal concentration-response relationship Y = 1/[1 + 10^{(logIC_{50} - X)/slope}], where X is the slope parameter, IC_{50} is the concentration producing 50% blockade, and Y is the drug concentration. Student's t test was used to compare fits of the data assuming a fixed or variable slope parameter, and for comparison of IC_{50} values.

**TABLE 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>τ_{fast} (ms)</th>
<th>τ_{slow} (ms)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>314.8 ± 50.2</td>
<td>1.4 ± 0.3</td>
<td>10</td>
</tr>
<tr>
<td>3 μM Cocaine</td>
<td>587.3 ± 88.8*</td>
<td>2.6 ± 0.4**</td>
<td>11</td>
</tr>
<tr>
<td>1 μM Cocaethylene</td>
<td>470.4 ± 32.0</td>
<td>2.2 ± 0.1</td>
<td>7</td>
</tr>
<tr>
<td>200 μM Methylecgonidine</td>
<td>766.5 ± 36.6*</td>
<td>4.0 ± 0.3**</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>628.2 ± 86.1</td>
<td>2.9 ± 0.4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>702.8 ± 139.1</td>
<td>4.3 ± 1.2</td>
<td>7</td>
</tr>
</tbody>
</table>

* P < 0.01, ** P < 0.001; # P < 0.0001 (by Student's paired t test).

**TABLE 2**

<table>
<thead>
<tr>
<th>Relative Time (min)</th>
<th>τ_{fast} (ms)</th>
<th>τ_{slow} (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>500.2 ± 63.1</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>511.8 ± 79.7</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>12</td>
<td>485.7 ± 72.5</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>18</td>
<td>486.8 ± 56.2</td>
<td>3.3 ± 0.3</td>
</tr>
</tbody>
</table>
in the presence of drug compared with cocaine. However, these effects could in theory also result from either time-dependent shifts in midpoint values for channel gating (Xu et al., 1994), or from drug effects. To distinguish between these possibilities, we conducted a separate series of experiments where we defined the voltage dependence of activation in the absence of drug at definite time intervals (that matched those used for serial drug applications) of 6, 12, and 18 min after first gaining access to the cell interior (i.e., since the onset of establishing “whole-cell” recording conditions). In these experiments we confirmed that there was a small, slow hyperpolarizing shift in the midpoint of this HERG activation as a function of time, with the mean shifts being $-2.3 \pm 0.7$ mV after 6 min, $-3.9 \pm 0.9$ mV after 12 min, and $-5.4 \pm 1.0$ mV after 18 min ($n = 7$). The amplitude of these shifts was statistically significant at each time point ($P < 0.05$) (repeated measures ANOVA and Tukey’s multiple comparison test). In time-matched experiments, we then compared the shift in $V_{mid}$ and slope values produced by time alone with those determined in the presence of cocaine, cocaethylene, and methylecgonidine at a drug concentration close to an IC$_{50}$. Exposure to either 3 $\mu$M cocaine, 1 $\mu$M cocaethylene, or 200 $\mu$M methylecgonidine did not produce a significantly different shift in $V_{mid}$ compared with time alone (Table 3). The differences in slope parameters were not significant, with the exception that a smaller shift was observed for methylecgonidine compared with control (Table 3). The reason for this difference is unclear and probably of little importance.

When the individual tail currents were normalized to their control amplitudes (Fig. 6), it became apparent that the percentage of block by cocaine and its metabolites was significantly smaller at voltages where HERG is less than maximally activated. This is consistent with the hypothesis that these drugs behave like state-dependent channel blockers, and they produce a progressively larger blockade as the fraction of activated or open channels is increased, until maximal level of HERG activation is achieved at voltages positive to 0 mV.

<table>
<thead>
<tr>
<th>Condition</th>
<th>$V_{mid}$ (mV)</th>
<th>Slope (mV)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6 min)</td>
<td>$-2.3 \pm 0.7$</td>
<td>$0.4 \pm 0.1$</td>
<td>7</td>
</tr>
<tr>
<td>3 $\mu$M Cocaine (6 min)</td>
<td>$-2.9 \pm 1.1$</td>
<td>$0.3 \pm 1.0$</td>
<td>7</td>
</tr>
<tr>
<td>Control (12 min)</td>
<td>$-3.9 \pm 1.0$</td>
<td>$1.1 \pm 0.6$</td>
<td>7</td>
</tr>
<tr>
<td>1 $\mu$M Cocaethylene (12 min)</td>
<td>$-5.8 \pm 0.7$</td>
<td>$0.3 \pm 0.4$</td>
<td>19</td>
</tr>
<tr>
<td>Control (18 min)</td>
<td>$-5.4 \pm 1.1$</td>
<td>$1.4 \pm 0.5$</td>
<td>7</td>
</tr>
<tr>
<td>200 $\mu$M Methylecgonidine (18 min)</td>
<td>$-8.0 \pm 1.5$</td>
<td>$-0.04 \pm 0.3^*$</td>
<td>7</td>
</tr>
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* $P < 0.05$.

**Discussion**

**Comparison with Previous Studies.** The results of this study document that both cocaine and cocaethylene are relatively potent blockers of HERG-encoded potassium channels, with cocaethylene having the lowest IC$_{50}$ of 1.1 $\mu$M compared with cocaine’s IC$_{50}$ of 4.4 $\mu$M (Fig. 4). This IC$_{50}$ value is in close agreement with that previously determined for both cocaine blockade of the rapid component of the delayed rectifier current (I$_{Ks}$) in guinea pig ventricular myocytes (Clarkson et al., 1996) as well as cocaine block of HERG channels expressed in tsA201 cells (IC$_{50}$ = 5.4 $\mu$M) (O’Leary, 2001). In contrast to cocaine, methylecgonidine exhibits a relatively low potency (IC$_{50}$ = 171 $\mu$M), and both benzoylecgonine and ecgonine methylester are essentially inactive at micromolar concentrations. A similar pattern of channel blocking potency has also been observed for the effects of cocaine and its metabolites on the cardiac sodium current,
with cocaethylene being more potent than cocaine (Xu et al., 1994), and both ecgonine methylester and benzoylecgonine being essentially inactive (Crumb and Clarkson, 1992). In addition we found in this study that methylecgonidine has relatively low potency for blocking both the HERG potassium current and the human sodium current. Interestingly, although we found methylecgonidine to be much (~39-fold) less potent than cocaine in blocking HERG current, a recent study has shown that methylecgonidine blocks the acetylcholine-activated potassium current with a 2-fold higher potency compared with cocaine (Xiao and Morgan, 1998). This indicates that cocaine’s metabolites do not show the same pattern of biological potencies for effecting all cocaine-sensitive ion channels.

Mechanism of HERG Channel Block. The block of HERG currents by cocaine and its two metabolites cocaethylene and methylecgonidine were similar to each other in terms of producing a more intense blockade as the prepulse voltage was made more positive over the range of HERG channel activation (Fig. 6). In contrast, at more positive voltages there was little, if any, voltage dependence of block. These results are consistent with the hypothesis that all three drugs have a selectively higher affinity for the HERG channels when they open or are activated, but the affinity of each drug for the channel is not markedly affected by changes in the transmembrane voltage field once HERG channels are fully activated (O’Leary, 2001). One possible explanation for this observation is that the binding site for these drugs is not located very deep within the channel lumen, in contrast to quinidine block of Kv1.5 (Yeola et al., 1996). Alternatively, the neutral form of the drug, which would not be sensitive to changes in transmembrane voltage, could be a major contributor to channel block. Additional experiments will be needed to distinguish between these possibilities. The ability of cocaine and cocaethylene to slow the rate of decay of tail currents and produce a “crossover” of tail currents when superimposed on control traces (Fig. 3; Table 1) is also consistent with open channel block and may reflect an inability of the channel to close prior to drug unbinding and exit from the channel (Yeola et al., 1996). Clinical Relevance. Understanding the effects of cocaine and its metabolites on cardiac ion channels is important because it provides insight into the cellular mechanisms underlying their adverse effects on the heart, which ultimately could have an impact on which therapeutic interventions may have the best chance for success in treating patients. Peak plasma concentrations more than 200 ng/ml (0.7 μM) cocaine have been documented in controlled studies of cocaine disposition in humans (Chow et al., 1985; Jeffcoat et al., 1989), whereas plasma levels of up to 421 ng/ml (1.4 μM) have been reported in (nonlethal) trauma victims (Bailey, 1993), and an average as high as 6 μg/ml (20 μM) has been detected in post-mortem blood samples (Mittleman and Welti, 1984). Under our experimental conditions, these levels of cocaine would block 14, 24, and 82% of the total HERG current, suggesting that significant cocaine blockade of HERG potassium currents may be achieved at clinically relevant concentrations. Our results also suggest that the presence of cocaethylene could contribute to QT prolongation in patients who coadminister cocaine and ethanol. This may be important because estimates of the combined use of cocaine and ethanol range from 60 to 85% (Grant and Harford, 1990). Plasma concentrations of 128 μg/l (0.4 μM) cocaethylene have been detected in hospitalized trauma patients that have tested positive for cocaine metabolites (Bailey, 1993), and concentrations up to 2.7 μg/ml (7 μM) cocaethylene have been measured in ante-mortem blood samples taken from patients admitted to a hospital emergency room (Hearn et al., 1991). Our data indicate that either of these concentrations should produce a substantial (33 versus 75%) block of
HERG channels (Fig. 4) and therefore could be expected to affect cardiac repolarization.

In contrast to cocaine and cocaethylene, we found that the other two major cocaine metabolites (benzoylgneline and ecgonine methylester) are relatively inactive at clinically relevant concentrations. Therefore, in theory, maneuvers to increase the rate of cocaine metabolism to benzoylcgonine or ecgonine methylester, such as the administration of catalytic antibodies or artificial enzymes (Cashman et al., 2000; DePrada et al., 2000) would be expected to reduce toxicity related to blockade of cardiac sodium channels or HERG-encoded potassium channels.

Our results also indicate that one of the primary pyrolysis products of cocaine, metylecgonidine, is unlikely to produce a significant effect on cardiac repolarization or conduction at clinically relevant concentrations. Plasma levels of metylecgonidine (3–34 ng/ml or 0.01–0.12 M) have been detected in patients after smoking crack cocaine (Toennes et al., 1999).

Our data indicate that this concentration of metylecgonidine will block less than 2% of available HERG-encoded potassium channels (Fig. 3) and have virtually no effect on sodium channels. Therefore, it is unlikely that this metabolite will contribute to conduction or repolarization abnormalities.

Finally, because cocaine and cocaethylene appear to be potent blockers of HERG potassium channels at clinically relevant concentrations, it seems likely that the use of other drugs such as ketonanazole or venlafaxine, which have been proposed for use in the treatment of cocaine disorders (Goeders et al., 1998; McDowell et al., 2000), should be monitored with caution, because they themselves have been reported to prolong, or have the potential for prolonging, the QT interval (Domaine et al., 1998; Physicians’ Desk Reference, 2000). The concomitant use of multiple drugs that prolong the QT interval, or conditions such as hypokalemia or bradycardia are known to increase the likelihood for the production of multifocal ventricular tachycardia such as torsade de pointes (Tamargo, 2000).

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References

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