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ORIGINAL RESEARCH

Methadone Blockade of Cardiac Inward Rectifier K+ Current Augments Membrane Instability and Amplifies U Waves on Surface ECGs: A Translational Study

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BACKGROUND: Methadone is associated with a disproportionate risk of sudden death and ventricular tachyarrhythmia despite only modest inhibition of delayed rectifier K^+ current (I_{Kr}), the principal mechanism of drug-associated arrhythmia. Congenital defects of inward rectifier K^+ current (I_{K1}) have been linked to increased U-wave amplitude on ECG and fatal arrhythmia. We hypothesized that methadone may also be a potent inhibitor of I_{K1}, contributing to delayed repolarization and manifesting on surface ECGs as augmented U-wave integrals.

METHODS AND RESULTS: Using a whole-cell voltage clamp, methadone inhibited both recombinant and native I_{K1} with a half-maximal inhibitory concentration IC50) of 1.5 µmol/L, similar to that observed for I_{Kr} block (half-maximal inhibitory concentration of 2.9 µmol/L). Methadone modestly increased the action potential duration at 90% repolarization and slowed terminal repolarization at low concentrations. At higher concentrations, action potential duration at 90% repolarization lengthening was abolished, but its effect on terminal repolarization rose steadily and correlated with increased fluctuations of diastolic membrane potential. In parallel, patient ECGs were analyzed before and after methadone initiation, with 68% of patients having a markedly increased U-wave integral compared with premethadone (lead V3; mean +38%±15%, P=0.016), along with increased QT and T_{Peak} to T_{End} intervals, likely reflective of I_{Kr} block.

CONCLUSIONS: Methadone is a potent I_{K1} inhibitor that causes augmentation of U waves on surface ECG. We propose that increased membrane instability resulting from I_{K1} block may better explain methadone's arrhythmia risk beyond I_{Kr} inhibition alone. Drug-induced augmentation of U waves may represent evidence of blockade of multiple repolarizing ion channels, and evaluation of the effect of that agent on I_{K1} may be warranted.

Key Words: I_{K1} ■ I_{Kr} ■ methadone ■ U waves ■ ventricular arrhythmia

See Editorial by Eckhardt

According to the Centers for Disease Control, nearly half a million people died from prescription and illicit opioids between 1999 and 2018.[1] While all opioids suppress ventilatory drive leading to respiratory arrest, synthetic opioids such as methadone and loperamide present an additional risk for sudden cardiac arrest caused by life-threatening ventricular arrhythmias.[2] Both drugs possess dual aromatic ring structures that interact within the distal human ether-à-go-go–related gene (hERG) channel, which, at least

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CLINICAL PERSPECTIVE

What Is New?

- Methadone treatment is associated with a disproportionate risk of sudden death caused by ventricular tachyarrhythmia. Methadone is a known but modest inhibitor of the delayed rectifier K^+ current.
- We demonstrate that methadone also blocks inward rectifier K^+ current, a critical K^+ ion current in the heart responsible for rapid repolarization and diastolic stability, and that suppression of inward rectifier K^+ current combined with known inhibition of the outward rectifier K^+ current prolongs the action potential duration, slows terminal repolarization, and results in diastolic instability in ventricular myocytes.
- The inhibition of inward rectifier K^+ current by methadone is also associated with augmentation of U waves among patients on methadone maintenance via delayed terminal repolarization.

What Are the Clinical Implications?

- Drugs such as methadone that inhibit multiple repolarizing ion currents likely present additional loss of repolarization reserve and therefore a higher risk of drug-induced proarrhythmia.
- The emergence of increased U waves on ECG should be viewed as a possible indicator of dangerous multichannel block. US Food and Drug Administration guidance does not currently recommend interrogation of the impact of inward rectifier K^+ current inhibition in new drug development, which should be reconsidered.

Nonstandard Abbreviations and Acronyms

AP	action potential
APD	action potential duration
DAD	delayed afterdepolarization
hERG	human ether-à-go-go–related gene
IC_{50}	half-maximal inhibitory concentration
I_{K1}	inward rectifier K^+ current
I_{Kr}	delayed rectifier K^+ current
QTc	corrected QT
QTcF	corrected QT with Fridericia formula
TRAP	trajectory of terminal repolarization of the myocyte action potential

in part, explains their proarrhythmic risk.[3,4] Methadone is recognized by the World Health Organization as an essential medication to treat opioid dependency and chronic pain.[5] Over the past decades, however, methadone has been disproportionately implicated in opioid-related fatalities despite representing a small fraction of prescription volume.[6] In a community-based forensic study of sudden cardiac death, methadone was present in 72 of 178 decedents while other opioids were found in only 11, suggesting a distinct proclivity to trigger cardiac arrest.[7] While causal inferences cannot be based solely on postmortem findings, this association was subsequently borne out in worldwide pharmacovigilance data, where methadone was among the most frequently reported drugs associated with cardiac arrest and ventricular arrhythmia.[8] To date, however, the mechanism of proarrhythmia caused by this compound has not been fully characterized.

In 2002, methadone was found to be a moderate inhibitor of the hERG channel responsible for outward rectifier K^+ current (I_{Kr}), the rapid component of the delayed rectifier potassium current, with a half-maximal inhibitory concentration (IC_{50}) of 9.8 μmol/L.[9] However, in an analysis of 5 clinical trials with 284 patients, <5% of patients manifested peak methadone concentrations exceeding 3 μmol/L.[10] Because of extensive protein binding, free methadone concentrations may be <1 μmol/L, substantially reducing the anticipated magnitude of I_{Kr} block. There are few data regarding methadone's impact on other ventricular ion channels that contribute to repolarization. In human embryonic kidney 293 (HEK-293) cells expressing human $Na_V1.5$ channels, methadone blocked the tonic and phasic components of the sodium current (IC_{50} of 11.2 and 5.5 μmol/L, respectively),[11] which would be predicted to shorten action potential (AP) duration (APD) by limiting the inward current and to reduce the impact of hERG inhibition. Current data, therefore, do not explain the observed delayed repolarization and torsade de pointes noted in both methadone case series[2] and pharmacovigilance data,[8,12,13] which suggest the importance of investigating additional contributory ion channels.

Methadone has been associated with the presence of U waves on 12-lead ECG,[14] which could reflect effects on terminal repolarization beyond its established actions on earlier, I_{Kr}-mediated repolarization. While the genesis of the U wave is debated,[15] recent clinical data suggest a relationship with the magnitude of the principal current of terminal repolarization: the inward rectifier K+ current (I_{K1}).[15] To our knowledge, no prior study has assessed the impact of methadone on I_{K1} nor quantified U waves before and after drug initiation. Given this background, we investigated electrophysiological mechanisms of delayed terminal repolarization, postulating blockade of both I_{Kr} and I_{K1} using the whole-cell patch-clamp method in isolated ventricular myocytes. We then assessed for corresponding ECG alterations in repolarization consistent with attenuation in I_{Kr} and I_{K1}.

METHODS

The in vitro experiments were approved by the Uniformed Services University's institutional review board. All animal procedures including the isolation of ventricular myocytes conformed to the *Guide for the Care and Use of Laboratory Animals* (8th edition, 2011) and were performed under protocols approved by the Institutional Animal Use and Care Committee of the Uniformed Services University. The Colorado Multiple Institutional Review Board approved the MAPS (Methadone Induction and U wave Amplitude, Area, Polarity and Spatial Features) study (protocol number 20-0533).

Data, Materials, and Code Disclosure Statement

The data and protocols that support the findings of this study are available from the first author on reasonable request.

Experimental Methods

Isolation of Ventricular Myocytes

Myocytes were isolated from the midmyocardial region of the left ventricle of explanted hearts of Yorkshire pigs following the procedures described in Klein et al.[4] The left anterior descending coronary artery of explanted pig heart was perfused with low calcium Tyrode (in mmol/L: NaCl 145, KCl 4.5, $MgCl_2$ 1.6, NaH_2PO_4 0.33, Glucose 10, HEPES 10, EGTA 0.5, pH 7.4, 37 °C, oxygenated with 100% O_2; 10 minutes), followed by Tyrode plus 0.75 mg/mL type II, 0.25 type I collagenase (Worthington) and 0.05 mg/mL type XIV protease (Sigma-Aldrich; 30 minutes), then by Tyrode plus 0.1 mmol/L $CaCl_2$ (10 minutes). Digested ventricular midwall was minced and strained through 200-µm nylon mesh. Cells were stored in chilled Kraft-Bruhe solution[16] and used for electrophysiology within 36 hours.

Cell Electrophysiology

Ionic currents under whole-cell patch-clamp were recorded using an Axopatch 200-B amplifier coupled to a Digidata 1440A interface and controlled by PClamp 10 software (Molecular Devices). The data were acquired at 10 kHz or 50 kHz, and low-pass filtered at 5 kHz or 10 kHz. Thick-walled microelectrodes were fire-polished (1–3 MΩ). For measurements of I_{K1}, the extracellular solution contained (in mmol/L): NaCl 140, KCl 5.4, HEPES 10, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 10, ouabain 0.001, nifedipine 0.001, pH 7.4, and 295 to 305 mOsm. The pipette solution contained: K^+ aspartate 100, KCl 30, HEPES 10, ATP 5, $MgCl_2$ 5.36, Na_2 creatine phosphate 5, EGTA 1, $CaCl_2$ 0.034, pH 7.2, and 290 to 295 mOsm. Concentrations of free Ca^{2+} and Mg^{2+}, calculated from MaxChelator, were 0.02 µmol/L and 1 mmol/L, respectively. I_{K1} was recorded after electronic compensation of series resistance (80%) and capacitance using a hyperpolarizing ramp command (+20 to –120 mV/s and –140 mV/s). The holding potential was –40 mV. Temperature was maintained at 37 °C using an in-line solution heater and temperature controller (Warner). Recorded voltages were corrected for a junction potential of –10 mV. I_{K1} magnitude was assessed as the maximum outward (repolarizing) current during a hyperpolarizing ramp voltage clamp sweep. All I_{K1} were corrected by subtracting a Ba^{2+}-insensitive (0.5–1 mmol/L) component. APs were recorded under whole-cell current-clamp using the perforated patch method. The pipette solution contained (in mmol/L) K^+ aspartate 100, KCl 30, NaCl 10, HEPES 10, $MgCl_2$ 5, K_2 EGTA 0.1, 240 µmol/L amphotericin B, and pH 7.2.

Constructs of hKir2 and hERG

Constructs with C-terminal conjugated green fluorescent protein (Rx Biosciences) were transiently expressed in COS cells. This construct was also stably expressed in HEK-293 cells using a serial transfection and selection procedure. hKir2.1 plasmid was purchased from Addgene. hKir2.2 plasmid was a generous gift from Dr Lee Eckhardt (University of Wisconsin). Cells were transiently transfected with hKir2.1 or hKir2.2 plus green fluorescent protein using lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific). A CHO-K1 cell line stably expressing the hERG product and I_{Kr} ionic current was a generous gift from Dr Alfred George (Northwestern University).

Cell Culture Methods

HEK-293 cells were obtained from the American Type Culture Collection and routinely cultured in T-75 tissue culture flask in DMEM (Thermo Fisher Scientific) supplemented with 10% FBS (Invitrogen) and 100 U/mL penicillin and streptomycin in a humidified atmosphere at 37 °C with 5% CO_2. The cells were subcultured once a week by trypsinization using 0.05% trypsin/EDTA. COS cells were grown in DMEM supplemented with 10% FBS (Invitrogen) and 100 U/mL penicillin and streptomycin in a humidified atmosphere at 37 °C with 5% CO_2. CHO-KI cells were grown in Ham's F-12 medium (Invitrogen), supplemented with 10% FBS, L-glutamine, 1% penicillin and streptomycin, and 0.2 mg/mL G418. Cells were passaged twice per week. For electrophysiological recording, the cells were plated on glass coverslips in a 6-well plate at *ca*. 50% density.

Drugs

For measurements of I_{K1} in swine myocytes, 1 µmol/L ouabain and 1 µmol/L nifedipine (Sigma) were added

to external solution from dimethylsulfoxide stock solutions. Racemic (R+S) methadone-HCl (Letco Medical) was dissolved to the desired concentration in external solution in myocyte experiments and dissolved in dimethylsulfoxide stock solutions for measurements of I_{K1} in heterologously expressed hKir2. Loperamide-HCl (Sigma) was dissolved in dimethylsulfoxide then added to external solution. The final dimethylsulfoxide concentration was <0.2%.

Analysis

Measurements of the trajectory of terminal repolarization of the myocyte AP (*TRAP*)[4] were obtained as the faster time constant of a nonlinear least-squares fit of a 2-exponential+constant function to phases 3 and 4 of the AP. dV_M/dt_{Max} was calculated from the first derivative of the AP upstroke (phase 0). Beat-to-beat variability of APD (BVR) was determined from $\sum|(APD_{90,i+1}-APD_{90,i})|/\sqrt{2}n$,[17] for n=30 consecutive beats. Fluctuations in the diastolic membrane potential following each AP (pacing at 0.3 Hz or 0.5 Hz) were expressed as diastolic variance, calculated as the variance of each diastolic interval (range, 1.5–2.5 seconds) after subtracting the ensemble average waveform. Delayed afterdepolarizations (DADs) were monitored in the same set of records during continuous pacing of APs. DADs were identified as transient depolarizations >2 mV from the mean diastolic potential.

Clinical Methods

Patient Management and ECG Acquisition

The impact of methadone on the ECG was assessed in a retrospective study of patients undergoing methadone induction for opioid dependence. Between January 2018 and August 2020, consecutive patients admitted to the Outpatient Behavioral Health Services (Denver, CO) were assessed for study eligibility. Patients were opioid dependent for ≥1 year, aged ≥18 years, and had a previous attempt at supervised ambulatory detoxification. All baseline ECGs were obtained before a first dose of oral methadone suspension (Methadose 10 mg/mL, Mallinckrodt Pharmaceuticals). Postinduction tracings were obtained ≈30 days thereafter based on cardiac safety consensus guidelines.[18] Per protocol, all patients commenced therapy with 10 mg/d to 30 mg/d with escalation to 50 mg/d over 5 days, with individualization thereafter. To adequately characterize the repolarization effects of methadone, we excluded patients if they self-reported methadone use in the 2 weeks before admission, if they had been transferred from another methadone program, or if admission urine toxicology demonstrated a detectable level of methadone. Utilization of prescription drugs associated with QT prolongation at baseline and postinduction were coded from the electronic medical record then cross-checked via a dynamic registry of QT-prolonging drugs.[19] Use of the illicit proarrhythmic drugs cocaine, an I_{Kr}-blocking compound,[20] and methamphetamine,[21] which is associated with corrected QT (QTc) prolongation, were tracked by urine toxicology using cloned enzyme donor immunoassay (Thermo Scientific Microgenics). ECGs were obtained at baseline and after methadone stabilization from a single Intellispace Tracemaster (Phillips Medical Systems) cart. ECG acquisition was performed in patients in the supine position, between 6:30 AM and 11 AM. All XML files were coded, deidentified, and exported for signal processing. The Fridericia formula was used for deriving the QTc interval: QTcF (ms) = QT interval (ms) divided by the cube root of the preceding RR interval(s). The mean QTcF interval was then reported for all records based on the summation of all 12 leads using the T-wave offset method. The T_{Peak} to T_{End} was calculated as the time interval between the maximal T-wave amplitude and T-wave offset at the electrical baseline using automated caliper detection of all fiducial points.[22] The change in T_{Peak} to T_{End} divided by the QT interval was also assessed.[23] All parameters were correlated with methadone dose.

U Waves

Characterization of U waves was determined by extracting individual ECG lead signals (500 Hz sampling rate, 0.5–150 Hz band-pass filter) from exported XML files. The mean single-cycle ECG complex for a given lead was extracted from each 10.6-second ECG record. The time course of late T- and U-wave components of the mean complex, y_{T+U}, was reproduced using a nonlinear least-squares fit of the function:

$$y_{T+U} = A_T \exp\left(-t/\tau_T\right) + A_U \exp\left(-\left(t-t_P\right)/\tau_U\right)\left(1+\exp\left(-\left(t-t_P\right)/\tau_U\right)\right)^{-2}, \quad \text{(Equation 1)}$$

where A_T and A_U are scaling constants for the T- and U-wave components, respectively, τ_T and τ_U are time constants for the T and U components, and t_P is a time delay. The first term corresponds to a declining exponential representing the terminal phase of the T wave. The second term is an approximation to a time-dependent normal distribution representing the U-wave component of the ECG signal. A fitting routine based on the Levenberg-Marquardt minimization algorithm optimized the 5 parameters A_T, A_U, τ_T, τ_U, and t_P to minimize the sum of the squared residuals of the fit. Parameters were constrained to be >0.

Statistical Analysis

Paired ECG samples (n=77) were evaluated for statistically significant differences using a paired, 2-tailed Student *t* test. Samples were tested (Shapiro-Wilk test) and found to be normally distributed. Correlations were calculated using Spearman *r* coefficient. Because of the expected diminution of the U-wave integral magnitude at higher heart rates, we prespecified a constrained analysis of ECG pairs limited to a heart rate <85 beats per minute (n=41). We compared pre-post changes with a 2-tailed paired Student *t* test. Bonferroni correction was used for multiple comparisons as appropriate. A paired t test was used to compare the use of prescription and illicit QTc-prolonging drug proportions before and after methadone induction and stabilization. All statistical analyses were performed using IDL software (L3Harris Geospatial). A $P<0.05$ was considered to represent statistical significance.

RESULTS

Methadone Blocks Recombinant I_{K1} and I_{Kr} Channels

The ion channels responsible for I_{K1} in mammalian ventricular myocytes are principally composed of Kir2.1 (*KCNJ2*) and Kir2.2 (*KCNJ12*).[24] To determine whether methadone inhibits I_{K1}, we first examined its effect on recombinant channels encoded by either hKir2.1 or hKir2.2 expressed heterologously. Figure 1A shows single current traces obtained during a hyperpolarizing ramp voltage clamp of hKir2.1-mediated I_{K1} before and after exposure to methadone (0.1 µmol/L, 1 µmol/L, and 10 µmol/L) applied in the extracellular solution. Methadone blocked I_{K1} in a concentration-dependent manner with an IC_{50} of 2.9 µmol/L and a Hill coefficient (n_H) of 0.8 (Figure 1B). Current-voltage (*I-V*) plots of averaged data (mean±SEM) are shown in Figure S1A. I_{K1} encoded by the hKir2.2 was similarly blocked by methadone (Figure 1C and 1D; mean *I-V* plots shown in Figure S1B). Loperamide, a structurally similar synthetic opioid, also blocked hKir2.2-mediated I_{K1} (Figure 1E and 1F; mean *I-V* plots in Figure S1C), in a concentration range similar to that of methadone (IC_{50}=1.2 µmol/L, n_H=0.8) consistent with I_{k1} blockade. Methadone blocked I_{Kr} outward tail-current (–50 mV) with an IC_{50} of 2.9 µmol/L, and a Hill coefficient of 0.8 (n=11 cells [see Figure 1G and 1H]; mean current records shown in Figure S1D), values comparable to those reported by Tran et al[25] but substantially lower than earlier reports.[9]

Methadone Blocks I_{K1} in Ventricular Myocytes

Since methadone inhibited recombinant I_{K1} channels, we next determined the effect of methadone on I_{K1} in isolated swine ventricular cardiomyocytes (Figure 2; mean *I-V* plot shown in Figure S2). Again, methadone inhibited I_{K1} in ventricular myocytes in a dose-dependent manner and in close agreement with the results from recombinant channels, the data are well described by a single-site binding scheme for I_{K1} inhibition by methadone with an IC_{50} of 1.5 µmol/L and Hill coefficient n_H of 0.7. Taken together, these results provide compelling evidence that methadone blocks I_{K1} with similar or higher affinity when compared with its inhibition of I_{Kr}.

Methadone Impact on APD

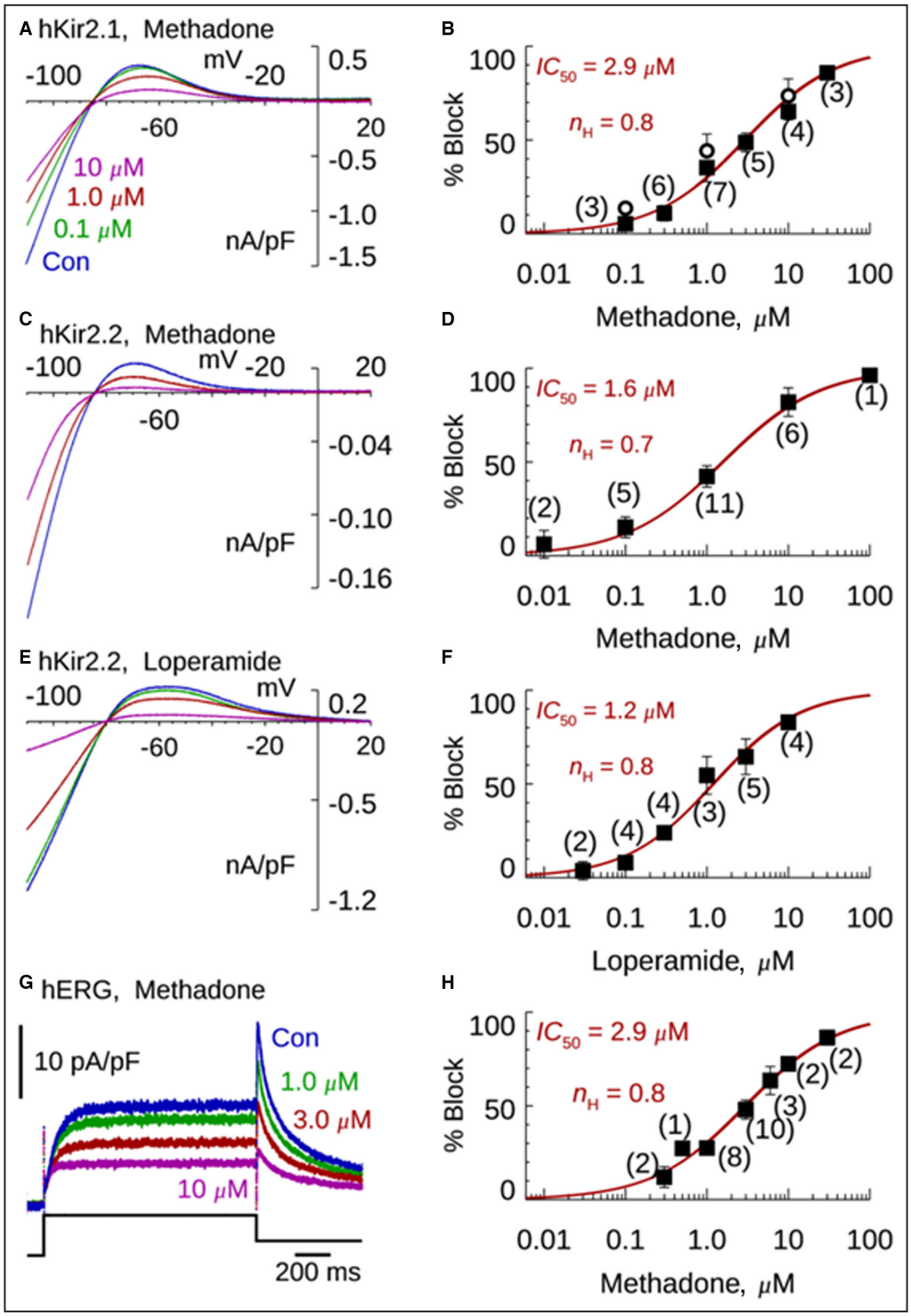
The effects of methadone on APD were quantified (Figure 3A through 3D) given the established association of AP prolongation with clinical QTc prolongation.[26] As expected given its known property of I_{Kr} inhibition,[9] methadone caused prolongation of the APD as compared with the preexposure controls at concentrations of 0.1 µmol/L and 1 µmol/L. Higher concentrations of methadone (10 µmol/L), however, significantly shortened the APD. Methadone also caused a concentration-dependent decline in the maximum upstroke rate of phase 0 of the AP, reflecting diminished Na^+ conductance (dV_M/dt_{Max}, Figure 3F). Figure S3 shows the effects of methadone dose on changes of APD_{10}, APD_{30}, and APD_{70}.

Methadone Prolongs *TRAP* and Increases Repolarization Instability

Inhibition of I_{K1} and slowing of terminal repolarization of the AP are associated with a marked decrease in postrepolarization refractoriness and an increase in diastolic instability that can make the heart more susceptible to reentrant arrhythmias.[4] Consistent with the blockade of I_{K1}, methadone slowed terminal repolarization and induced an increase in *TRAP* in a concentration-dependent fashion (Figure 3E; Figure S4 shows the evaluation of *TRAP* in each AP of Figure 3A through 3C). The beat-to-beat variability of APD_{90} (Figure 3G), variance of diastolic voltage fluctuations (DV, Figure 3H), and mean number of delayed afterdepolarizations per diastolic interval (DADs, Figure 3I) also increased in the presence of methadone in a dose-dependent manner. Figure S5 shows examples of increased diastolic voltage fluctuations, and DADs, caused by 1 µmol/L or 10 µmol/L of methadone. Consistent with inhibition of both I_{K1} and I_{Kr}, increases in the latter quantities represent markers of proarrhythmia in isolated cells that track the effects on terminal repolarization and *TRAP* (Figure 3E) more closely than does APD_{90} (Figure 3D).

Impact of Methadone on Surface ECG

The sociodemographic and clinical characteristics of the patients were consistent with a vulnerable population with ongoing opioid-use disorder. The median



age was 33 years (interquartile range, 28–44 years), 44% were women, and 37% were of Hispanic ethnicity. Cardiovascular disease comorbidities were uncommon (1% had a history of coronary artery disease). No patient developed ventricular arrhythmia or sudden death during the study period. The proportion of patients receiving prescription drugs known to prolong the QTc interval did not differ from baseline to postinduction (3.9%±4.6% to 3.3%±4.0%, P=0.65). As expected, a greater proportion of patients manifested positive toxicology for illicit drugs associated with I_{Kr} blockade/QTc prolongation at baseline (P=0.006). QTcF intervals were longer in women (418.8±3.2 versus 414.5±2.9, P=0.31). Conduction and repolarization parameters before and following initiation of methadone are shown in the Table.

Figure 1. The effects of methadone and loperamide on inward rectifier K⁺ current (I_{K1}) in heterologous cells expressing isoforms of hKir2.1 and hKir2.2.
A, Inhibition of I_{K1} mediated by hKir2.1. Each record is a single-sweep from a hyperpolarizing ramp command voltage, in control (Con) and during serial exposure to increasing methadone concentrations, coded by line and label color. Records are corrected for Ba^{2+}-insensitive current. **B**, Concentration-dependence of hKir2.1-mediated I_{K1} inhibition by methadone. Filled squares are means±SEM for the indicated number of stably transfected human embryonic kidney 293 (HEK-293) cells. Open symbols represent current inhibition from transiently transfected COS cells. The red line indicates the fit of a Hill binding equation to the HEK-293 cell data, with a half-maximal inhibitory concentration (IC_{50}) of 2.9 µmol/L and Hill coefficient (n_H) of 0.7. A total of 23 cells from 10 preparations. **C** and **D**, Blockade of I_{K1} currents in COS cells transiently transfected with hKir2.2. A total of 13 cells from 5 preparations. **E** and **F**, Blockade by loperamide of I_{K1} in COS cells transiently transfected with hKir2.2. A total of 15 cells from 4 preparations. **G** and **H**, Block of human ether-à-go-go–related gene (hERG) current expressed in CHO-K1 cells by methadone. A total of 23 cells from 6 preparations. Data points in (**B**, **D**, and **F**) represent the percent inhibition of outward (repolarizing) I_{K1} current by drug, and in (**H**) the peak outward current during a voltage step from +20 to −50 mV (voltage pulse, **G**).

As expected, there was a modest increase in the QTcF interval from 417 to 428 ms (+11 ms, n=77 pairs, P<0.001) at a mean methadone dose of 64±24 mg/d. Moreover, the QTcF interval was positively and significantly correlated with methadone dose (Figure 4A), as was the T_{Peak} to T_{End} interval (Figure 4B) (but not T_{Peak} to T_{End}/QT; Figure 4C), indicative of methadone-induced slowing of terminal repolarization.

Preinduction U waves (baseline) were largest in leads V2 and V3 (Figure 5A) but were often readily observable and amenable to quantitative analysis in the other precordial and limb leads. U waves at baseline and after methadone stabilization for each patient were extracted from the T-U segment of mean-beat ECG records (Figure 5B) by fitting the waveform (Equation 1, Methods), and is illustrated in Figure 5C.

U-wave integrals (∫U wave) from all limb and precordial leads were larger following methadone induction. ∫U wave increased distinctly following methadone treatment (lead V3) in 68% of patients with a heart rate <85 beats per minute (28/41); the net increase in ∫U wave compared with premethadone measures averaged 38%±15% (P=0.016). The mean difference in the ∫U wave magnitude (methadone–baseline) was significantly increased after methadone induction in precordial leads V3 to V6 (Figure 5D). However, the change in ∫U wave did not show an apparent dose dependence (Figure 5E).

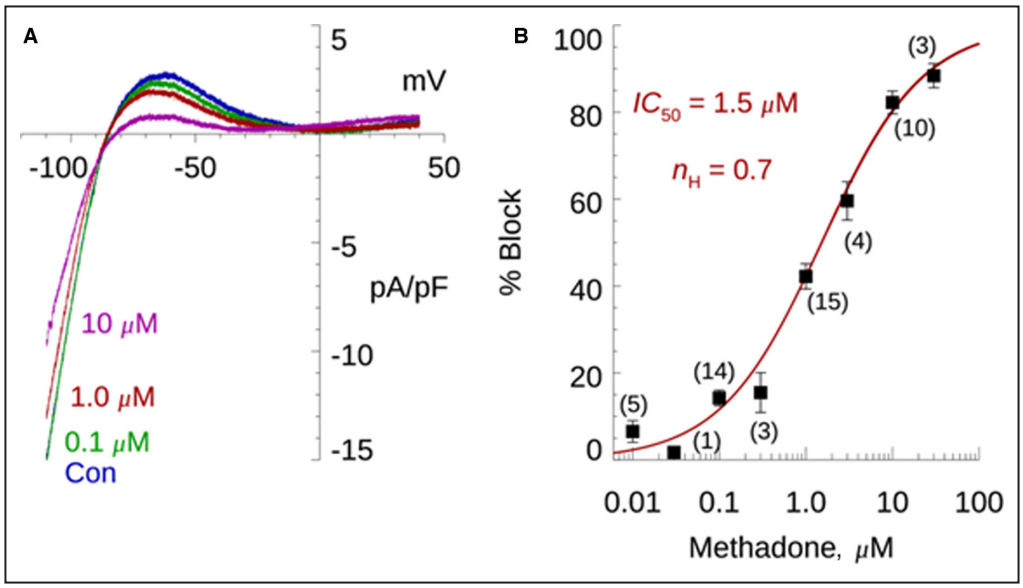


Figure 2. Methadone blockade of inward rectifier K⁺ current (I_{K1}) ionic current in swine ventricular cardiomyocytes.
A, I_{K1} currents in an isolated ventricular myocyte in control (Con) and at indicated increasing concentrations of methadone in extracellular solution. Each record is a single-sweep during a hyperpolarizing ramp command voltage. Currents are corrected for Ba^{2+}-insensitive current (1 mmol/L) and normalized to cell membrane capacitance. **B**, Inhibition of I_{K1} outward current vs methadone concentration from the indicated number of cells. A total 23 total myocytes from 9 animals. Values are means±SEM. The red line is a fit of the Hill binding equation, with a half-maximal inhibitory concentration (IC_{50}) of 1.5 µmol/L and Hill coefficient (n_H) of 0.7.

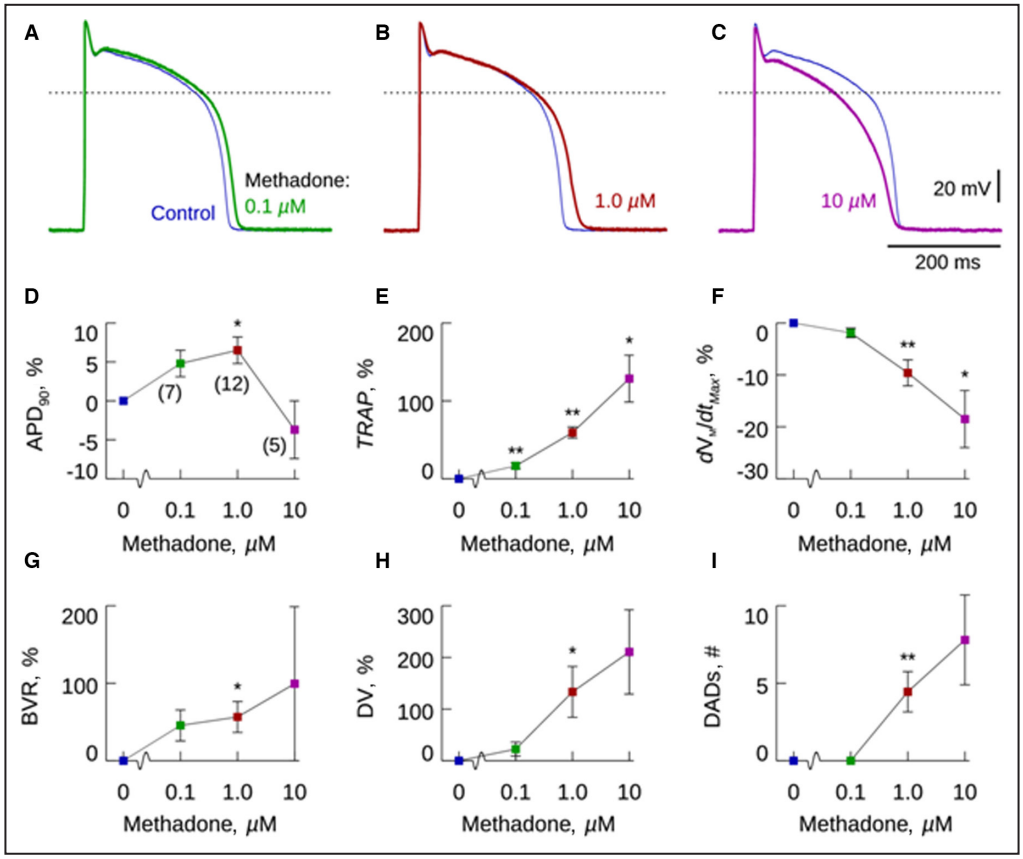


Figure 3. Effects of methadone on action potential (AP) and membrane stability properties. **A**, Methadone (0.1 μmol/L) prolongs the AP duration (APD; green) compared with control (blue). **B**, Methadone 1 μmol/L further prolongs APD and significantly slows terminal repolarization (phase 3). **C**, Methadone 10 μmol/L causes AP shortening, delayed terminal repolarization, and triangularization. **D** through **I**, Show impact of methadone on APD_{90}, terminal repolarization of the AP (*TRAP*), dV_M/dt_{Max}, beat-to-beat APD variability (BVR), diastolic variance (DV), and the mean number of delayed afterdepolarizations (DADs; 1.5- to 2-second interval range) at different methadone doses. respectively. Values are expressed as percent change (±SEM) from pretreatment baselines in (**D** through **H**) and mean number±SEM (**I**). Number of cells indicated in (**D**). **P*<0.05, ***P*<0.02.

DISCUSSION

In this translational investigation combining the study of recombinant ion channels, freshly isolated cardiac myocytes, and human patients, several novel observations emerge that could explain a disproportionate occurrence of ventricular arrhythmia among methadone-treated patients. To our knowledge, this is the first study to demonstrate methadone blockade of I_{K1} at clinically relevant concentrations comparable to its effect on I_{Kr} current. In agreement with this inhibitory action on I_{K1}, higher doses of methadone caused progressive slowing of terminal repolarization and increased the incidence of delayed afterdepolarizations consistent with reduced cardiac membrane stability. On ECG analysis, methadone induction resulted in significantly prolonged terminal repolarization as manifested by U-wave augmentation and lengthening of the T_{Peak}–T_{End} interval. Pursuing parallel tracks of investigation, the current study therefore offers new insight into the potential mechanism of methadone's distinct arrhythmia risk, strengthens the proposed association between I_{K1} blockade and U-wave augmentation, and, more broadly, suggests that the U-wave integral could serve as a novel parameter for evaluation of drug-induced loss of repolarization reserve.

Methadone Potently Inhibits I_{K1}

I_{K1} is found in both atrial and ventricular myocytes but is expressed in greater density within the ventricles,[26] suggesting potential clinical relevance for the genesis of ventricular arrhythmia. The I_{K1} is the dominant conductance in terminal repolarization and phase 4 of the AP. It functions to hold the membrane potential near the equilibrium potential for potassium.[26,27] Conductance of I_{K1}, therefore, is thought to "stabilize" the resting membrane potential at strongly negative values, preventing premature

Table 1. Conduction and Repolarization Parameters Before and After Methadone Induction

Parameter	Pretreatment	Postinduction	Change	*P* value
HR, beats per min	73.1±1.7	70.1±1.6	−3	0.137
PR interval, ms	146.8±2.5	150.9±2.4	4.1	0.010
QRS interval, ms	89.0±1.2	90.4±1.2	1.4	0.073
QT interval, ms	393.9±3.8	410.0±3.8	16.1	<0.001
Corrected QT interval with Fridericia formula, ms	416.6±2.1	427.8±2.3	11.2	<0.001
U-wave amplitude, μV*	51.2±4.0	60.0±4.0	9.1	<0.002
U-wave integral, mV·ms Lead V3, n=41*	7.7±0.6	9.2±0.7	1.5	<0.01
T_{Peak} to T_{End}				
All pairs n=77	70.3±1.7	84.0±2.6	14.0	<0.001
HR <85 beats per min, n=41*	79.4±3.6	98.0±4.4	18.3	<0.001

*Reflects analysis sample with a heart rate (HR) of <85 beats per minute.

depolarizations that could trigger extrasystoles or lead to reentry, thus moderating overall ventricular excitability.[28] Contrary to our findings of a significant inhibition of I_{K1} by methadone, Kuryshev et al[11] previously reported no significant effect at room temperature on I_{K1} mediated by hKir2.1 expressed in CHO cells. This discrepancy may reflect the impact of lower temperature on the development of channel block.[29] At physiologic temperatures, we observed suppression of I_{K1} by both methadone and the structurally similar compound loperamide in both isoforms at low micromolar concentrations. Importantly, we confirmed methadone's potent block of I_{K1} in freshly isolated pig myocytes assessed at physiological temperature, and conclude that methadone blocks the I_{K1} at concentrations likely to be both clinically relevant and equally potent in their influence on I_{Kr} and I_{K1}. Peak free drug concentrations in methadone users are ≈0.3 μmol/L, lower than methadone's IC_{50} for suppression of I_{Kr} and I_{K1}. Serum values may be unreliable indicators of myocyte receptor interactions, however. Directly measured cardiac concentrations of methadone averaged 4.7-fold higher than serum concentrations at autopsy,[30] and therefore appear comparable to the IC_{50} for suppression of I_{K1}. These data suggest that ordinary use of methadone will often result in substantial inhibition of I_{K1}. Assessing the drug impact on myocyte APs, higher doses of methadone caused progressive slowing of terminal repolarization, increased spontaneous diastolic membrane fluctuations, and resulted in greater frequency of afterdepolarizations, with each finding consistent with a decrease in cardiac membrane stability and confirming the physiologic significance of a reduction in I_{K1}.

Role of Combined I_{K1} and I_{Kr} Blockade in Arrhythmogenesis

While nearly all drugs that prolong the QTc interval block I_{Kr} to some extent, susceptibility to arrhythmia may be augmented by concomitant blockade of I_{K1}. Loss of I_{K1} is a consistent finding in animal models of heart failure caused by reduced ejection fraction,[31] and the addition of I_{Kr} inhibitors is associated with increased proarrhythmia during heart failure treatment in humans.[32] While human heart failure presents a complex phenotype beyond an isolated reduction in I_{K1}, a more specific, albeit rare, condition known as Andersen-Tawil syndrome type 1 (ATS-1) is associated with isolated loss-of-function mutations in the *KCNJ2* gene encoding the Kir2.1 channel.[33] Zhang et al[34] reviewed ECG manifestations and found a significant increase in U-wave amplitude in 71% and a delay in the terminal portion of the T wave in 70% of those with ATS-1, with changes proportionately similar to those associated with methadone treatment observed in the current study. Moreover, a natural history study of 118 patients with ATS-1 revealed a 7.9% incidence of life-threatening arrhythmia within only 5 years.[35] In a multivariate analysis, they found that treatment with amiodarone, a potent I_{Kr} inhibitor, increased the risk of life-threatening arrhythmia by >200-fold. These results are consistent with the notion that while loss of I_{K1} function itself confers modest arrhythmia liability, combined inhibition of I_{K1} and I_{Kr} may be particularly hazardous with regard to triggering malignant arrhythmia.

Further support for the importance of combined I_{Kr} and I_{K1} blockade was shown in a transgenic rabbit model.[36] In atrioventricular node–ablated, excised hearts with an LQT2 phenotype and genetically reduced I_{Kr}, spontaneous arrhythmic activity was infrequent (<5% of premature ventricular beats). However, the addition of barium chloride, a known I_{K1} inhibitor, resulted in spontaneous ventricular tachycardia and fibrillation, and arrhythmic activity was present for >50% of infusion duration. In aggregate, these data bolster the contention that the combination of I_{Kr} and I_{K1} blockade, as demonstrated by methadone, would be anticipated to have far greater proarrhythmic

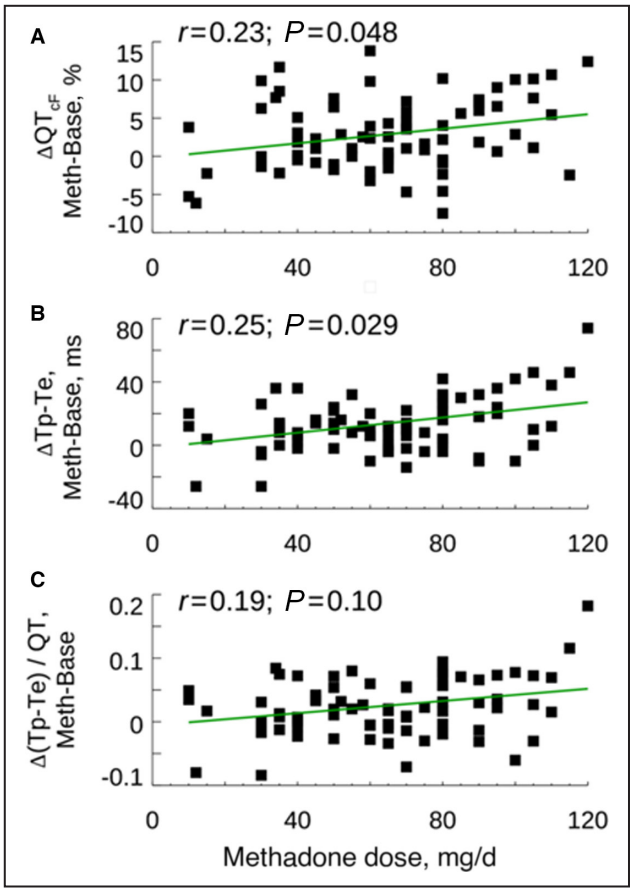


Figure 4. Repolarization changes with methadone. **A**, Squares represent the percent change of corrected QT with Fridericia formula (lead V5, n=77) in baseline and methadone pairs vs methadone dose. **B**, Change of T_{Peak} to T_{End} interval (methadone-baseline, ms, lead V5) vs methadone dose. **C**, Change of T_{Peak} to T_{End} normalized by QT interval vs methadone dose. In each panel the green line represents the linear regression to the data, and Spearman *r* and corresponding *P* value are indicated.

impact than either alone. Current US Food and Drug Administration guidance does not mandate interrogation of the inward rectifier as part of preclinical testing for new drug applications, and this may reduce the prognostic utility of preclinical testing.[37]

U Wave in Drug-Associated Arrhythmia Risk Assessment

In our study of ECG changes in humans, methadone induction resulted in significantly prolonged terminal repolarization as manifested by U-wave augmentation and lengthening of the T_{Peak} to T_{End} interval. The current study therefore introduces proof-of-concept for a potentially new paradigm for understanding the mechanism of methadone's distinct arrhythmia risk. Additionally, the data strengthen the proposed association between I_{K1} blockade and U-wave amplitude, which suggests that the U-wave integral could serve as a novel tool for the evaluation of drug-induced loss of repolarization reserve when assessing drug safety.

The International Conference on Harmonization guidance for assessing the proarrhythmic potential for nonantiarrhythmic drugs recommends reporting changes in U-wave morphology, but not their magnitude or duration, during new drug development.[38] This lack of emphasis was attributed to uncertainty regarding the predictive value of the U wave.[38] This document, released in 2005, reflected the prevailing belief that U waves are a normal finding of unclear provenance. Using clinical data from individuals having either gain- or loss-of-function variants in *KCNJ2*, however, Postema et al[15] showed that a genetically determined reduction in I_{K1} resulted in an increase in U-wave magnitude, while an increase in I_{K1} had the opposite effect. When combined with our findings, the totality of experimental and clinical data support the contention that the U wave represents a voltage gradient persisting in the terminal phase of repolarization, ie, beyond 90% repolarization of the ventricles, and that its amplitude reflects the time course of the terminal portion of phase 3. Whether inclusion of the U wave in future arrhythmic prediction models is justified will depend on the results of studies employing quantitatively precise methods similar to those presented here.

Limitations

This study has several limitations. We did not measure serum methadone levels in patients undergoing methadone induction, as this is not routinely performed in clinical practice. Therefore, we cannot precisely correlate pharmacokinetics with either the QT interval or the degree of suppression of I_{K1} or I_{Kr} in the heart. Published values of the maxium concentration for methadone in patients undergoing opioid replacement therapy are 5- to 10-fold lower than the IC_{50} for suppression of I_{K1} and I_{Kr} as reported here; however, cardiac tissue levels of methadone may be significantly higher than measured blood levels.[30] Indeed, we observed QT prolongation in patients following methadone induction, indicative of significant suppression of I_{Kr}, and likely I_{K1} as well. However, we observed no cardiac arrhythmias or arrest in patients involved in the study. Additionally, the ECGs were obtained under clinical conditions and not as part of a focused QT study. We observed an increase in the mean number of DADs in a dose-dependent manner. While the appearance of DADs is not pathognomonic for membrane instability, the confluence of APD prolongation, increased beat-to-beat variability of APD, increased diastolic variance, and increased DADs promote phase 4 instability and enhanced arrhythmogenicity. Finally, we did not examine methadone's effects in a whole-heart or intact animal model of chronic use, which would permit the induction of sustained arrhythmias.

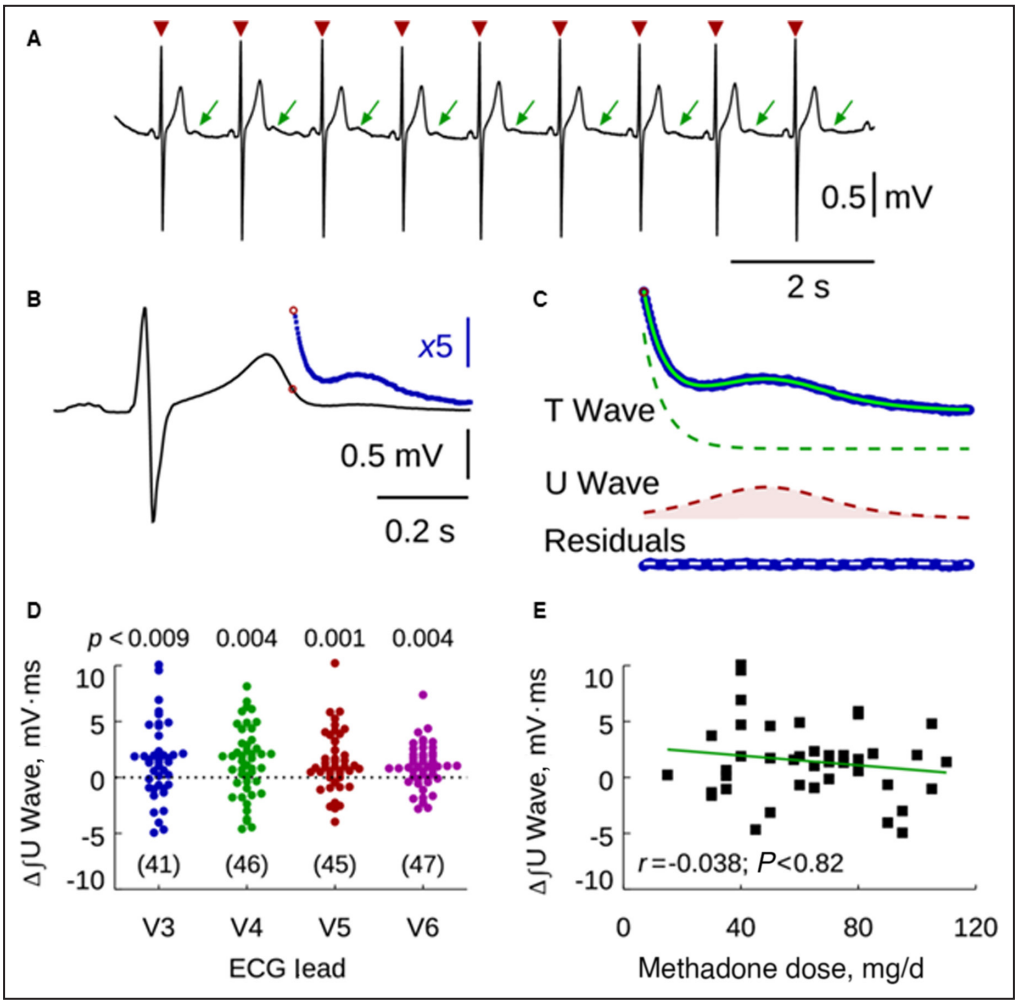


Figure 5. Ascertainment and quantification of U-wave integral changes with methadone induction.

A, A 10.6-second patient ECG record (lead V3) following methadone induction. A threshold algorithm identified R waves (red arrowheads). U waves are evident (green arrows) following each T wave. The mean heart rate in the example was 54 beats per minute, corrected QT with Fridericia formula was 402 ms, and methadone dose was 30 mg/d. **B**, Nine individual beats aligned at the R wave and averaged to produce a single mean beat. The segment containing the terminal T wave plus U wave is shown (blue) on a 5-fold expanded vertical scale. Red open symbols indicate the start of the fitted region. **C**, The T- and U-wave segments (blue) after fitting text Equation 1 to the data (green, superimposed). The resulting contribution of the terminal T wave (green dashed) and U wave (red dashed) comprising the fit are shown. The red shaded region represents the U-wave integral, 10.8 mV·ms. The residuals of the fit (lower blue record) uniformly span the zero line (white dashed), indicating an excellent fit to the record. The parameter values of the fit of Equation 1 were: A_T=204.2 μV, τ_T=62.0 ms, t_P=90.3 ms, A_U=370.6 μV, τ_U=50.6 ms, and reduced χ^2 of fit 0.069. **D**, U-wave integral differences (methadone−baseline) as dot plots for leads V3, V4, V5, and V6. Number of pairs (n) and *P* value of differences are indicated (paired *t* test). Dot values >0 indicate an increase of U-wave integral after methadone induction. **E**, Change of U-wave integrals (methadone−baseline, lead V3) vs methadone dose, showing no correlation with daily dose (Spearman *r* coefficient and corresponding *P* value).

In conclusion, we have demonstrated that methadone blocks both the inward rectifier and rapid component of the delayed rectifier K^+ currents, resulting in the slowing of terminal repolarization and an increase in U-wave integral on the surface ECG. This dual inhibitory action may figure importantly in methadone's evident and unique association with lethal arrhythmia. Neither the inward rectifier current nor the U wave are currently interrogated in drug development. We believe that consideration should be given to the addition of these complementary in vitro and in vivo pharmacodynamic targets in the evaluation of proarrhythmic liability.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Figures S1–S5

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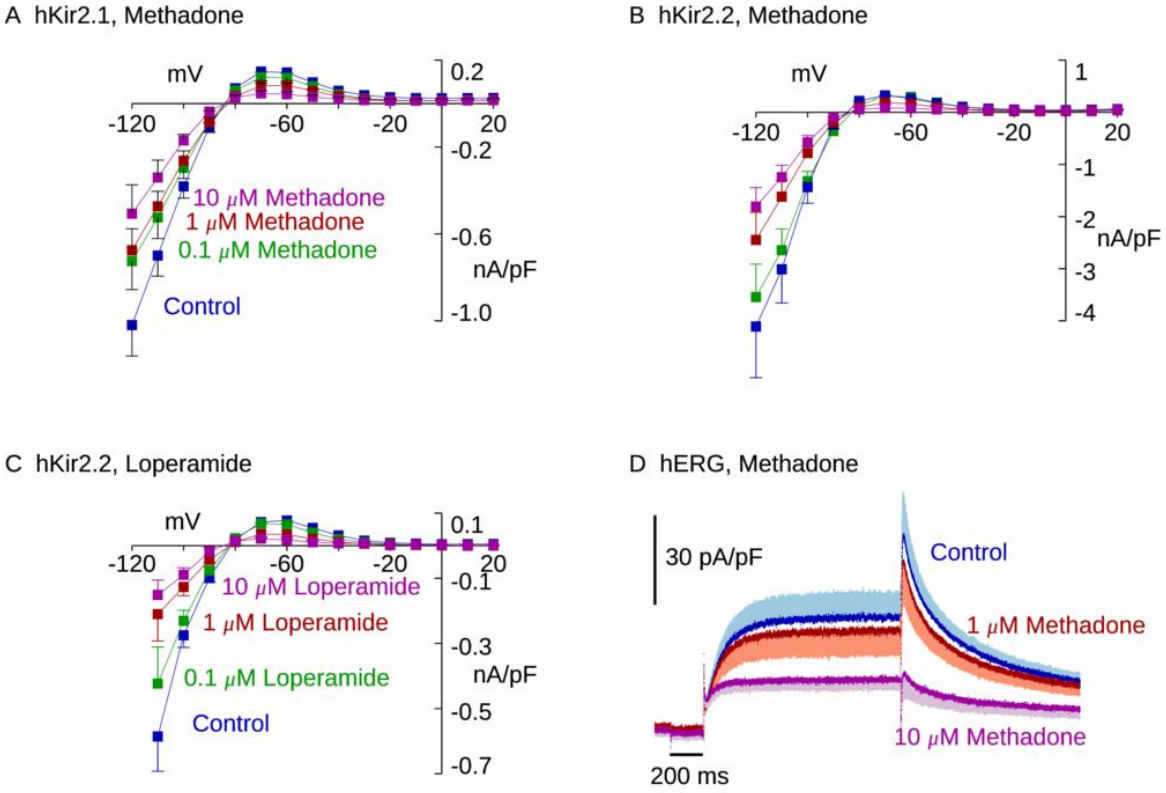
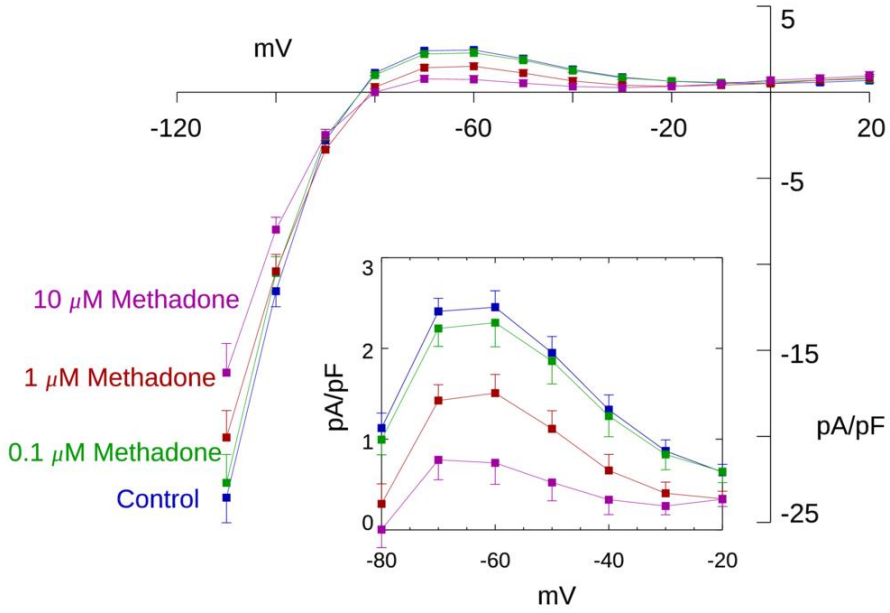
Supplemental Material

Figure S1. Effects of methadone (A, B, D) and loperamide (C) on IK1 and hERG ionic currents.



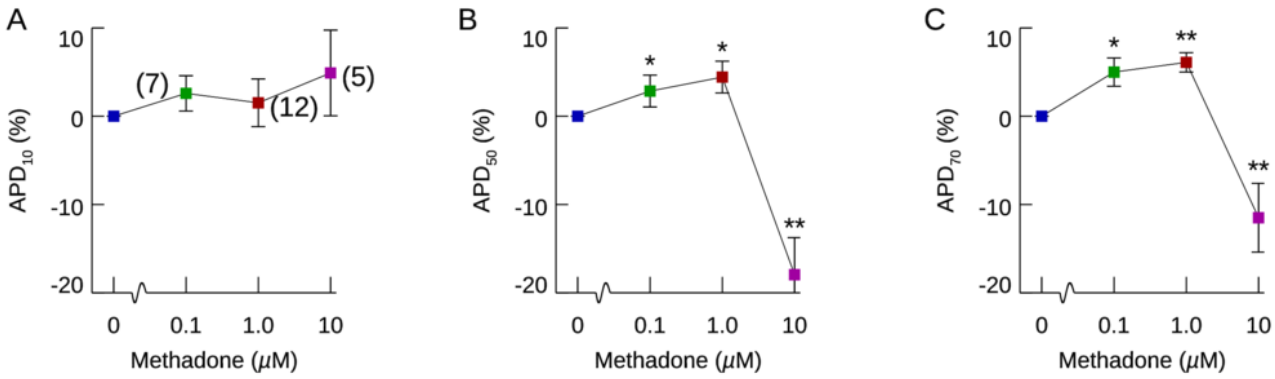
The points represent the current-voltage (*I-V*) relationship of mean (± SEM) values of data, normalized by cell capacitance, used to construct the concentration-inhibition plots in Fig. 1 for Kir2 isoforms hKir2.1 (A) and hKir2.2 (B, C). Panel D shows the inhibition of hERG ionic current by indicated concentrations of methadone. Each record is the mean of 3 cells with the SEM represented by shaded regions.

Figure S2. Current-voltage relationship of IK1 and inhibition by methadone in ventricular myocytes from swine.



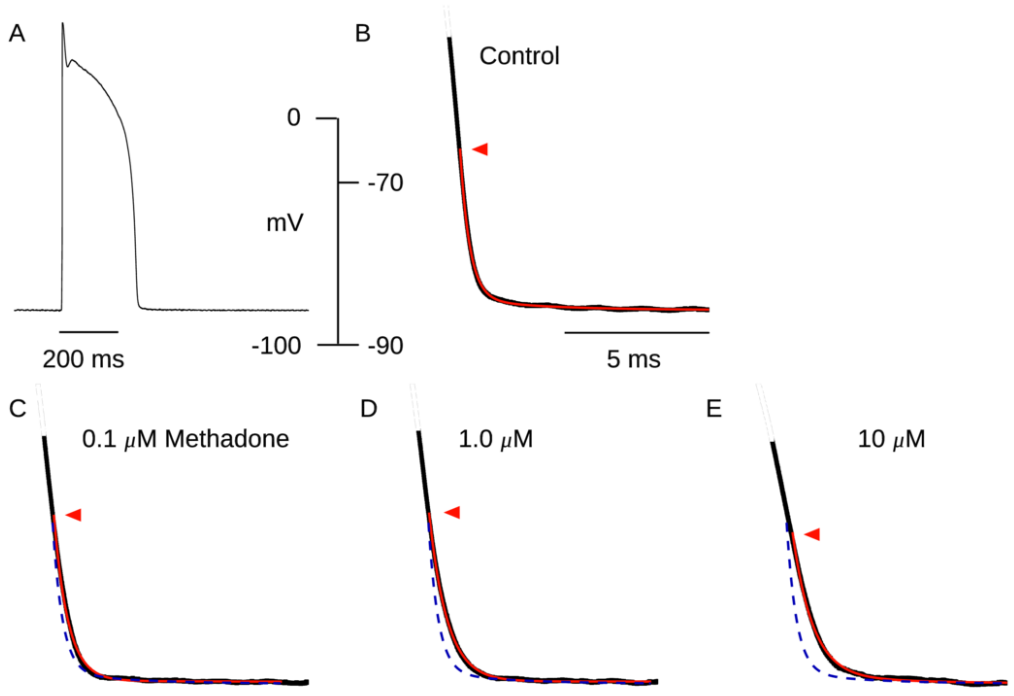
The points are mean (± SEM) values from 20 myocytes and 8 animals. The inset shows an expanded region of outward (repolarizing) ionic current between -80 and -20 mV.

Figure S3. Effect of methadone on action potential duration (APD) at 10% repolarization (A), 50% (B) and 70 % (C).



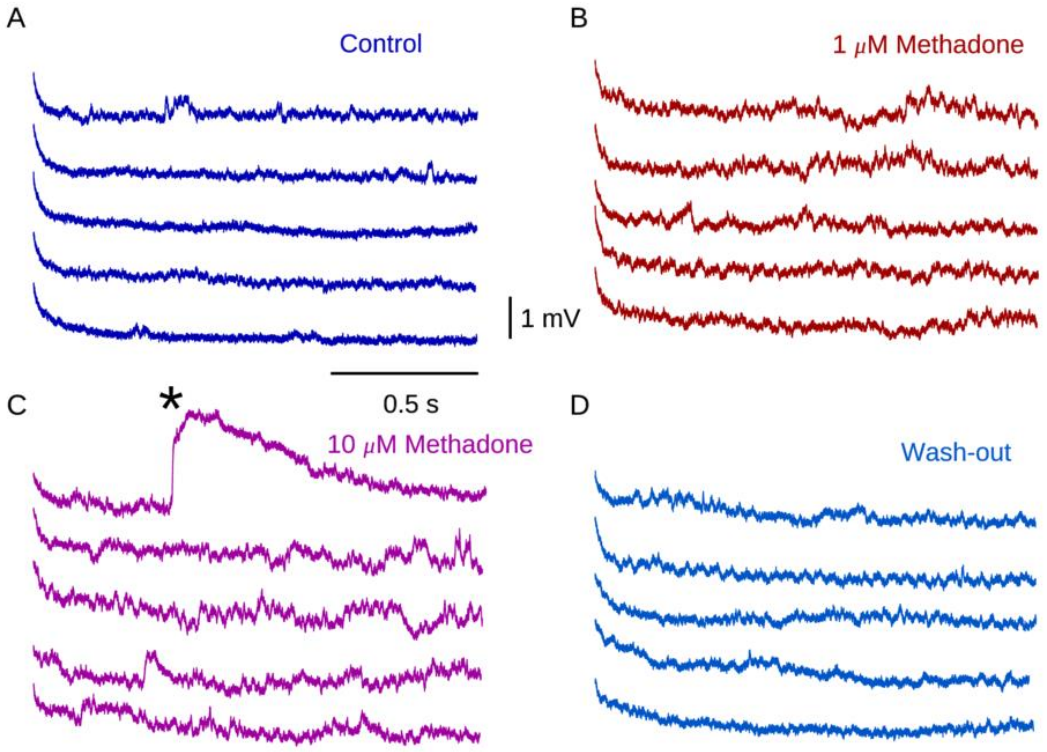
Points are mean (± SEM) percent difference from control. Lower concentrations of methadone (0.1, 1 µM) caused AP prolongation in panels B and C while 10 µM methadone caused significant AP shortening. * $p < 0.05$; ** $p < 0.02$.

Figure S4. Evaluation of the *TRAP* parameter during terminal repolarization of the APs shown in text Fig. 3A-C.



A, Control AP. B, Terminal repolarization of the AP of panel A, showing AP phases 3 and 4 at higher amplitude and time scale. A non-linear least-squares fit of a 2-exponential plus constant function to this phase is superimposed on the record (red line). The red arrowhead indicates the start of the fit. The *TRAP* parameter is the time constant of the faster exponential, 3.3 ms in this example. Note that the fit is contained within the noise of the record. C-E, Terminal phase of APs during exposure to methadone; 0.1 µM (C), 1.0 µM (D) and 10 µM (E). The *TRAP* parameters are, respectively, 4.7 ms, 6.1 ms and 8.8 ms. The fit to the control record is superimposed on the records in methadone as dashed blue lines.

Figure S5. Effect of methadone on diastolic membrane voltage instability.



Each panel shows 5 stacked records of membrane voltage following paced (0.3 Hz) action potentials. A, Control; B, during exposure to 1 µM methadone; C, during exposure to 10 µM methadone; D, following wash-out. Note the increase in instability caused by exposure to drug (B, C) and reversal upon wash-out (D). In C a delayed after-depolarization is indicated by *, representing a transient depolarization greater than 2 mV above the mean diastolic potential.