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Comparative Evaluation of *HERG* Currents and QT Intervals following Challenge with Suspected Torsadogenic and Non-torsadogenic Drugs

Alexander N. Katchman, John Koerner, Toshimasa Tosaka, Raymond L. Woosley, and Steven N. Ebert

Department of Pharmacology, Georgetown University Medical Center, Washington, DC (A.N.K. and T.T.); United States Food and Drug Administration, Rockville, MD (J.K.); The C-Path Institute, Tucson, AZ (R.L.W.); and Biomolecular Science Center, The University of Central Florida, Orlando, FL (S.N.E.)

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Corresponding Author:

Steven N. Ebert, PhD

Associate Professor

Biomolecular Science Center

4000 Central Florida Blvd.

University of Central Florida

Building 20 (BMS)

Orlando, FL 32816-2364

Tel: (407) 823-4609

Fax: (407) 823-0956

e-mail: ebert@mail.ucf.edu

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Abbreviations:

As_2O_3	Arsenic trioxide
ECG:	Electrocardiogram
HERG:	Human ether-a-go-go related gene
HEK:	Human embryonic kidney
i.v.:	intravenous
TdP	Torsade de pointes
I_{Kr}	Rapid component of delayed rectifier K^+ current

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ABSTRACT

The purpose of the present study was to comparatively evaluate *HERG* currents and QT Intervals following challenge with suspected torsadogenic and non-torsadogenic drugs. Various concentrations of 14 different drugs were initially evaluated in terms of their relative potency to block I_{HERG} in stably-transfected HEK cells. Four general categories of drugs were identified: (1) High-potency blockers (IC_{50} < 0.1 μM) included lidoflazine, terfenadine, and haloperidol; (2) Moderate-potency blockers (0.1 μM < IC_{50} < 1 μM) included sertindole, thioridazine, and prenylamine; (3) Low-potency blockers (IC_{50} > 1 μM) included propafenone, loratadine, pyrilamine, lovastatin, and chlorpheniramine; and (4) Ineffective blockers (IC_{50} > 300 μM) included cimetidine, pentamidine, and arsenic trioxide. All measurements were performed using similar conditions and tested acute drug effects only (< 30 mins of drug exposure per measurement). Since two of the drugs that were ineffective I_{HERG} blockers, arsenic trioxide and pentamidine, have been associated with cardiac repolarization delays (QT interval lengthening) and torsades de pointes ventricular arrhythmias in patients, we chose to evaluate them further using the isolated perfused rabbit heart model. Neither arsenic trioxide nor pentamidine had any significant effect on QT intervals in this model, even at relatively high (micromolar) concentrations. Similar results were obtained for loratadine in this model. When the hearts were challenged with a known torsadogenic drug such as cisapride, significant QT-lengthening was rapidly induced. These results demonstrate that arsenic trioxide and pentamidine are essentially devoid of direct acute effects on cardiac repolarization or inhibition of I_{HERG}.

Introduction

QT interval prolongation and an associated severe life-threatening ventricular arrhythmia, *torsade de pointes* (TdP), are a high priority cause for concern in drug development and regulatory safety evaluation (Fenichel et al., 2004). Of the drugs recently removed from the U.S. market, one of the most common causes has been QT interval-related cardiac toxicity. This toxicity was discovered either after approval during clinical use or in late stage clinical trials rather than in early drug development, with significant resultant difficulties. Notably, women have tended to be much more susceptible than men to these cardiotoxic drug effects (Makkar et al., 1993; Lehmann et al., 1997).

Given the medical and economic consequences of this issue, the International Conference on Harmonization established an Expert Working Group to draft guidance recommending the incorporation into drug development of preclinical models predictive of QT interval prolongation and proarrhythmia. This draft guidance, ICH S7B, was published in revised form for comment in the Federal Register in June, 2004 (Attachment 5). ICH S7B recommends a testing strategy comprised of both *in vitro* and *in vivo* assays considered likely to be predictive for drug-induced QT interval prolongation and proarrhythmia.

A large number of drugs from a wide variety of classes, including antihistamines, antipsychotics, antiarrhythmics, antibiotics, and gastrointestinal prokinetic agents have been associated with the syndrome of TdP, a potentially fatal form of ventricular cardiac

arrhythmia. Many of the drugs that have been associated with drug-induced development of TdP arrhythmias have also been shown to block the rapid component of the delayed rectifier repolarizing potassium current, I_{Kr}, in ventricular cardiomyocytes (Nattel, 1999). The major channel protein responsible for I_{Kr} is encoded by the *human ether-a-go-go-related gene* (HERG) gene, also known as KCNH2 (Curran et al., 1995). Zhou et al. (1998) demonstrated that expression of the HERG gene in stably transfected HEK cells produces a current, I_{HERG}, that has the characteristics of I_{Kr}. This cell line has proved to be useful for studies examining pharmacological blockade of I_{Kr}. Since HEK cells produce few other currents, the results obtained from these cells are typically much easier to acquire and interpret than are those from studies that use freshly isolated ventricular cardiomyocytes. There are a growing number of drugs being monitored by the FDA and others because of their suspected involvement in the development of TdP-like cardiac arrhythmias. At present, there are limited systematic data available regarding the influence of many suspected arrhythmogenic compounds on I_{HERG}. Thus, the purpose of the present study was to determine whether or not a subset of these compounds with a range of risk for TdP arrhythmias can block I_{HERG}, and if so, what their relative potency is.

We comparatively evaluated 14 compounds for their ability to block I_{HERG} in a stably-transfected cell culture model system. Many of these compounds, including lidoflazine, terfenadine, haloperidol, sertindole, thioridizine, prenylamine, propafenone, pyrilamine, pentamidine, and arsenic trioxide, have been associated with clinical Long QT Syndrome and TdP arrhythmias in patients. A subset of these drugs (lidoflazine,

terfenadine, haloperidol, sertindole, thioridazine, and propafenone) have previously been shown to be relatively potent blockers of I_{HERG} (Hanley and Hampton, 1983; Drolet et al., 1999; Connolly et al., 1983), whereas there were limited and sometimes conflicting reports regarding the effects of loratadine, prenylamine, pyrilamine, pentamidine, and arsenic trioxide on I_{HERG} (Guijarro et al., 1976; Drolet et al., 2004; Ficker et al., 2004). The present study, therefore, set out to conduct a systematic comparison of all of these compounds to determine their relative potencies for blocking I_{HERG}. Several 'negative control' compounds, including, lovastatin, chloropheniramine, and cimetidine were also evaluated under similar conditions.

Two compounds in particular, arsenic trioxide and pentamidine, had little or no effect on I_{HERG}, though both of these compounds have been associated with Long QT Syndromes and TdP-like arrhythmias in patients (Wharton et al., 1987; Bibler et al., 1988; Stein et al., 1991; Eisenhauer et al., 1994; Ohnishi et al., 2000; Unnikrishnan et al., 2001; Barbey and Soignet, 2001). Thus, to further evaluate the potential for these compounds to influence cardiac repolarization and cause arrhythmias, we also examined their influence in the isolated perfused (Langendorff) rabbit heart model. The isolated rabbit heart has been shown previously to be sensitive to torsadogenic drugs (Asano et al., 1997; Drici et al., 1999; Liu et al., 1999) and hence, would fit into the preclinical strategy of early detection of torsadogenic hazard. Our results suggest that neither arsenic trioxide nor pentamidine have any significant acute effect on cardiac repolarization.

Materials and Methods

Drugs and Chemicals

Racemic methadone was obtained from Eli Lilly & Co. (Indianapolis, IN). Lidoflazine was obtained from Research Diagnostics Inc. (Flanders, NJ). Loratadine was obtained from Schering-Plough (Kenilworth, NJ). All other drugs and chemicals were purchased from Sigma-Aldrich (St. Louis, MO). For each compound tested, a concentrated stock solution (>10 mM) was prepared by dissolving the powder in deionized Milli-Q water or DMSO. Small aliquots of the concentrated stocks were immediately frozen and stored at −80°C. Aliquots were thawed immediately before use and diluted to the desired final concentration in Tyrode's solution.

Cell Culture

HEK 293 cells that stably express HERG were obtained from Dr. Craig January (Zhou et al., 1998). These cells were maintained using the culture conditions previously described by Zhou et al. (1998).

Whole-Cell Patch-Clamp

Coverslips with stably transfected HERG-expressing HEK cells were placed in a recording chamber (ΔT3 Dish, Bioptechs, Butler, PA), mounted on the stage of an inverted microscope (Olympus IX50). The superfusion rate was 0.5 ml/min. The time needed to change the solution near the cell was estimated to be less than 0.2 s. Output signals from the amplifier were digitized using a DigiData 1200 A/D, D/A board in

conjunction with an IBM PC-compatible computer. This system was used to generate command pulses and to acquire and analyze the data.

Whole-cell voltage-clamp measurements were obtained by ruptured patch clamp technique. For this purpose the Axopatch 200B Amplifier (Axon Instruments Inc.,Union City, CA) was used. Recording electrodes, 2-5 MOhm resistance, were made from thin walled borosilicate glass TW150F (WPI, Sarasota, FL). Measurements were checked for possible rundown during the experiment. Creation of voltage-clamp command pulse protocols and data acquisition were controlled by pCLAMP software (Version 8.01, Axon Instruments Inc., Foster City, CA) installed on a personal computer (Compaq Deskpro 4000).

The bath solution consisted of (in mM): NaCl ,137; KCl, 5.1; $CaCl_2$, 2; HEPES, 10; $MgCl_2$, 1; and Glucose ,10. The pH was adjusted to 7.4 by addition of NaOH. The pipette filling solution contained: (in mM): KCl ,140; Mg-ATP, 4; EGTA, 5; $MgCl_2$,1; HEPES, 10, pH adjusted to 7.2 with KOH. Experiments were performed at room temperature: $22.0 \pm 0.5\ ^{o}C$.

To study dependence of steady-state block of HERG channels on drug concentration in HEK cells membrane potential was switched from holding –80 mV to +20 mV for 2 s following return to –50 mV for 6 s in intervals of 15-30 s. Tail currents were measured at –50 mV in control and in the presence of the drug at concentrations to be determined empirically.

For each batch of cells, this protocol was applied for 3 minutes to rule out possible run down of HERG currents. Drug testing was performed only on cells showing less than 5% of decline from initial value of tail current. In addition, we performed vehicle control experiments in parallel with drug testing to ensure that the repeated recordings and the duration of the experiment did not significantly influence the amount of HERG current produced.

Each cell served as its own control. All raw measurements of tail currents were performed using CLAMPFIT program, a part of pCLAMP software (Version 8.1, Axon Instruments Inc., Union City, CA). Results were transferred to Origin 6.1 and/or Microsoft Excel spreadsheets for further analysis.

Animals

New Zealand White rabbits (3-4 months old, weight 3 to 3.5 kg) were obtained from Covance Laboratories, Inc. (Denver, PA). This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996), and all experiments were conducted in accordance with the guidelines of the Georgetown University Animal Care and Use Committee.

Isolated Perfused Rabbit Heart Preparation

The isolated perfused rabbit heart preparation was performed essentially as we

have described previously (Liu et al., 2003). Following anesthesia with pentobarbital (30 mg/kg, i.v.), heparin (10,000 units, i.v.) was administered to prevent clotting. The heart was then rapidly removed through a median sternotomy incision and placed in an ice cold Tyrode's solution. After the removal of extraneous fat and connective tissue, the aortic root was cannulated for retrograde perfusion. The heart was then mounted in a Langendorff–perfusion apparatus. The right atrium was removed and the AV node was cauterized to ablate endogenous pacing. The heart was paced from an electrode positioned in the ventricular endocardium. The perfusate was maintained at 37ºC by immersion in a temperature-controlled bath containing the perfusion solution and delivered to the aortic inflow cannula by a Masterflex™ pump at a constant flow rate of 10 ml/min in a noncirculating manner. Hearts were paced at a fixed cycle length of 400 ms (twice diastolic threshold) using a Pulsemaster A300 cardiac stimulator and the Stimulus Isolator A360 (World Precision Instruments, Sarasota, FL). Four silver-silver chloride electrode pellets were positioned in a simulated "Einthoven" configuration with the reference and "foot" electrodes fixed beneath the heart on the walls of a tissue bath. All hearts were perfused with Tyrode's solution in order to equilibrate the preparation for 30-60 min prior to the baseline measurements.

The signals were amplified by an ECG amplifier, that allowed the simultaneous recording of three signals, filtered selectively for 60-Hz noise, and ECG recordings were acquired on a strip chart with a paper speed of 100 mm/sec. All signals were digitized at sampling rate 2 kHz and stored on a computer hard drive and CD for later analysis.

After baseline ECG recordings were obtained, the QT interval and QRS duration were observed until three separate recordings 2-3 minutes apart with stable measurements (<5% change) have been obtained. Each test drug was evaluated over a range of concentrations, beginning with a low concentration and sequentially increasing the concentration after 30-minutes. All measurements were made during the last 5-minutes of perfusion with each drug concentration. The QT values recorded at the end of each perfusion period were used for comparative analysis.

Statistical Analyses

Unless otherwise noted, all data are expressed as Mean±S.E.M. One-way analysis of variance (ANOVA) was used for multiple comparisons, and the Student's t-test was used when only two groups were compared. A *p*-value of less than 0.05 was required to reject the null hypothesis.

Results

We tested 14 drugs for their ability to block cardiac HERG K^+ currents in a stably transfected HEK cell line (Zhou et al., 1998). The drugs were tested using the whole-cell patch-clamp recording technique as described previously (Katchman et al., 2002). For each drug tested, we calculated the concentration that inhibited 50% of I_{HERG} (IC_{50}) by comparing the amount of I_{HERG} present in the presence of a given concentration of drug relative to the amount of I_{HERG} present in the same cell prior to the administration of drug. These results are summarized in Table 1.

The drugs are listed in order of potency for blockade of I_{HERG}. Lidoflazine, terfenadine, and haloperidol were by far the most potent HERG inhibitors of the compounds tested, with IC_{50} values in the mid-nanomolar range. Notably, all three drugs blocked I_{HERG} in a use-dependent manner, as exemplified by the results shown for terfenadine in Fig. 1. For comparison, control tracings recorded under similar conditions but in the absence of any drugs are shown in Fig. 2. In contrast to the recordings performed in the presence of terfenadine and other use-dependent blockers of I_{HERG} (Fig. 1 & Table 1), there was no significant change in peak I_{HERG} tail current density following repeated (n=20-30) measurements in the absence of drugs (Fig. 2).

Some of the drugs showing intermediate potency for I_{HERG} block, such as sertindole and prenylamine, were also found to act in a use-dependent manner. Most of the less potent drugs tested did not show any signs of use-dependency. For example,

lovastatin blocked I_{HERG} in a concentration-dependent manner that showed no signs of use-dependency (Fig. 3).

For three of the drugs tested, cimetidine, pentamidine, and arsenic trioxide, we were unable to derive IC_{50} values because little or no inhibition of I_{HERG} was observed in the presence of these drugs. In the cimetidine example, partial block (< 50%) of I_{HERG} was achieved when the drug was applied at the highest concentration tested (10 mM). Upon removal of cimetidine (washout), nearly complete recovery of current was observed (data not shown).

Pentamidine, on the other hand, appears to represent a special case. Although there was no significant inhibition of I_{HERG} at concentrations up to 1 mM pentamidine ($p > 0.05$, n=11), we observed a much faster decay of the tail currents in the presence of pentamidine (Fig. 4). Upon removal of pentamidine ('Washout'), we observed nearly complete recovery of the decay rate (Fig. 4, lower set of tracings in upper panel). Subsequent application of the known I_{HERG} blocker, haloperidol (1 μM), caused strong suppression of I_{HERG} tails within seconds after administration. Although we could not derive an IC_{50} for pentamidine block of peak HERG tail currents due to the weak effect of this drug on I_{HERG} tail amplitude (Fig. 4, middle panel), we were able to calculate an IC_{50} of 205 μM for its effect on the decay rate, τ, of I_{HERG} deactivation (Fig. 4, lower panel).

Of the compounds tested in this study, arsenic trioxide appeared to be the least effective blocker of I_{HERG}. At the highest concentration tested (300 μM), weak inhibition of I_{HERG} tail currents was seen, but this was not found to be significant (p>0.05, n=9). There was no question that I_{HERG} could be blocked in these cells by other drugs since application of either terfenadine (1 μM) or haloperidol (1 μM) readily blocked I_{HERG} in the same cells (Fig. 5). Thus, arsenic trioxide does not appear to directly influence I_{HERG} at concentrations ≤ 300 μM.

To determine if arsenic trioxide and pentamidine may directly cause repolarization delays through blockade of endogenous cardiac K^+ currents or other mechanisms, we examined the effects of these drugs on QT interval duration in the isolated perfused (Langendorff) rabbit heart preparation. Examples of our ECG recordings for these experiments are shown in Fig. 6. Arsenic trioxide (10 μM) had little effect on the ECG, yielding QT durations that were not distinct from control (Fig. 6A). In contrast, subsequent perfusion with methadone (3 μM) led to a small but noticeable 4.4% increase in QT duration from 225 ms (control) to 235 ms. Methadone's effect could be partially reversed by removal of the drug (washout). When the same heart was then challenged with cisapride (1 μM), QT duration increased to 255 ms (13.3% increase relative to control). Thus, the heart was clearly responsive to known HERG-blocking drugs such as methadone (Katchman et al., 2002) and cisapride (Bran et al., 1995), but not to arsenic trioxide.

A similar finding was observed for pentamidine (Fig. 6B). Although a mild increase of 5 ms in QT duration was observed in the presence of pentamidine in this experiment, there was little concentration-dependency associated with this response since QT duration was similar in the presence of both concentrations (3 and 10 μM) of pentamidine tested in this experiment. In contrast, subsequent perfusion with prenylamine clearly lengthened QT duration in a concentration-dependent manner, thereby indicating that the QT interval was responsive to a known HERG-blocking drug (prenylamine) whereas pentamidine had little effect under similar conditions. Subsequent experiments supported these examples by showing that neither arsenic trioxide, nor pentamidine elicited any significant fluctuations in QT interval duration across broad concentration ranges, extending to 50 and 30 μM, respectively (Fig. 7A & B, respectively).

Because loratadine also showed some use-dependent ability to block I_{HERG} at micromolar concentrations, and because there are conflicting reports about loratadine's effects on I_{HERG} in different laboratories (Crumb, Jr., 2000; Davie et al., 2004; Ducic et al., 1997), we also evaluated loratadine using the Langendorff-perfused rabbit heart model. Our data show that loratadine has no significant effect on QT length at concentrations up to and including 50 μM (Fig. 7C). The only apparent effect was a tendency for the QT to become shorter in the presence of loratadine, but this effect was not found to be significant ($p > 0.05$, n=4). No arrhythmias or other ECG abnormalities were observed during perfusion with loratadine in these experiments.

In contrast, methadone, which was previously shown to block I_{HERG} in a concentration-dependent fashion (IC_{50}=10-20 μM) (Katchman et al., 2002; Kornick et al., 2003) led to significant increases in QT interval duration in this model (Fig. 8). A clear trend towards increased QT duration was observed in the presence of methadone at concentrations as low as 1-3 μM (10-12% increase relative to control, Fig. 8B), with significant increases apparent at 10 μM (21±5%, n=4, p<0.05) and 30 μM (44±12%, n=4, p<0.001). No arrhythmias or other ECG abnormalities were observed following methadone perfusion in these experiments.

Discussion

We tested 14 drugs for their ability to block I_{HERG} in a stably transfected cell line. Our data suggest that these drugs could generally be segregated into 4 groups based on their potency and mechanism of I_{HERG} inhibition. For example, the most potent I_{HERG} blockers (IC_{50} < 100 nM) were lidoflazine, terfenadine, and haloperidol, which all interfered with I_{HERG} in a use-dependent manner. A second category of compounds (sertindole, thioridizine, and prenylamine) blocked I_{HERG} with moderate potency (IC_{50}=0.1-1 μM) with some use-dependency. The third and largest category consisted of low-potency I_{HERG} blockers (IC_{50}=1-100 μM) that, with one exception (loratadine), did not induce any apparent use-dependency with respect to I_{HERG} inhibition. This third category includes propafenone, loratadine, pyrilamine, lovastatin, and chlorpheniramine. The fourth and final category consists of 3 compounds: cimetidine, pentamidine, and arsenic trioxide, which were essentially inactive with respect to I_{HERG} blockade in our assays even when evaluated at excessively high concentrations. While several of the drugs tested here have been shown to block I_{HERG} in previous studies, this is the first report showing that prenylamine, pyrilamine, and chlorpheniramine can block I_{HERG} in a concentration-dependent manner.

Although prenylamine has long been associated with clinical QT-lengthening and TdP-like arrhythmogenic events (Guijarro et al., 1976; Oakley et al., 1980) there do not appear to be any published reports evaluating its influence on I_{HERG} directly. Thus, the present study provides the first quantitative assessment of prenylamine with respect to

I_{HERG}. The IC_{50} for this block was 590 nM, similar to that of sertindole and thioridazine, suggesting that it could be contributory to the observed cardiac repolarization and arrhythmogenic problems associated with prenylamine use. Although we did not perform comprehensive evaluation of prenylamine's actions using the Langendorff model, we did observe QT-lengthening by prenylamine in each of the three experiments that were performed with this drug (e.g., see Fig. 6B).

There are few reports of QT problems or ventricular arrhythmias, and no reports of I_{HERG} blockade by pyrilamine or chlorpheniramine. Like chlorpheniramine, we included lovastatin as a 'negative control', but found that it also blocked I_{HERG} in a concentration-dependent manner with an IC_{50} of 7 μM, a value that is remarkably close to that (12.5 μM) recently reported for I_{HERG} block in another study (Wible et al., 2005). While each of these compounds did, in fact, block I_{HERG} in a concentration-dependent manner, the concentrations required to achieve such inhibition are far above (>100-fold) typical plasma concentrations for these drugs in patients. Thus, the I_{HERG} results reported for these compounds are consistent with their cardiac safety record clinically.

Loratadine is a widely used non-sedating antihistamine that is thought to be generally 'safe' clinically (Woosley, 1996) despite apparently conflicting reports about its ability to block I_{HERG} and I_{Kr} (Crumb, Jr., 2000; Davie et al., 2004; Ducic et al., 1997). Our I_{HERG} results with loratadine (IC_{50}=4μM) are remarkably similar to those reported by Davie et al. (2004) (IC_{50}=5μM). In contrast, Crumb (2000) reported that loratadine had much greater potency with respect to block of I_{HERG} amplitude (IC_{50}=173 nM), whereas

Ducic et al. (1997) reported that loratadine had little or no effect on I_{HERG} in microinjected *Xenopus* oocytes and I_{Kr} in isolated ventricular myocytes at concentrations up to 1 μM. To help resolve the issue of whether or not loratadine has a significant effect on cardiac repolarization, we used the isolated Langendorff-perfused rabbit heart model to assess ECG effects. As our results showed (Fig. 7C), loratadine did not significantly alter QT duration even during challenges with relatively high (micromolar) concentrations, thereby indicating that it had little or no effect on the duration of cardiac repolarization in this model. These results are reminiscent of those reported by Davie et al. (2004), who showed that despite micromolar blockade of I_{HERG} in transfected cells, loratadine had no appreciable effect on either action potential duration or I_{Kr} in guinea pig ventricular myocytes.

The fourth category of compounds (cimetidine, pentamidine, and arsenic trioxide) was relatively ineffective at blocking I_{HERG}. Despite these observations, both pentamidine (Wharton et al., 1987; Bibler et al., 1988; Eisenhauer et al., 1994) and arsenic trioxide (Ohnishi et al., 2000; Unnikrishnan et al., 2001; Barbey and Soignet, 2001) have been associated with cardiac repolarization delays (QT-lengthening) and TdP-like arrhythmias in patients. Recent reports have suggested that while these drugs may not interfere with I_{HERG} directly, they may nevertheless cause reductions in I_{HERG} via interference with trafficking of HERG proteins to the membrane (Ficker et al., 2004); (Kuryshev et al., 2005; Cordes et al., 2005). In the case of pentamidine, our results are consistent with these previously published data. Although we observed a dose-dependent decline in the τ of I_{HERG} deactivation with pentamidine, this effect required

extremely high concentrations (IC_{50}=205 μM) that are far above the maximal plasma concentration (C_{max}=10-30 nM) (Vohringer and Arasteh, 1993) reported in patients taking recommended therapeutic doses of pentamidine.

In the case of arsenic trioxide, however, there are independent reports suggesting that it lengthens action potential duration and rate-corrected QT intervals (QTc) in vivo in guinea pig hearts (Chiang et al., 2002) and that it blocks I_{HERG} in transfected CHO cells at low micromolar concentrations (Drolet et al., 2004). In contrast, our data suggest that arsenic trioxide has no significant acute effect on I_{HERG} in stably-transfected HEK cells at concentrations up to 300 μM.

The lack of a QT response for pentamidine or arsenic trioxide in the isolated perfused rabbit heart model is consistent with the our finding that neither of these compounds had much effect on I_{HERG} at relevant concentrations, and suggests that neither of these compounds cause any direct acute effect to block I_{HERG}. In contrast, (Drolet et al., 2004) have published data suggesting that submicromolar concentrations of arsenic trioxide were sufficient to achieve significant inhibition of I_{HERG}. We did not see this effect in our experiments, though certain differences in assay systems exist. For example, the Drolet study (2004) used transiently transfected CHO cells whereas we used stably-transfected HEK cells in the present study. Conceivably, these differences and/or other methodological differences could account for the apparent discrepancy in our results with respect to direct blockade of I_{HERG} by arsenic trioxide. Further testing is required to resolve this apparent discrepancy.

In contrast, our arsenic trioxide results are consistent with those recently published by Ficker et al. (2004). Both we (present study) and Ficker et al. (2004) showed that arsenic trioxide has no significant direct acute effect on I_{HERG} in transfected cells. Although we have not examined the effects of prolonged exposure to arsenic trioxide, Ficker et al. (2004) demonstrated that 24-hr exposure to arsenic trioxide leads to a concentration-dependent decrease in I_{HERG}, with the IC_{50} for this effect being ~1.5 μM. From these and other data, Ficker et al. (2004) have suggested that arsenic trioxide may reduce I_{HERG} by interfering with HERG protein trafficking. As indicated above, similar observations regarding HERG protein trafficking interference have recently been made with pentamidine (Kuryshev et al., 2005; Cordes et al., 2005), thus suggesting that these two drugs may act through similar mechanisms with respect to their arrhythmogenic potential (Eckhardt et al., 2005).

Alternatively, it is possible that drugs like arsenic trioxide and pentamidine have arrhythmogenic activity that is independent of I_{HERG} and QT. It has recently been suggested, for example, that QT interval may not be the best marker for ventricular proarrhythmia potential (Brown, 2004; Shah and Hondeghem, 2005). Shah and Hondeghem (2005) have instead proposed a novel “TRIaD” (Triangulation, Reverse use-dependency, Instability of action potential, and Dispersion) analytical method. Interestingly, there does appear to be T-wave morphology changes that could be indicative of “triangulation” during some of our drug perfusions (e.g., prenylamine - see Fig. 6B). It may be interesting to see if the TRIaD method will be predictive of

arrhythmogenic potential for arsenic trioxide and pentamidine, but additional data are needed to make such a determination. Other potential mechanisms such as indirect effects that could lead to proarrhythmic conditions should also be evaluated.

Conclusions

In summary, our data show that unlike many other torsadogenic and suspected torsadogenic drugs, neither pentamidine nor arsenic trioxide appear to directly block the channel to interfere with I_{HERG} or cause QT prolongation acutely in well-established model systems, even at relatively high non-therapeutically relevant concentrations. A possible mechanistic explanation for the clinical QT and torsadogenic effects of these compounds may reflect impaired trafficking of HERG proteins (Eckhardt et al., 2005), as suggested by recent studies (Ficker et al., 2004; Kuryshev et al., 2005; Cordes et al., 2005). Further evaluation of how these and other non-HERG blockers may elicit dangerous cardiac arrhythmias is warranted.

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Footnotes

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Figure Legends

Figure 1. Effect of terfenadine on I_{HERG}. (Upper panel) Example of I_{HERG} repeatedly recorded in the presence of 30 nM terfenadine (inset). The time-dependent decrease in I_{HERG} tail currents is illustrated graphically. The time between pulses was 30 s. (Lower panel) Derivation of IC_{50} for I_{HERG} block by terfenadine. Relative I_{HERG} was determined under steady-state conditions (~12 pulses).

Figure 2. Control recordings of I_{HERG} in stably-transfected HEK cells. Example of I_{HERG} repeatedly recorded in the absence of any drugs (inset). The graph shows that under control conditions, there was little change in HERG tail currents in these cells over 10-12 pulses, with 30 s between pulses.

Figure 3. Effect of lovastatin on I_{HERG}. (Upper panels) Example of HERG tail current block by increasing concentrations of lovastatin. The second panel (right) illustrates lack of use-dependency. Note that all three traces recorded in the presence of 10 μM lovastatin are superimposed on each other, indicating that there does not appear to be further decline in this current with repeated stimulation in the presence of lovastatin. (Lower panel) Derivation of IC_{50} for lovastatin with respect to I_{HERG} block.

Figure 4. Effect of pentamidine on I_{HERG}. (Upper panel) Examples of I_{HERG} recorded in the absence (Control, arrow) and presence of 300 μM pentamidine (arrow). Note that while there is little change in tail current amplitude in the presence of pentamidine, a decrease in the rate of current decay is clearly evident. Upon drug removal (Washout),

I_{HERG} returned to approximately control levels (lower set of tracings). When 1 μM haloperidol was applied, I_{HERG} was strongly and rapidly suppressed. (Middle panel) Graph showing that pentamidine (up to at least 300 μM) has no significant effect on HERG tail current amplitudes (p>0.05, n=11). (Lower panel) Graph showing derivation of IC_{50} derivation for τ of I_{HERG} deactivation by pentamidine.

Figure 5. Effect of arsenic trioxide (As_2O_3) on I_{HERG}. Example of I_{HERG} recorded in the absence (Control) and presence of As_2O_3 (100 μM). In this experiment, I_{HERG} was recorded within 5 mins of drug application, but similar results were found in other experiments when the length of As_2O_3 (100 μM) exposure was increased to 20-30 mins (not shown). In contrast, addition of 1 μM terfenadine to the same cells rapidly abolished I_{HERG}.

Figure 6. Sample ECG recordings from isolated perfused rabbit hearts. Data from two different hearts (A & B) are shown. The traces are aligned vertically with the solid line marking the beginning of the QRS complex. The vertical dashed line marks the end of the T-wave in the Control (no drug) tracings, and is superimposed on the lower tracings for reference. The angled dashed lines mark the point where the end of the T-wave intersects the isoelectric line. Baseline (Control) recordings were performed after an initial stabilization period of 30-40 mins for each heart. The hearts were then challenged with the indicated drugs for 30 min intervals each in the order shown (from top to bottom). Each tracing shown was collected from recordings made during the last 20-30 mins of perfusion with the indicated drug. Note that neither (A) arsenic trioxide

(As_2O_3, 10 μM), nor (B) pentamidine (10 μM) had much effect on QT in these experiments. Scale, each square on the grid is equivalent to 50 ms (note that the scale for 'B' is slightly compressed relative to 'A'). The QT duration is given for each trace.

Fig. 7. Lack of QT change in isolated perfused rabbit hearts following challenge with (A) Arsenic trioxide, (B) Pentamidine, or (C) Loratadine. The number (n) of hearts tested at each concentration is indicated. No significant differences in QT duration were observed in these experiments. Please note that each graph depicts data for only one drug, and that the hearts were not exposed to any other drug prior to perfusion with the indicated drug.

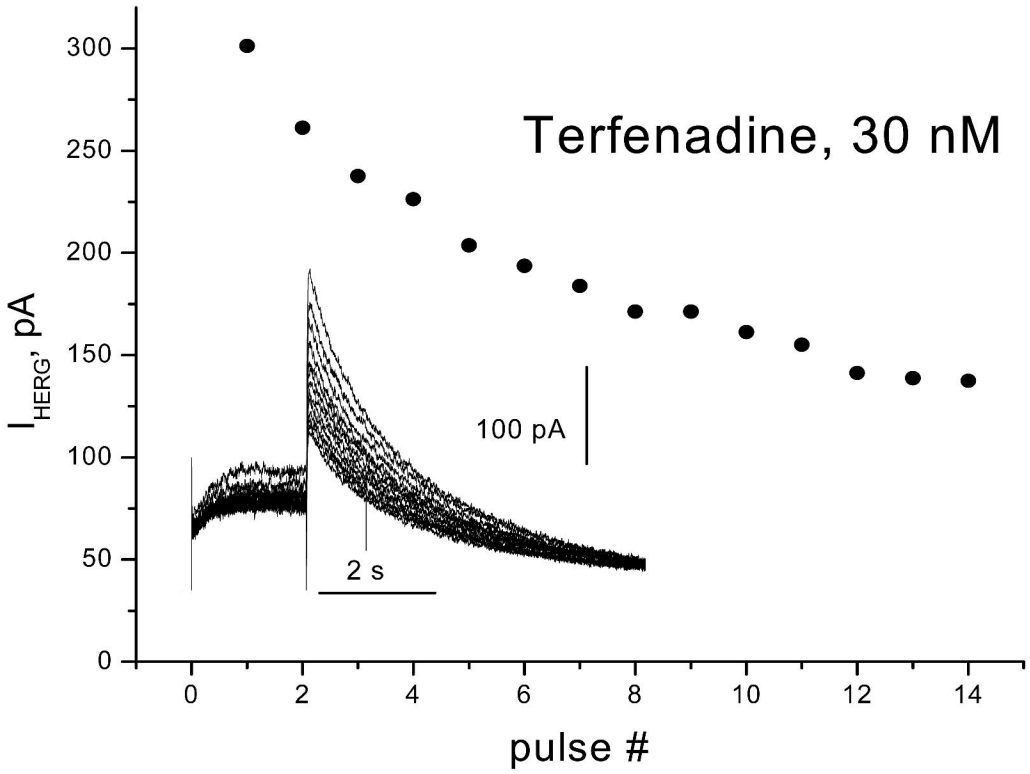
Figure 8. Effects of methadone on QT intervals in isolated perfused rabbit hearts. (Left panel) Absolute QT vs. methadone concentration. The number (n) of hearts tested at each concentration is indicated. (Right panel) The degree of QT difference (%QT Change) observed at each concentration of methadone tested. *$p<0.05$, ***$p<0.001$ relative to control (absence of drug).

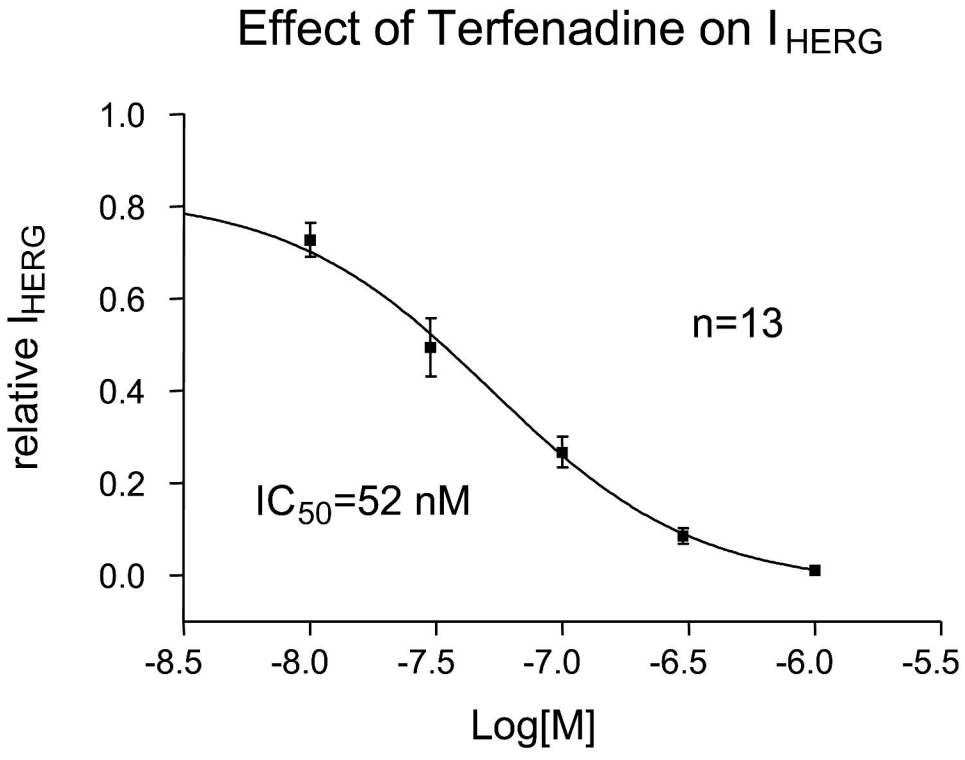
Table 1. Rank order of potency for blockade of I_{HERG}.

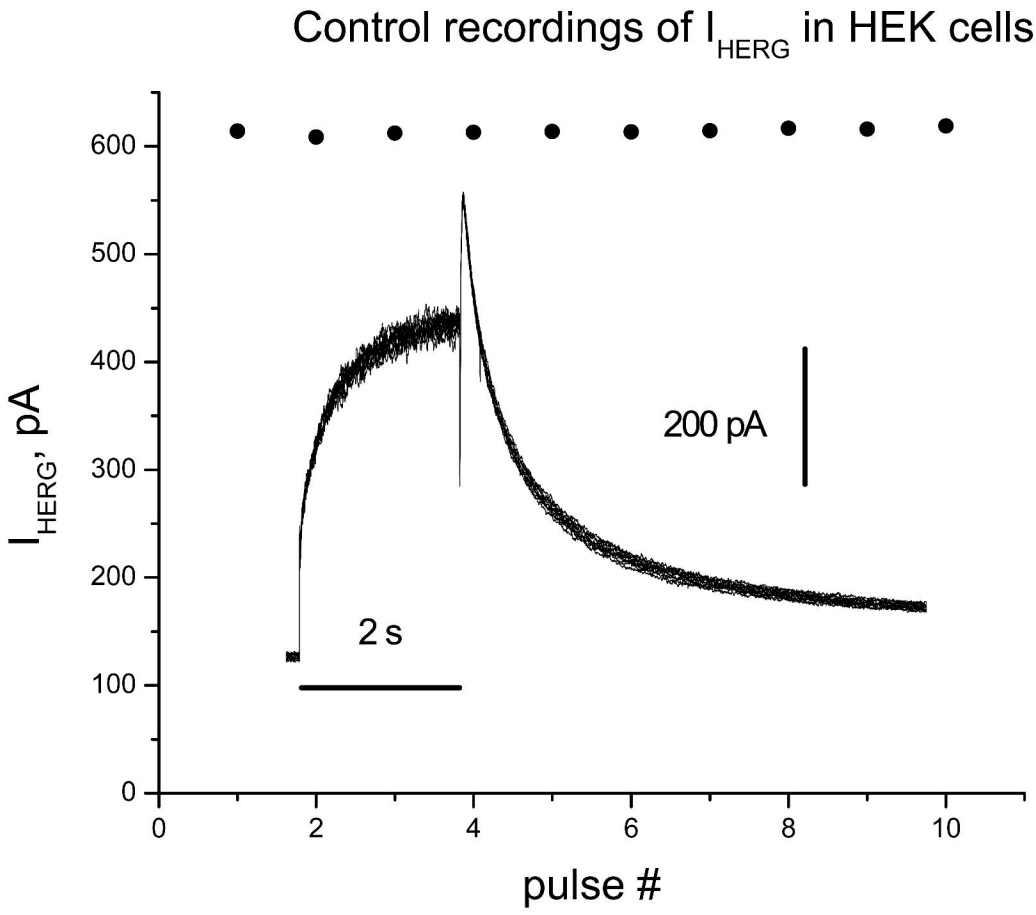
Category[a]	Drug	IC_{50} for I_{HERG} Block	Use-dependence	Maximum Concentration[b] Tested on I_{HERG}	n
	Lidoflazine	≤ 37 nM	+++		13
1	Terfenadine	≤ 52 nM	+++		13
	Haloperidol	63 nM	+		8
	Sertindole	210 nM	+		13
2	Thioridazine	390 nM	-		9
	Prenylamine	590 nM	+		19
	Propafenone	2 μM	-		6
	Loratadine	4 μM	+		10
3	Pyrilamine	6 μM	-		8
	Lovastatin	7 μM	-		15
	Chlorpheniramine	13 μM	-		9
	Cimetidine	≥10 mM	-	10 mM	8
4	Pentamidine	-	-	1 mM	11
	Arsenic Trioxide	-	-	300 μM	9

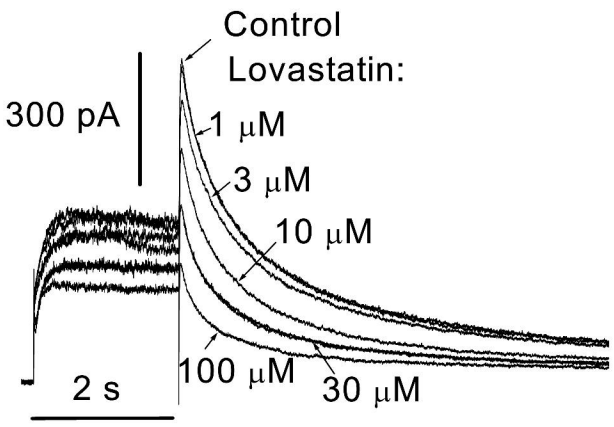
[a]Category was arbitrarily assigned based on relative potency of I_{HERG} block.

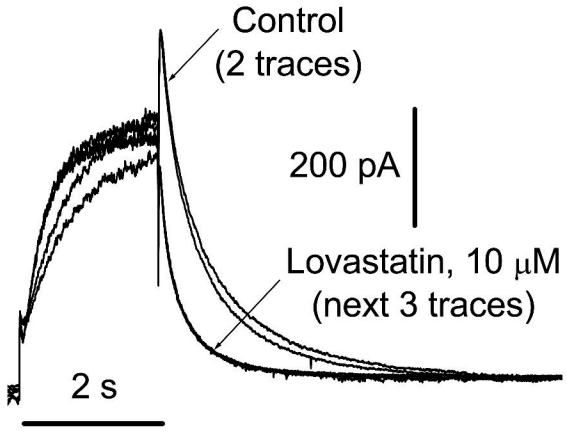
[b]Maximum test concentrations are shown only for those drugs where there was little or no inhibition of I_{HERG} observed.

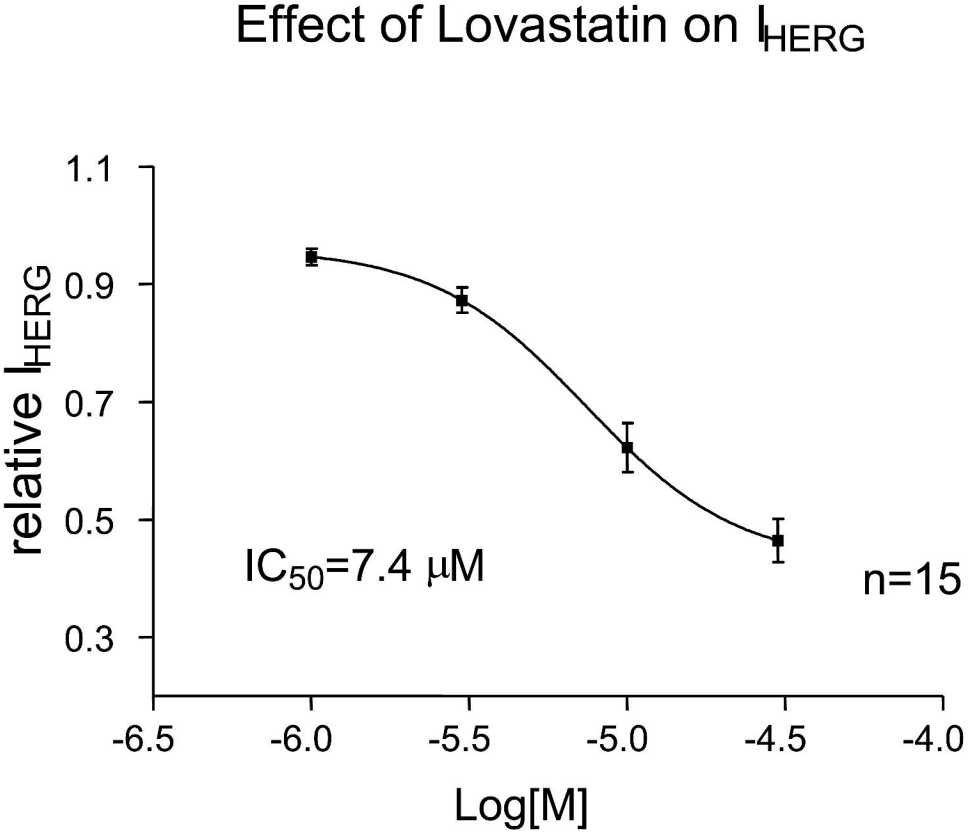

Terfenadine, 30 nM
I_{HERG}, pA
300
250
200
150
100
50
0
100 pA
2 s
0
2
4
6
8
10
12
14
pulse #

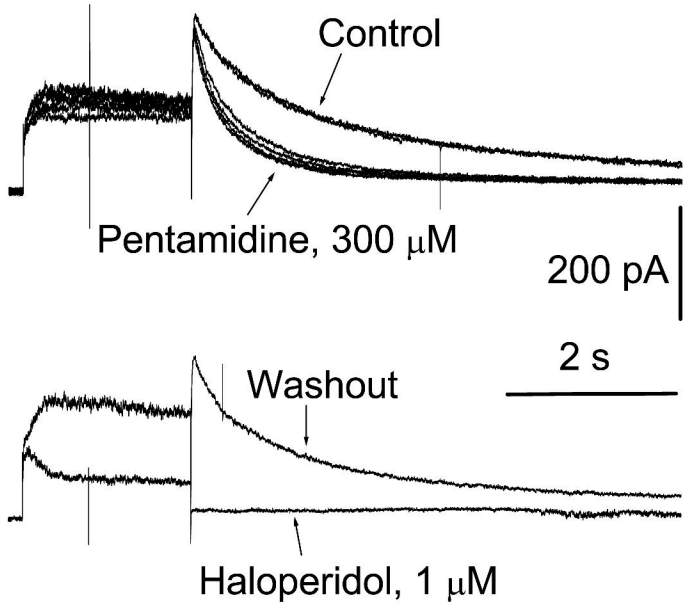

Effect of Terfenadine on I_{HERG}
relative I_{HERG}
1.0
0.8
0.6
0.4
0.2
0.0
n=13
IC_{50}=52 nM
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
-5.5
Log[M]

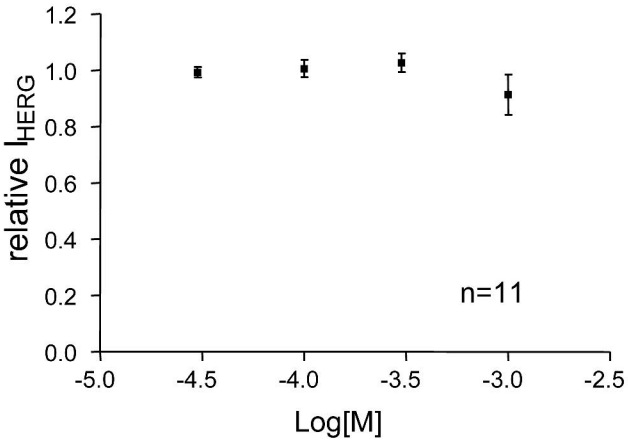

Control recordings of I_{HERG} in HEK cells
I_{HERG}, pA
600
500
400
300
200
100
0
200 pA
2 s
0
2
4
6
8
10
pulse #

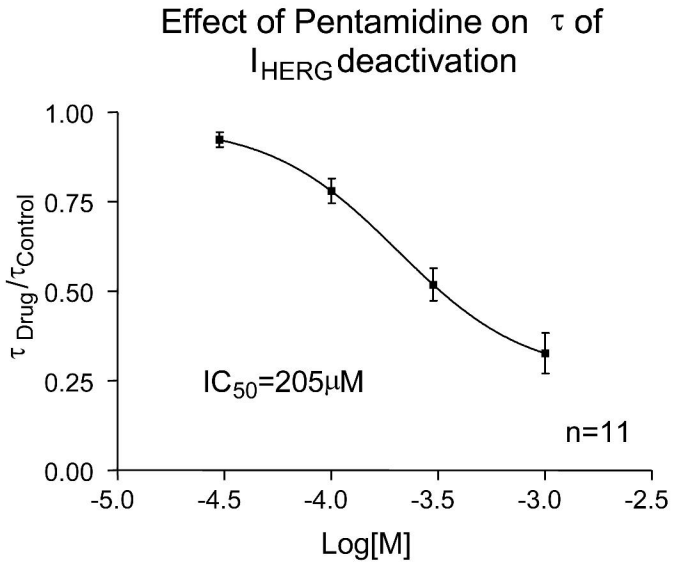

Control
Lovastatin:
300 pA
1 μM
3 μM
10 μM
100 μM
30 μM
2 s

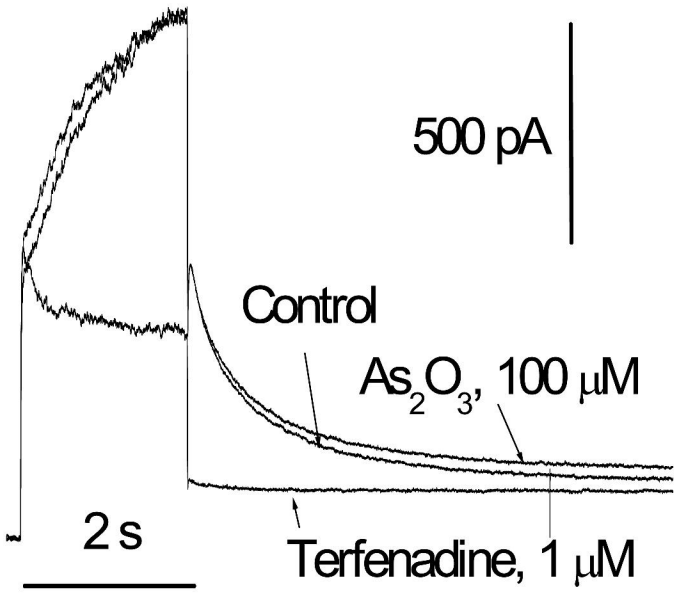

Control
(2 traces)
200 pA
Lovastatin, 10 μM
(next 3 traces)
2 s

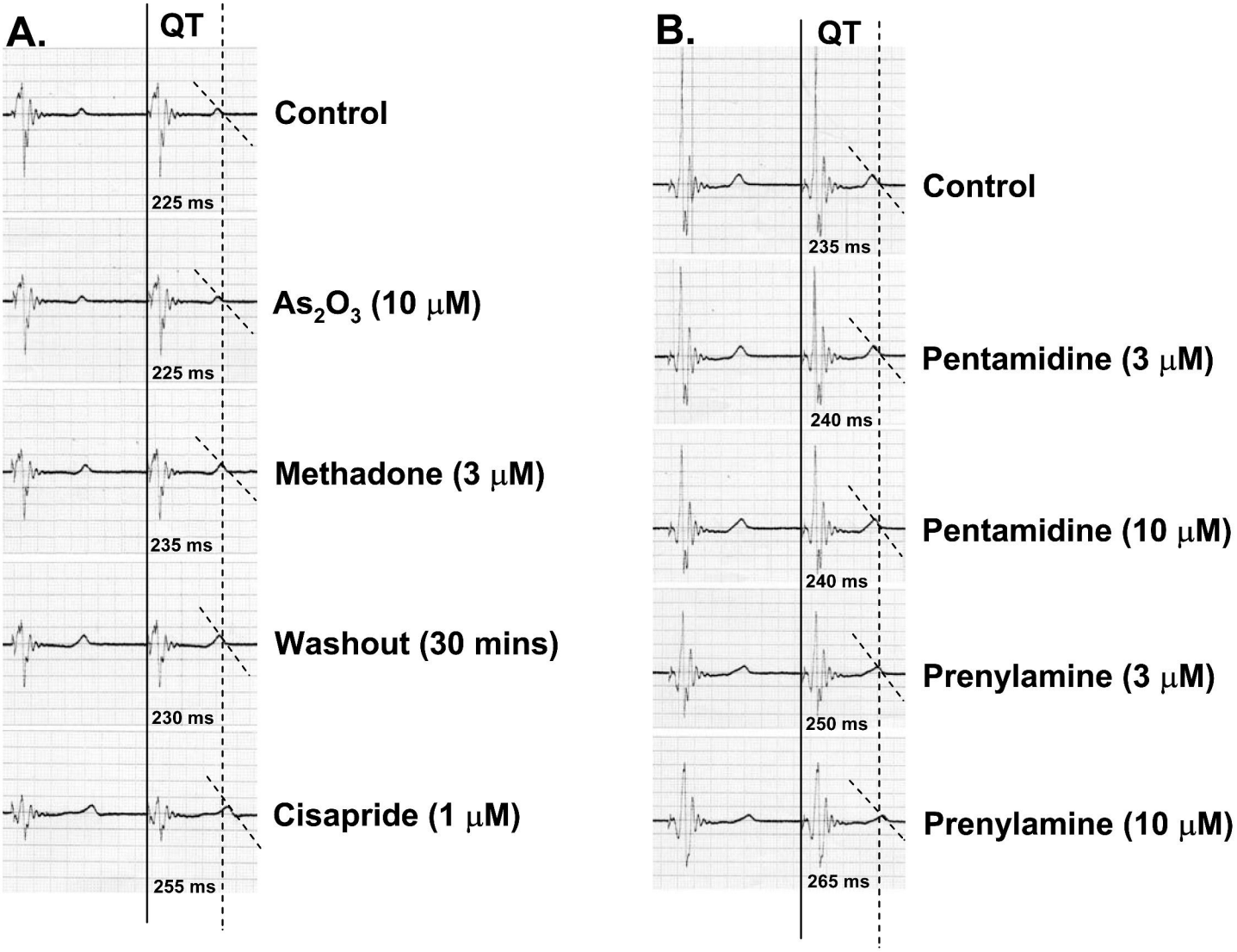

Effect of Lovastatin on I$_{HERG}$
relative I$_{HERG}$
1.1
0.9
0.7
0.5
0.3
IC$_{50}$=7.4 μM
n=15
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
Log[M]

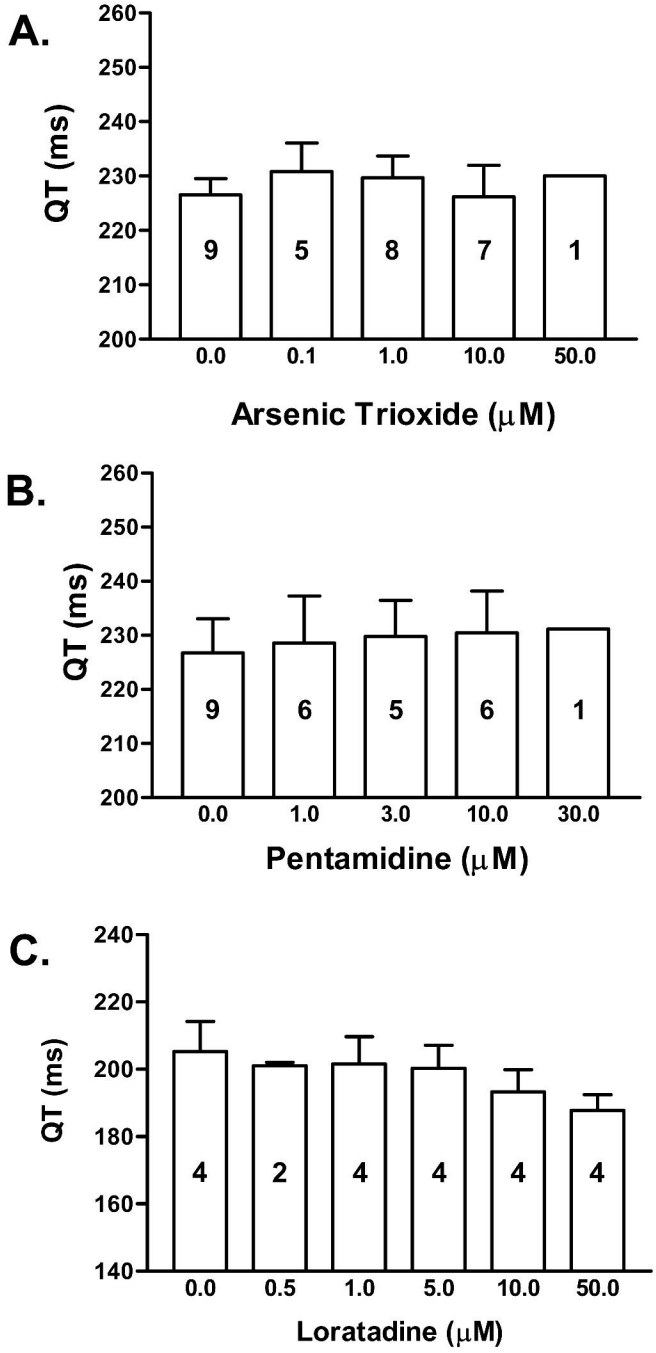

Control
Pentamidine, 300 μM
200 pA
2 s
Washout
Haloperidol, 1 μM

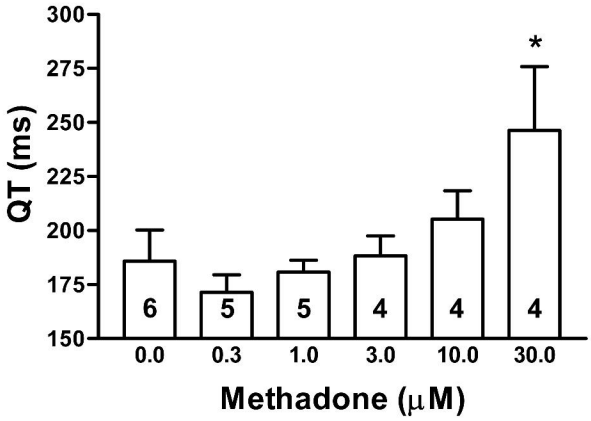

Effect of Pentamidine on I HERG peak tail
relative I HERG
1.2
1.0
0.8
0.6
0.4
0.2
0.0
n=11
-5.0
-4.5
-4.0
-3.5
-3.0
-2.5
Log[M]

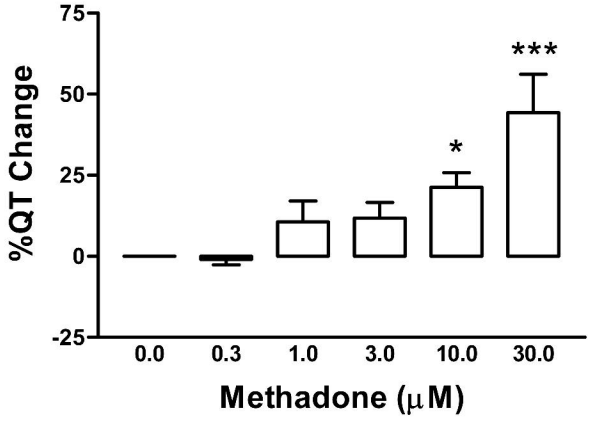

Effect of Pentamidine on τ of I HERG deactivation
τ Drug/τ Control
1.00
0.75
0.50
0.25
0.00
IC50=205μM
n=11
-5.0
-4.5
-4.0
-3.5
-3.0
-2.5
Log[M]


500 pA
Control
As2O3, 100 μM
2 s
Terfenadine, 1 μM


A.
QT
Control
225 ms
As_2O_3 (10 μM)
225 ms
Methadone (3 μM)
235 ms
Washout (30 mins)
230 ms
Cisapride (1 μM)
255 ms
B.
QT
Control
235 ms
Pentamidine (3 μM)
240 ms
Pentamidine (10 μM)
240 ms
Prenylamine (3 μM)
250 ms
Prenylamine (10 μM)
265 ms


A.
QT (ms)
260
250
240
230
220
210
200
9
5
8
7
1
0.0
0.1
1.0
10.0
50.0
Arsenic Trioxide (μM)
B.
QT (ms)
260
250
240
230
220
210
200
9
6
5
6
1
0.0
1.0
3.0
10.0
30.0
Pentamidine (μM)
C.
QT (ms)
240
220
200
180
160
140
4
2
4
4
4
4
0.0
0.5
1.0
5.0
10.0
50.0
Loratadine (μM)


QT (ms)
300
275
250
225
200
175
150
6
5
5
4
4
4
*
0.0
0.3
1.0
3.0
10.0
30.0
Methadone (μM)


%QT Change
75
50
25
0
-25
*
***
0.0
0.3
1.0
3.0
10.0
30.0
Methadone (μM)