

## Pentamidine-Induced Long QT Syndrome and Block of hERG Trafficking

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### ABSTRACT

The diamidine pentamidine is used to treat leishmaniasis, trypanosomiasis, and *Pneumocystis carinii* pneumonia. Treatment may be accompanied by prolongation of the QT interval of the electrocardiogram and torsades de pointes tachycardias. Up to now, it has been thought that therapeutic compounds causing QT prolongation are associated with direct block of the cardiac potassium channel human ether a-go-go-related gene (hERG), which encodes the  $\alpha$  subunit of cardiac  $I_{Kr}$  currents. We show that pentamidine has no acute effects on currents produced by hERG, KvLQT1/mink, Kv4.3, or SCNA5. Cardiac calcium currents and the guinea pig cardiac action potential were also not affected. After overnight exposure, however, pentamidine reduced hERG currents and inhibited trafficking and maturation of hERG with  $IC_{50}$  values of 5 to 8  $\mu$ M similar to

therapeutic concentrations. Surface expression determined in a chemiluminescence assay was reduced on exposure to 10, 30, and 100  $\mu$ M pentamidine by about 30, 40, and 70%, respectively. These effects were specific for hERG since expression of hKv1.5, KvLQT1/minK, and Kv4.3 was not altered. In isolated guinea pig ventricular myocytes, 10  $\mu$ M pentamidine prolonged action potential duration  $APD_{90}$  from  $374.3 \pm 57.1$  to  $893.9 \pm 86.2$  ms on overnight incubation.  $I_{Kr}$  tail current density was reduced from  $0.61 \pm 0.09$  to  $0.39 \pm 0.04$  pA/pF. We conclude that pentamidine prolongs the cardiac action potential by block of hERG trafficking and reduction of the number of functional hERG channels at the cell surface. We propose that pentamidine, like arsenic trioxide, produces QT prolongation and torsades de pointes in patients by inhibition of hERG trafficking.

The antiprotozoal agent, pentamidine isethionate, is used in developing countries for the treatment of parasitic diseases such as trypanosomiasis and antimony-resistant visceral leishmaniasis (Nacher et al., 2001; Burchmore et al., 2002). In the United States, it is used in the treatment of *Pneumocystis carinii* pneumonia, a common opportunistic infection in patients who have contracted the human immunodeficiency virus or in patients immunosuppressed during chemotherapy (Sands et al., 1985; Goa and Campoli-Richards, 1987). Therapy with pentamidine is often accompanied by prolongation of the QT interval on the electrocardiogram (ECG) and, in some instances, by torsades de pointes (TdP) tachycardias that can degenerate into ventricular fibrillation

and cause sudden cardiac death (Wharton et al., 1987; Bibler et al., 1988; Girgis et al., 1997).

Prolongation of the QT interval and torsades de pointes are usually seen in patients with inherited (congenital) long QT syndrome (Keating and Sanguinetti, 2001) or in association with a wide variety of structurally diverse medications including antiarrhythmic, antihistamine, antibiotic, and psychotropic compounds (Fermini and Fossa, 2003). Most drugs known to cause acquired long QT syndrome do so by direct blockade of the cardiac potassium channel hERG (Pearlstein et al., 2003; Redfern et al., 2003), which underlies the rapid component of the delayed rectifier potassium current  $I_{Kr}$  in the human heart (Sanguinetti et al., 1995; Keating and Sanguinetti, 2001). Although some of these drugs such as class III antiarrhythmics were designed to prolong cardiac repolarization with hERG as the intended target (Vaughn Williams et al., 1982), for the vast majority, block of hERG constitutes an unwanted adverse side effect. To determine drug-related cardiac toxicity, hERG block is usually detected

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**ABBREVIATIONS:** TdP, torsades de pointes; hERG, human ether a-go-go-related gene;  $I_{Kr}$ , rapidly activating delayed rectifier K current; ER, endoplasmic reticulum; HA, hemagglutinin; HEK, human embryonic kidney; APD, action potential duration.

directly by patch-clamp electrophysiology on the cloned hERG channel. Although hERG/ $I_{Kr}$  is most extensively studied, other cardiac potassium currents, e.g., the slow component of the delayed rectifier current  $I_{Ks}$  (encoded by KvLQT1/minK), the ultra-rapidly activating delayed rectifier current  $I_{Kur}$  (encoded by Kv1.5), or the transient outward current  $I_{to}$  (encoded by Kv4.3) may provide additional plausible substrates for acquired long QT syndrome.

Recently, we have reported a completely different mechanism associated with acquired long QT syndrome and TdP. We have shown that arsenic trioxide ( $As_2O_3$ ) used in the treatment of acute promyelocytic leukemia reduced hERG/ $I_{Kr}$  currents not by direct block, but by inhibiting the processing of hERG protein in the endoplasmic reticulum (ER) thereby decreasing surface expression of hERG (Ficker et al., 2004). Another compound that reduces hERG currents by trafficking inhibition is geldanamycin, a benzoquinoid antibiotic that specifically inhibits function of the cytosolic chaperone Hsp90 (Ficker et al., 2003). Derivatives of geldanamycin are in clinical trials for the treatment of various forms of cancer with no reports of adverse cardiac events currently available. In the present report, we examine a novel compound class represented by the antiprotozoal agent pentamidine which has been associated clinically with QT prolongation and TdP and show that the aromatic diamidine pentamidine acts via inhibition of hERG channel trafficking.

## Materials and Methods

**Cellular Electrophysiology.** HEK293, L cells, or Chinese hamster ovary cells stably expressing hERG, hKv1.5, KvLQT1/minK, or Kv4.3 potassium channels were studied using the whole-cell configuration of the patch-clamp technique. Patch pipettes were filled with 100 mM potassium aspartate, 20 mM KCl, 2 mM  $MgCl_2$ , 1 mM  $CaCl_2$ , 10 mM EGTA, and 10 mM HEPES (pH 7.2). The extracellular solution was 140 mM NaCl, 5 mM KCl, 1 mM  $MgCl_2$ , 1.8 mM  $CaCl_2$ , 10 mM HEPES, and 10 mM glucose (pH 7.4). Stably transfected cell lines used in this study were regularly checked for responses to positive control compounds; cisapride was used for hERG, 4-aminopyridine for hKv1.5, chromanol 293B for KvLQT1/minK, flecainide for Kv4.3, and lidocaine for *SCNA5* expressing cells. In guinea pig ventricular myocytes, nisoldipine was used to validate L-type calcium currents. To study delayed effects of pentamidine on heterologously expressed hERG, hKv1.5, KvLQT1/minK, and Kv4.3 potassium currents, drug was added to stable cell lines for 16 to 20 h (overnight) prior to recording. Sodium currents were recorded in HEK293 cells stably expressing the human cardiac  $Na^+$  channel gene *SCN5A* using the following extracellular solution: 40 mM NaCl, 55 mM *N*-methyl-D-glucamine, 20 mM CsCl, 5.4 mM KCl, 2 mM  $MgCl_2$ , 0.02 mM  $CaCl_2$ , 30 mM tetraethylammonium chloride, 5 mM 4-aminopyridine, and 10 mM HEPES (pH 7.4). The intracellular solution was: 120 mM CsCl, 2 mM  $MgCl_2$ , 1 mM  $CaCl_2$ , 11 mM EGTA, 10 mM HEPES, 10 mM glucose, and 1 mM  $MgATP$  (pH 7.2). Cardiac L-type calcium currents were recorded in freshly isolated guinea pig ventricular myocytes using the following extracellular solution: 137 mM NaCl, 5.4 mM CsCl, 1.8 mM  $MgCl_2$ , 1.8 mM  $CaCl_2$ , 10 mM glucose, and 10 mM HEPES (pH 7.4). The intracellular solution was: 130 mM  $CsMeSO_4$ , 20 mM tetraethylammonium chloride, 1 mM  $MgCl_2$ , 10 mM EGTA, 10 mM HEPES, 4 mM  $MgATP$ , 14 mM Tris-phosphocreatine, 0.3 mM Tris-GTP, and 50 U/ml creatine phosphokinase (pH 7.2). Action potentials and cardiac  $I_{Kr}$ /hERG currents were recorded in ventricular guinea pig myocytes either freshly isolated or cultured overnight in M199 medium using the following intracellular solution: 119 mM potassium gluconate, 15 mM KCl, 3.75 mM  $MgCl_2$ , 5 mM EGTA, 5 mM HEPES, 4 mM K-ATP,

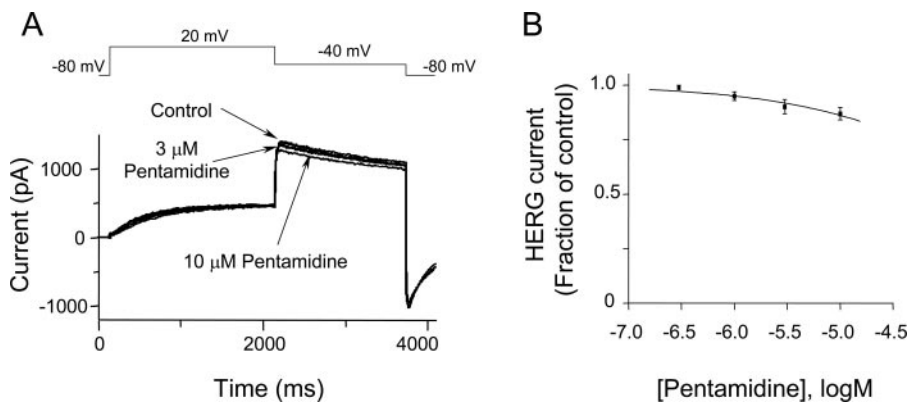
14 mM phosphocreatine, 0.3 mM Tris-GTP, and 50 U/ml creatine phosphokinase (pH 7.2). The extracellular solution was 132 mM NaCl, 4 mM KCl, 1.2 mM  $MgCl_2$ , 1.8 mM  $CaCl_2$ , and 5 mM HEPES (pH 7.4). Measurements of cardiac  $I_{Kr}$  currents were performed in the presence of 1  $\mu$ M nisoldipine to block L-type  $Ca^{2+}$  currents. The specific blocker, E4031, was used to pharmacologically isolate  $I_{Kr}$ . To analyze current densities, membrane capacitances were measured using the analog compensation circuit of an Axon 200B patch-clamp amplifier (Axon Instruments Inc., Foster City, CA). pCLAMP software (Axon Instruments) was used to generate voltage-clamp protocols and for data acquisition. All recordings were performed at room temperature (20–22°C). Data are expressed as means  $\pm$  S.E.M.

**Western Blot Analysis.** The HEK/hERG cell line, the L/hKv1.5 cell line, and antibodies used in the present study have been described previously (Ficker et al., 2003). Briefly, stably transfected cells expressing either hERG or hKv1.5 were solubilized for 1 h at 4°C in a lysis buffer containing 1% Triton X-100 and protease inhibitors (Complete; Roche Diagnostics, Indianapolis, IN). Protein concentrations were determined by the BCA method (Pierce Chemical, Rockford, IL). Proteins were separated on SDS polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and developed using appropriate antibodies and ECL Plus (Amersham Biosciences Inc., Piscataway, NJ). For quantitative analysis, chemiluminescence signals were captured directly on a Storm PhosphorImager (Amersham Biosciences Inc.). Normalized image densities are expressed as means  $\pm$  S.E.M.

**Chemiluminescence Detection of Cell Surface hERG Protein.** A hemagglutinin (HA) tag was inserted into the extracellular loop of hERG between transmembrane domains S1 and S2 (Ficker et al., 2003). Stably transfected HEK/hERG WT HA<sub>ex</sub> cells were plated at 40,000 cells/well in a 96-well plate. After overnight incubation with pentamidine, cells were fixed with ice-cold 4% paraformaldehyde, blocked by incubation with 1% goat serum, and incubated for 1 h with rat anti-HA antibody (Roche Diagnostics). After washing, horseradish peroxidase-conjugated goat anti-rat IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and the dsDNA stain SYBR Green (Molecular Probes, Eugene, OR) were added for 1 h (Myers, 1998; Margeta-Mitrovic et al., 2000). SYBR Green fluorescence was measured to determine cell numbers. Chemiluminescent signals were developed using SuperSignal (Pierce Chemical) and captured in a luminometer. If necessary, luminescence signals in pentamidine-treated wells were corrected for cell loss as measured by SYBR Green fluorescence with the data presented as normalized surface expression relative to control (means  $\pm$  S.E.M.). For correction of cell loss, a standard curve of SYBR Green fluorescence was generated using four different amounts of cells per well (10, 20, 30, or 40  $\times 10^3$ ,  $n = 3$ ).

## Results

**Pentamidine Does Not Directly Block hERG Currents.** Direct block of the cardiac potassium channel hERG is the most common mechanism underlying acquired long QT syndrome. Therefore we first studied the effects of acute application of extracellular pentamidine on hERG currents activated with depolarizing pulses to +20 mV from a holding potential of –80 mV. Pentamidine block was evaluated by analyzing peak tail current amplitudes on return to –40 mV (Fig. 1A). Pentamidine demonstrated no significant inhibition of hERG currents with ascending concentrations ranging from 0.3 to 10  $\mu$ M (Fig. 1B). With 10  $\mu$ M pentamidine in the extracellular perfusate, we observed a maximum inhibition of  $13 \pm 3\%$ , which was similar to current run-down observed over the same time period ( $12 \pm 5\%$ ,  $n = 6$ ). To exclude the possibility that access of the dicationic pentamidine to an intracellular blocking site was hindered, we per-



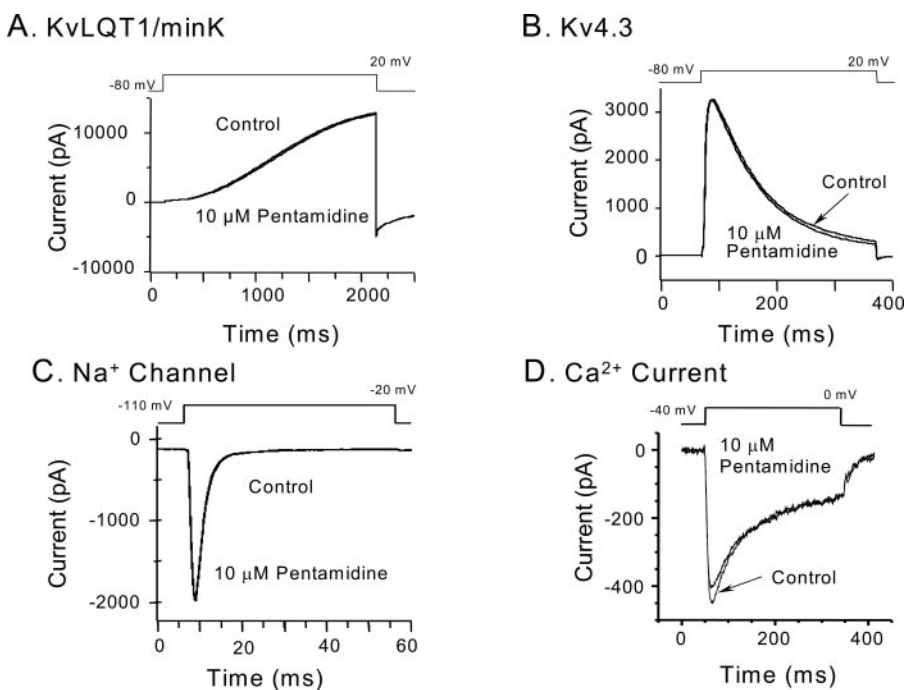
**Fig. 1.** Effects of pentamidine on hERG currents. A, whole-cell hERG currents were elicited by 2-s depolarizing pulses to +20 mV from a holding potential of  $-80$  mV at 40-s intervals. The membrane potential was then returned to  $-40$  mV to generate large outward tail currents. The effects of 3 and 10  $\mu\text{M}$  pentamidine are shown. B, dose-response relationship of pentamidine block. Pentamidine produced  $13 \pm 3\%$  inhibition of hERG currents at the highest concentration tested (10  $\mu\text{M}$ ,  $n = 6$ ), a value similar to the current run-down observed over the same time period (approximately 10 min).

formed experiments with 10  $\mu\text{M}$  pentamidine added to the intracellular pipette solution. With intracellular application of pentamidine, hERG currents showed a small time-dependent run-down averaging  $14 \pm 5\%$  when measured 10 min after initiation of whole-cell recordings ( $n = 4$ ). This value was similar to that observed for vehicle-treated (0.1% dimethyl sulfoxide) control cells ( $15 \pm 5\%$  reduction after 10 min,  $n = 3$ ).

Since block of other repolarizing cardiac potassium currents such as Kv4.3 or KvLQT1/minK may also prolong the cardiac action potential (Nerbonne, 2000), we tested whether those currents were affected by pentamidine. Both KvLQT1/minK and Kv4.3 channels were activated by depolarizing step pulses to +20 mV from a holding potential of  $-80$  mV. Currents were continuously recorded whereas 10  $\mu\text{M}$  pentamidine was applied for 10 min with the extracellular bath solution. Neither KvLQT1/minK nor Kv4.3 currents were blocked at this concentration ( $p > 0.05$ , paired  $t$  test;  $n = 5-6$ ; Fig. 2, A and B). In addition, we studied a possible contribution of cardiac inward currents to the proarrhythmic effects exerted by pentamidine. We elicited sodium currents in HEK293 cells stably expressing the cardiac sodium channel gene *SCN5A* using depolarizing pulses to  $-20$  mV from a

holding potential of  $-110$  mV while perfusing 10  $\mu\text{M}$  pentamidine for 10 min. At this concentration, neither peak currents nor current kinetics were affected ( $p > 0.05$ , paired  $t$  test,  $n = 6$ , Fig. 2C). Since cardiac calcium currents are composed of multiple subunits and are not easily reconstituted in heterologous expression systems, we used freshly isolated guinea pig ventricular myocytes to evaluate pentamidine effects. Cardiac calcium currents were activated from a holding potential of  $-40$  mV with depolarizing step pulses to 0 mV. In these experiments, we detected a small reduction in peak current amplitudes of  $4.4 \pm 3.7\%$  ( $n = 3$ ) whereas steady-state currents remained stable in the presence of 10  $\mu\text{M}$  pentamidine (Fig. 2D).

**Prolonged Exposure to Pentamidine Inhibits Maturation of hERG.** Since pentamidine did not directly block cardiac membrane currents, we explored whether prolonged exposure to pentamidine might interfere with hERG processing as reported for the antineoplastic drugs geldanamycin and  $\text{As}_2\text{O}_3$  (Ficker et al., 2003, 2004). To this end, we exposed stably transfected HEK/hERG cells overnight (16–20 h) to increasing concentrations of pentamidine. We found that hERG currents were reduced in a dose-dependent manner with an  $\text{IC}_{50}$  of 5.1  $\mu\text{M}$  as determined by analyzing changes

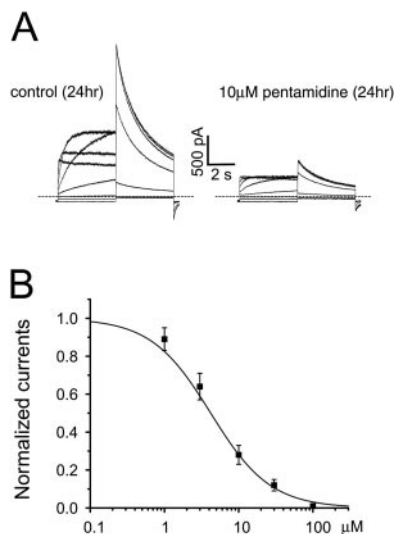


**Fig. 2.** Pentamidine has no effect on other major cardiac membrane currents. A, heterologously expressed KvLQT1/minK currents recorded under control conditions and after extracellular application of 10  $\mu\text{M}$  pentamidine. Pentamidine had no effect on KvLQT1/minK ( $n = 5$ ). B, effects of pentamidine on heterologously expressed Kv4.3 currents and cardiac  $\text{Na}^+$  currents (expressed from *SCN5A* gene) recorded under control conditions and in the presence of 10  $\mu\text{M}$  pentamidine (C). As in the case of KvLQT1/minK, 10  $\mu\text{M}$  pentamidine has no effect on either Kv4.3 or  $\text{Na}^+$  currents ( $n = 6$ ). D, effect of 10  $\mu\text{M}$  pentamidine on native L-type calcium currents recorded in guinea pig ventricular myocytes. Exposure to 10  $\mu\text{M}$  pentamidine failed to significantly reduce cardiac  $\text{Ca}^{2+}$  currents ( $n = 3$ ). Voltage protocols for each experiment are shown above current traces.

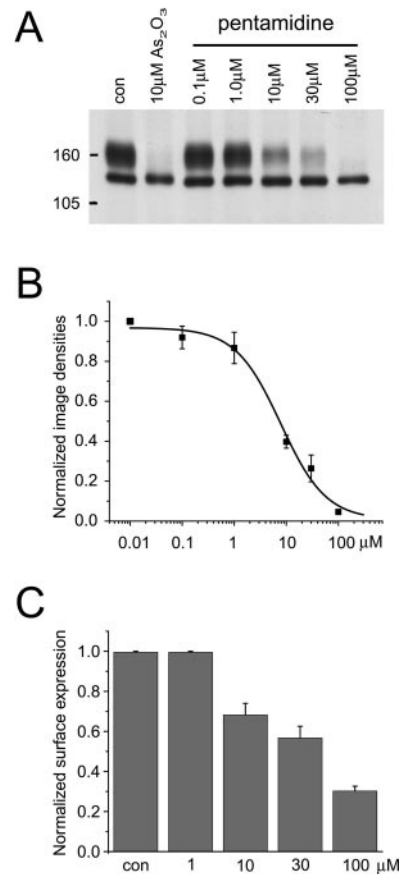


in tail current amplitudes ( $n = 8-10$ , Fig. 3, A and B). To validate our electrophysiological analysis, we performed Western blots of hERG protein isolated under control conditions and after overnight exposure to increasing concentrations of pentamidine. hERG channels are synthesized as a core-glycosylated, immature ER form of about 135 kDa and as a mature, fully glycosylated cell surface form of about 160 kDa. Incubation with pentamidine produced a dose-dependent decrease in the amount of mature fully glycosylated hERG protein (Fig. 4A). Expression of the mature cell surface form of hERG was suppressed with an  $IC_{50}$  of  $7.8 \mu\text{M}$  ( $n = 3$ ; Fig. 4B), which is similar to the  $IC_{50}$  of  $5.1 \mu\text{M}$  determined in electrophysiological experiments on chronic exposure to pentamidine. To quantify pentamidine-induced changes in surface expression of hERG protein more directly, we used a chemiluminescence assay. This assay was performed with HEK293 cells stably expressing a modified hERG protein with an extracellular HA epitope tag inserted in the extracellular S1-S2 linker (Ficker et al., 2003). HEK/hERG-HA<sub>ex</sub> cells were treated overnight with 10, 30, or 100  $\mu\text{M}$  pentamidine, and surface expression of hERG-HA<sub>ex</sub> was reduced by 30, 40, and 70%, respectively (Fig. 4C).

**Specificity of Pentamidine Effects on hERG Trafficking.** Since processing of hERG may be handled by proteins shared between different ion channels, the question arises whether the observed pentamidine effect is specific for hERG or whether other cardiac potassium channels are affected in a similar manner. To test for specificity, we exposed L cells stably expressing the ultrarapid delayed rectifier hKv1.5 overnight (16–20 h) to increasing concentrations of pentamidine and performed Western blots. For all concentrations tested, we detected hKv1.5 protein as a core-glycosylated, immature ER form of about 68 kDa and as a mature, fully glycosylated cell surface form of about 75 kDa (Fig. 5A). Incubation with pentamidine did not alter the expression pattern of hKv1.5 (Fig. 5B). In line with our biochemical



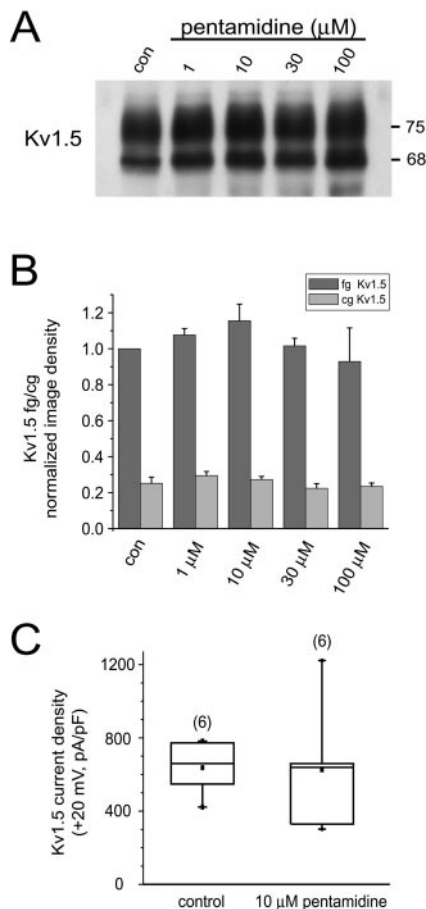
**Fig. 3.** Prolonged exposure to pentamidine suppresses hERG currents heterologously expressed in HEK293 cells. A, representative hERG current recordings obtained under control conditions (left panel) and from an HEK/hERG cell incubated overnight with 10  $\mu\text{M}$  pentamidine (right panel). Currents were elicited from a holding potential of  $-80 \text{ mV}$  with pulses from  $-60$  to  $+60 \text{ mV}$  in  $20 \text{ mV}$  increments. Tail currents were recorded on return to  $-50 \text{ mV}$ . B, concentration dependence of pentamidine effect (24 h treatment).  $IC_{50}$  is  $5.1 \mu\text{M}$  ( $n = 8-10$ ).



**Fig. 4.** Pentamidine inhibits maturation of hERG. A, Western blot showing effects of overnight treatment with pentamidine on hERG channel protein stably expressed in HEK293 cells. On Western blots, pentamidine reduces fully glycosylated, mature 160-kDa hERG in a concentration-dependent manner. Treatment with 10  $\mu\text{M}$   $\text{As}_2\text{O}_3$  was used as positive control. B, concentration-dependent reduction of fully glycosylated 160-kDa hERG after overnight exposure to pentamidine. Image densities on Western blots were quantified using a Storm PhosphorImager and normalized to control.  $IC_{50}$  is  $7.8 \mu\text{M}$  ( $n = 3$ ). C, overnight treatment with pentamidine reduces, in a concentration-dependent manner, surface expression of HA<sub>ex</sub>-tagged hERG protein stably expressed in HEK293 cells as determined by chemiluminescence measurements. Surface expression levels were normalized relative to control.

data, we found that hKv1.5 current densities were not significantly altered upon overnight incubation with 10  $\mu\text{M}$  pentamidine (Fig. 4C). We measured  $636.6 \pm 60 \text{ pA/pF}$  under control conditions and  $625.6 \pm 135.5 \text{ pA/pF}$  after overnight exposure to pentamidine ( $p > 0.05$ , Student's  $t$  test,  $n = 6$ ). We also recorded currents from HEK293 cells stably expressing KvLQT1/minK and Kv4.3 channels under control conditions and after overnight exposure to pentamidine (Fig. 6, Aa and Ba) and found for both channels that current densities were not altered ( $p > 0.05$ , Student's  $t$  test). For KvLQT1/minK, we measured current densities of  $40.5 \pm 8.7 \text{ pA/pF}$  ( $n = 6$ ) under control conditions and  $48.1 \pm 13.2 \text{ pA/pF}$  ( $n = 8$ ) after overnight exposure to 10  $\mu\text{M}$  pentamidine (Fig. 6Ab). In Kv4.3-expressing cells, we measured  $240 \pm 38.5 \text{ pA/pF}$  ( $n = 16$ ) under control conditions and  $195.7 \pm 88.3 \text{ pA/pF}$  ( $n = 10$ ) in the presence of 10  $\mu\text{M}$  pentamidine (Fig. 6Bb).

**Prolonged Exposure to Pentamidine Prolongs the Cardiac Action Potential and Reduces  $I_{Kr}$  in Guinea Pig Ventricular Myocytes.** To test whether our results obtained in heterologous expression systems can also be ap-



**Fig. 5.** Pentamidine does not alter the expression pattern of hKv1.5. **A**, Western blot showing steady-state levels of hKv1.5 protein stably expressed in L cells after prolonged exposure to increasing concentrations of pentamidine. hKv1.5 is expressed as a fully glycosylated protein of 75 kDa and as a core-glycosylated protein of 68 kDa. **B**, image densities of fully glycosylated and core-glycosylated hKv1.5 protein were quantified as a function of pentamidine concentrations using a PhosphorImager. All image densities were normalized to the fully glycosylated 75-kDa protein form measured under control conditions ( $n = 3$ ). **C**, hKv1.5 current densities measured after overnight culture under control conditions and in the presence of 10  $\mu\text{M}$  pentamidine ( $n = 6$ ). Current densities are presented in statistical box charts and were not significantly different (Student's  $t$  test). In box charts, asterisks represent outliers, whiskers determine the 5th and 95th percentiles, boxes determine the 25th and 75th percentiles, and means are represented by square symbols.

plied to cardiomyocytes, we studied the effects of pentamidine on the cardiac action potential. Acute application of 10  $\mu\text{M}$  pentamidine failed to alter action potentials measured in freshly isolated guinea pig ventricular myocytes (Fig. 7). In these experiments,  $\text{APD}_{90}$  was  $535 \pm 32$  ms under control conditions (0 min) and  $522 \pm 31$  ms after extracellular perfusion of 10  $\mu\text{M}$  pentamidine. Action potentials were further studied by culturing myocytes overnight in the absence or presence of 10  $\mu\text{M}$  pentamidine. We found that 10  $\mu\text{M}$  pentamidine prolonged  $\text{APD}_{90}$  significantly from  $374.3 \pm 57.1$  to  $893.9 \pm 86.2$  ms ( $p < 0.05$ ; Student's  $t$  test,  $n = 10-11$ , Fig. 8, A and B). This indicated that chronic drug exposure induces changes compatible with clinically observed QT prolongation and TdP. Since our experiments in heterologous expression systems pointed toward a reduction of the native  $I_{\text{Kr}}$ /hERG current as a possible cause for the observed action potential prolongation, we determined  $I_{\text{Kr}}$  current densities in voltage-clamp experiments performed in guinea pig ven-

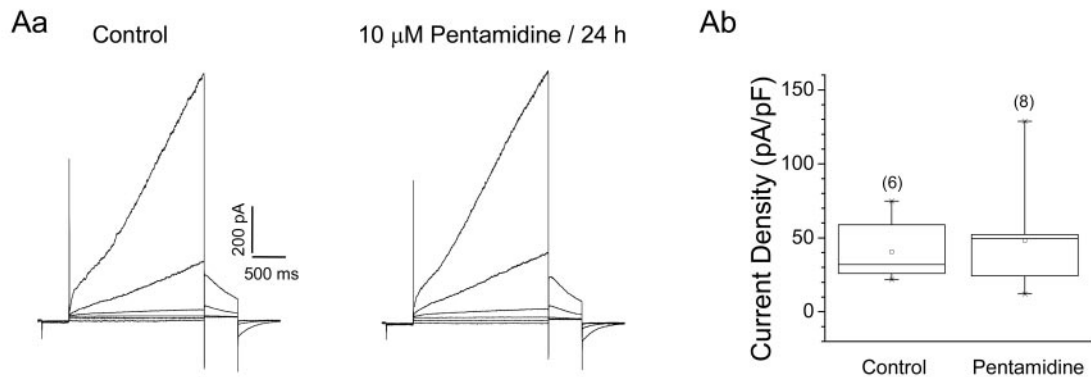
tricular myocytes.  $I_{\text{Kr}}$  currents were elicited in myocytes cultured overnight using a ramp protocol and isolated as E4031 sensitive tail current component upon return to  $-40$  mV (Fig. 8C). In these experiments, chronic exposure to 10  $\mu\text{M}$  pentamidine significantly reduced  $I_{\text{Kr}}$  tail current density by about 35% from  $0.61 \pm 0.09$  to  $0.39 \pm 0.04$  pA/pF ( $p < 0.05$ , Student's  $t$  test,  $n = 6-7$ , Fig. 8D).

## Discussion

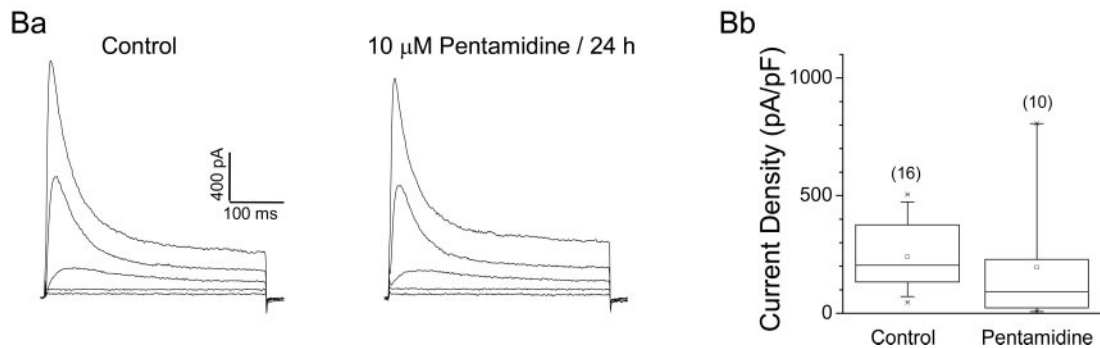
This report is the first to describe the effects of the antiprotazoal drug pentamidine on cardiac ion channels. Pentamidine is known to produce QT prolongation and TdP in clinical use. Most drugs that produce adverse cardiac events do so via a direct block of the cardiac potassium channel hERG that is easily detected with patch-clamp techniques (Pearlstein et al., 2003; Redfern et al., 2003). We were, therefore, surprised to find that acute administration of pentamidine had no immediate effect on hERG currents. Similarly, acute administration of pentamidine failed to affect four different, major cardiac ion channels including KvLQT1/minK, Kv4.3, *SCN5A*  $\text{Na}^+$  channels, and L-type calcium channels. By contrast, prolonged treatment of hERG-expressing cells resulted in a dose-dependent reduction of hERG currents with an  $\text{IC}_{50}$  of about 5  $\mu\text{M}$ . The effect was specific since current densities of heterologously expressed hKv1.5, KvLQT1/minK, and Kv4.3 channels were not altered. Western blots revealed that the reduction in hERG current density was associated with a decrease in the fully glycosylated cell surface form of the hERG protein. Accordingly,  $I_{\text{Kr}}$  density was reduced, and the cardiac action potential was prolonged in cardiomyocytes. Based on these data, we propose that QT prolongation and ventricular tachycardias observed in patients treated with pentamidine are not caused by direct block of hERG or other cardiac ion channels, but rather are the result of a reduction in  $I_{\text{Kr}}$  current density due to an acquired trafficking block of hERG/ $I_{\text{Kr}}$  channels.

Pentamidine has been widely used in the treatment of *P. carinii* pneumonia in patients infected with human immunodeficiency virus (Goa and Campoli-Richards, 1987). The drug is also used in developing countries to treat a variety of parasitic diseases including trypanosomiasis and leishmaniasis (Nacher et al., 2001; Burchmore et al., 2002). Typically, the drug is administered via daily intramuscular injection or slow intravenous infusion at a dose of 4 mg/kg b.wt. These doses result in peak serum levels that range from about 1 to 5  $\mu\text{M}$  (Sands et al., 1985; Conte et al., 1986; Lidman et al., 1994), similar to the concentrations required for inhibition of hERG trafficking in vitro. Prolongation of the QT interval on the electrocardiogram and the development of TdP tachycardias are well documented adverse events associated with pentamidine treatment (Wharton et al., 1987; Bibler et al., 1988; Girgis et al., 1997; Kroll and Gettes, 2002). However, QT prolongation is not immediately evident in these patients and generally takes several days to develop (Stein et al., 1991; Eisenhauer et al., 1994; Otsuka et al., 1997). We believe that this slow time course is consistent with a pentamidine-induced decrease in the expression of functional hERG channels in the heart, rather than a direct blocking effect of the drug, since QT prolongation by direct hERG channel blockers such as dofetilide are evident immediately upon administration (Lande et al., 1998).

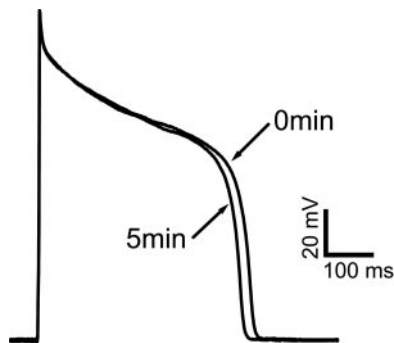
## A. KvLQT1/minK



## B. Kv4.3



**Fig. 6.** KvLQT1/minK and Kv4.3 channels are not sensitive to overnight treatment with 10  $\mu$ M pentamidine. A, KvLQT1/minK channels stably expressed in HEK293 cells. Aa, representative current recordings obtained from control (left) and pentamidine-treated (right) cell. Currents were elicited from a holding potential of  $-80$  mV with 2-s pulses from  $-60$  to  $+20$  mV in 20-mV increments. Tail currents were recorded on return to  $-50$  mV. Ab, current density measured in cells cultured overnight under control conditions and in the presence of 10  $\mu$ M pentamidine. Current densities were determined from current amplitudes at  $+20$  mV and normalized to cell capacitance. Data are presented in statistical box charts as described in the legend to Fig. 5. B, Kv4.3 channels stably expressed in HEK293 cells. Ba, representative current recordings obtained from control (left) and pentamidine-treated (right) cells. Currents were elicited from a holding potential of  $-80$  mV with depolarizing 400-ms voltage steps from  $-60$  to  $+20$  mV in increments of 20 mV. Bb, current densities were measured in the absence and presence of 10  $\mu$ M pentamidine as peak currents at 0 mV and normalized to cell capacitance. Data are presented in statistical box charts.



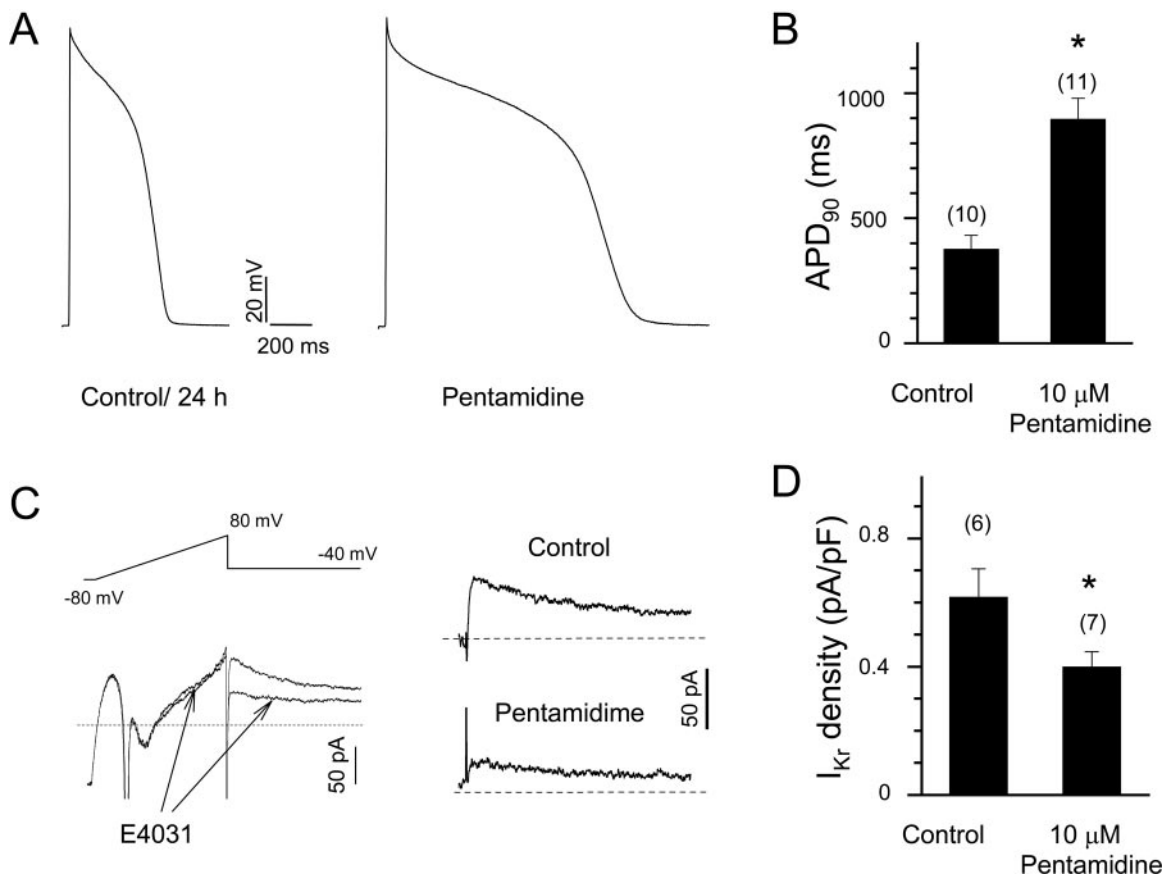
**Fig. 7.** Acute application of pentamidine does not prolong cardiac action potentials. Cardiac action potentials elicited in a freshly isolated ventricular myocyte immediately after establishing whole-cell configuration (0 min) and 5 min after start of extracellular perfusion with 10  $\mu$ M pentamidine. Membrane potential was  $-80$  mV.

At present, it is not clear how pentamidine interferes with hERG processing and maturation. In microbial cells, pentamidine has been reported to inhibit topoisomerase activity and decrease intracellular ATP content, whereas in mammalian cells, it has been shown to inhibit several tyrosine phosphatases (Reddy et al., 1999; Pathak et al., 2002). Other antimicrobial agents such as quinolone antibiotics, which also function as topoisomerase inhibitors in microbial sys-

tems, have been shown to directly block hERG currents (Cirioni et al., 1997; Anderson et al., 2001; Larsen et al., 2003). Similarly, the antineoplastic topoisomerase inhibitor, amsacrine, which has been associated with adverse cardiac events, reduces hERG currents by direct block (Thomas et al., 2004). Both quinolone antibiotics (sparfloxacin, ofloxacin, and ciprofloxacin) and amsacrine do not affect hERG trafficking (L. Wang and E. Ficker, unpublished data). Given these observations, we speculate that the pentamidine-induced trafficking block of hERG is not due to inhibition of topoisomerases but rather to inhibition of a protein or proteins required for successful maturation of hERG potassium channels. Further studies will be necessary to elucidate the biochemical mechanism(s) responsible for pentamidine's effects on hERG protein trafficking.

In summary, the present study demonstrates that the proarrhythmic effects of pentamidine are not due to a direct blockade of hERG currents, but are consistent with inhibition of hERG trafficking and a reduction in the number of functional hERG channels in the heart. Thus, pentamidine joins arsenic trioxide, another nonantiarrhythmic compound that is proarrhythmic as a result of an acquired hERG trafficking defect. Geldanamycin, an antineoplastic Hsp90 inhibitor currently in clinical trials, may be proarrhythmic for similar reasons (Ficker et al., 2003, 2004). In this regard,





**Fig. 8.** Prolonged exposure to pentamidine prolongs cardiac action potentials and reduces  $I_{Kr}$  in cultured guinea pig ventricular myocytes. **A**, representative cardiac action potentials recorded from ventricular myocytes cultured overnight (24 h) under control conditions (left panel) and after incubation with 10  $\mu$ M pentamidine (right panel). **B**, APD<sub>90</sub> measured in control and pentamidine-treated (10  $\mu$ M; 24 h) cardiomyocytes. **C**, isolation of E4031-sensitive  $I_{Kr}$  current in a ventricular myocyte cultured overnight. Current traces were elicited with a 1-s voltage ramp from  $-80$  to  $+80$  mV. Holding potential was  $-80$  mV.  $I_{Kr}$  currents were isolated as E4031-sensitive tail current component on return to  $-40$  mV. E4031-sensitive subtraction currents are shown in right part of panel for a control myocyte and for a myocyte cultured overnight in the presence of 10  $\mu$ M pentamidine. Dashed lines indicate zero current levels. **D**,  $I_{Kr}$  current densities quantified in control and pentamidine (10  $\mu$ M, 24 h)-treated myocytes ( $n = 6-7$ ). \*, significant difference at  $p < 0.05$  (Student's  $t$  test).

chemotherapeutic agents that disrupt protein synthesis as their intended mechanism of action may be of special concern. Likewise, drugs that produce QT prolongation in the clinic only after prolonged treatment may suggest similar effects on the processing of ion channel proteins. At present, the detection of cardiac risk as a result of acquired trafficking defects has not been considered, and the hERG assay presently recommended by the International Conference of Harmonization [ICH S7B—the nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals, European Medicines Agency EMEA, CHMP/ICH/423/02 (June 2004)] will fail to identify trafficking inhibitors.

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