[K⁺]₀-dependent change in conformation of the HERG1 long QT mutation N629D channel results in partial reversal of the in vitro disease phenotype

Guo Qi Teng, James P. Lees-Miller, Yanjun Duan, Bao-Tsen Li, Pin Li, Henry J. Duff

Department of Medicine, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

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Abstract

Objectives: We hypothesized that exposure of N629D/wildtype channels to transient increases in [K⁺]₀ could alter the conformation of the outer vestibule and thus reverse the disease phenotype. N629D is a recently described mutation of the HERG1 gene that causes familial long QT syndrome. This mutation alters the pore signature sequence resulting in loss of K⁺ selectivity. Previous studies have reported that enforced occupancy of [K⁺]₀ at sites near the selectivity filter alters the conformation/folding of the outer vestibule of the Kv2.1 channel. Methods: Since the long QT syndrome is manifest in individuals who are heterozygous for this HERG trait, we co-expressed N629D and the wildtype at equimolar concentrations. Results: Co-expression of N629D/wildtype in Xenopus oocytes and mammalian cells resulted in a channel with a positive shift in reversal potential and a loss in the outward tail current, relative to the wildtype. Exposure of the N629D/wildtype to transient increases in [K⁺]₀ from 5 to 40 mM/l changed the tail current from inward to outward during repolarization and restored the reversal potential to values similar to the wildtype. These findings in Xenopus oocytes were also seen when N620D/wildtype channels were expressed in mammalian cells. These [K⁺]₀-dependent changes persisted for hours after the [K⁺]₀ was returned to 2.5 mM. This potential therapeutic effect began with increases in [K⁺]₀ from 2.5 to 5 mM. Conclusions: This study reports a novel therapeutic strategy and mechanism to partially restore physiologic function in this HERG LQTS mutation.

Keywords: Arrhythmia (mechanisms); Intra/extracellular ions; Ion channels; K-channel; Long QT syndrome

1. Introduction

Mutations of the human ether a-go-go-related gene, HERG1 [1–10], cause one form of the long QT syndrome. The N629D HERG mutation [9,10] is of particular interest as it alters the pore selectivity signature sequence GFGN to GFGD. Action potential clamp studies have examined the physiologic relevance of this mutation [9]. The important consequence of the N629D mutation is a loss of selectivity for K⁺ over Na⁺, resulting in a shift in the reversal potential from approximately −90 to −50 mV and a loss of the property of inward rectification. A shift in the reversal potential, resulting from a change in selectivity, results in an inward depolarizing Na⁺ current during repolarization between −20 and −70 mV [10]. This inward sodium current likely reflects occupancy by Na⁺ ions in the K⁺ binding sites. The presence of an inward Na⁺ current [11] along with a loss of outward K⁺ current during late repolarization likely contributes to the prolonged QT interval in patients with the N629D mutation. A loss of inward rectification results in a larger potassium current early during the action potential, but this is of little physiologic consequence, due to the redundancy of K⁺ channel currents during the early phases of the action potential.

[K⁺]₀-dependent changes in occupancy of binding sites, near the selectivity filter in the outer vestibule of the Kv2.1 channel, modulate its folding, K⁺ selectivity and sensiti-

*Corresponding author. Tel: +1-403-220-6841; fax: +1-403-270-0313.
E-mail address: hduff@ucalgary.ca (H.J. Duff).

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ty to TEA [12–14]. From this data, we hypothesized that changes in occupancy of K\(^+\) for sites near the selectivity filter could alter the conformation of the external vestibule of the N629D/wildtype (WT) channel normalizing potassium selectivity. Accordingly, the specific objectives of this study were to assess the effects of exposure of the N629D/WT to transient increases in [K\(^+\)]\(_o\) on ion selectivity, inactivation properties and sensitivity to dofetilide. Herein, we report a change in the properties of N629D/WT channels probably resulting from a conformational change that occurs with transient exposure to increases in [K\(^+\)]\(_o\) and persists even when the [K\(^+\)]\(_o\) is lowered within the physiologic range.

2. Methods

The investigation conforms with 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).

2.1. Oocyte isolation

Oocytes were surgically removed from anesthetized female frogs (Xenopus laevis, Nasco, Fort Atkinson, WI, USA) and defolliculated with 1 mg/ml collagenase B (Boehringer-Mannheim, Indianapolis, IN, USA). A Drummond microinjector was used to inject the oocytes. Oocytes were stored for 1–7 days at 17°C in standard ND-96 solution (in mM: 96 NaCl, 2 KCl, 1 MgCl\(_2\), 1.8 CaCl\(_2\), and 5 HEPES, pH 7.4) supplemented with 10 μg/ml gentamycin sulfate.

2.2. Heterologous expression

HERG1 A in a pSP64 was obtained from M.T. Keating [1]. Site-directed mutagenesis was carried out by overlap extension using the polymerase chain reaction [15]. WT and mutant N629D were transcribed in vitro from the SP6 promoter. The RNA transcripts were injected into Xenopus oocytes and the expressed currents were analysed after 1–3 days using two-microelectrode voltage clamp techniques. Since the long QT syndrome is manifested in individuals (at zero potential) was considered the reversal potential.

2.3. Electrophysiologic recordings

The oocytes were perfused with modified frog Ringer’s solution at room temperature 21–22°C containing (mM): NaCl, 114; KCl, 2.5; MgCl\(_2\), 1; CaCl\(_2\), 1.8; HEPES 10; pH 7.2 adjusted with NaOH. Niflumic acid was included (0.15 mmol/l) to block chloride currents. Glass microelectrodes were filled with 3 M KCl with tip resistances of 1.0–1.5 MΩ. Oocytes were clamped with a Geneclamp 500 amplifier and voltage clamp protocols were generated with pClamp software (Axon Instruments), a Pentium computer and a Digidata 1200 interface board (Axon Instruments). Currents were sampled at a rate of 2 kHz and filtered at 1 kHz using a 4-pole Bessel filter. The oocyte membrane was held at −80 mV between pulses. The interpulse interval was 30 s.

The oocyte bath had a volume of 2.2 ml and a flow rate of 1 ml/min. To determine how quickly the oocyte is in equilibrium with a change in [K\(^+\)]\(_o\), the N629D/WT channel which manifests an inward tail current at baseline was first exposed to an elevated [K\(^+\)]\(_o\) of 40 mM/l and then returned to 2.5 mM/l to elicit an outward tail current (see Section 3). The oocytes were then exposed to very low [K\(^+\)]\(_o\) (30 μm/l) for a variable time to assess how long it would take to recapitulate the control phenotype. The control phenotype was reconstituted within 3.5 min.

In this study, 6 ng of cRNA encoding WT and N629D were co-injected [10]. As previously reported, co-injection of equimolar concentrations of N629D and WT yielded three phenotypes: a HERG-like phenotype, an N629D-like phenotype and an intermediate phenotype [10]. This study focuses on oocytes manifesting the intermediate phenotype with reversal potentials of −50±10 mV at a [K\(^+\)]\(_o\) of 2.5 mM.

The current–voltage relationships for the time-dependent currents were studied by applying voltage clamp steps from a holding potential of −80 mV to different depolarizing levels. Each outward current was elicited by a 4-s depolarizing pulse to potentials of −70 to +30 mV in 10-mV steps. To assess kinetics of deactivation and to assess reversal potential, a double pulse protocol was followed. The first pulse was introduced from a holding potential of −80 mV to a potential of +20 mV for 1.6 s, followed by a second 10-s pulse to a variety of test potentials ranging from −30 to −120 mV to elicit tail currents. To assess the reversal potential, current–voltage relationships of the tail currents were plotted. The point at which the current–voltage relationship crossed the x-axis (at zero potential) was considered the reversal potential.

Control measurements were obtained at [K\(^+\)]\(_o\) of 2.5 mM/l. Oocytes were then transiently (10 min) exposed to altered [K\(^+\)]\(_o\) and then returned to a [K\(^+\)]\(_o\) of 2.5 mM/l. Electrophysiologic measurements were not made during the transient exposure to changes in [K\(^+\)]\(_o\), but rather measurements were made at least 15 min after a return to a [K\(^+\)]\(_o\) of 2.5 mM/l. Thus, all measurements were made at a constant [K\(^+\)]\(_o\) of 2.5 mM.

2.4. Statistics

Analysis of variance was used to compare the elec-
trophysiologic data. Tukey’s test was used for multiple comparisons. NS indicates non-significant. For paired comparisons in the same cells, the paired Student’s t-test was used.

3. Results

3.1. Transient exposure of N629D/WT to 40 mM/l $[K^+]_o$ restores outward tail currents and inward rectification which are features characteristic of WT

Fig. 1 shows typical control N629D/WT currents expressed in Xenopus oocytes before (panel A) and after (panel C) transient exposure to a $[K^+]_o$ of 40 mM/l. Control measurements were obtained at a $[K^+]_o$ of 2.5 mM/l. Oocytes were then transiently (10 min) exposed to increased $[K^+]_o$ and then returned to a $[K^+]_o$ of 2.5 mM/l for at least 15 min. All measurements were made at a constant $[K^+]_o$ of 2.5 mM. Mean current–voltage data are shown in panels D and E. At control, the mean tail currents (panel D) of N629/WT were dominantly inward and the time-dependent current (panel E) showed no apparent inward rectification. After transient exposure to a $[K^+]_o$ of 40 mM/l, the tail currents of N629/WT became outward and the inward rectification of the time-dependent current became manifest. Panel B shows a similar but less striking effect after exposure to 5 mM/l $[K^+]_o$. These data indicate that transient exposure to a $[K^+]_o$ of as little as 5 mM/l can partially restore the phenotype towards WT. These changes in phenotype were stable for as long as we made measurements in a single oocyte (2 h).

Since WT and N629D were co-expressed, the formation of tetramers likely follows a binomial distribution. Indeed, a small proportion of the channels formed during co-expression must be homomultimeric WT and N629D. To determine whether the responses to transient increases in $[K^+]_o$ shown in Fig. 1 could be explained by changing the electrophysiologic features of homomultimeric N629D or WT, we assessed the response of homotetrameric channels to transient exposure to a $[K^+]_o$ of 40 mM/l. Fig. 2 shows that the tail currents of WT and homomultimeric N629D were not altered by transient increases in $[K^+]_o$ ($n=5$) relative to control. This finding suggests that the channels

![Fig. 1](image-url). Transient exposure to elevated $[K^+]_o$ permanently restores outward tail currents and inward rectification of N629D/WT channels expressed in Xenopus oocytes when returned to a $[K^+]_o$ of 2.5 mM for at least 15 min. Representative example of families of currents elicited by the protocol (inset) (A) at control, (B) after transient exposure to a $[K^+]_o$ of 5 mM, and (C) after 40 mM in Xenopus oocytes. All measurements were made at a constant $[K^+]_o$ of 2.5 mM. After transient exposure to a $[K^+]_o$ of 40 mM, the time-dependent current manifests inward rectification. Panel D and E shows mean current–voltage relationships of the tail and time-dependent currents (end of pulse), respectively. At control, the tail-currents were inward (D). After transient exposure to a $[K^+]_o$ of 5 or 40 mM, the tail current becomes outward. In panel E, the time-dependent current–voltage relationship is virtually linear at baseline, whereas after transient exposure to a $[K^+]_o$ of 40 mM, the current shows inward rectification over the voltage range from −30 to +10 mV. * Indicates a statistically significant change compared to control; $P<0.05$, $n=5$. 
Fig. 2. Transient exposure to elevated [K\(^+\)]\(_o\) does not alter the outward tail currents and inward rectification of WT or the character of the homomultimeric N629D channels expressed in Xenopus oocytes. Representative examples of (panels A,B) WT and (panels C,D) homomultimeric N629D are shown when expressed in Xenopus oocytes before and after transient exposure to a [K\(^+\)]\(_o\) of 40 mM. The magnitude of the WT tail current and the character inward rectification was never altered after transient exposure to a K\(^+\) of 40 mM. Similarly, the inward tail currents characteristic of homomultimeric N629D was never altered by transient exposure to elevated [K\(^+\)]\(_o\), \((n=9)\).

WT, N629D and N629D/WT were heterologously expressed in both Xenopus oocytes and mammalian cells (L-cells). Fig. 3A,B shows the dofetilide-sensitive WT and N629D homomultimeric currents when expressed in L-cells. The electrophysiologic features of WT and N629D currents are virtually identical in L-cells and Xenopus oocytes. Specifically, the tail currents of homomultimeric N629D were dominantly inward (B) and the time-dependent current showed no apparent inward rectification.

To address whether the responses to transient exposure to elevated [K\(^+\)]\(_o\) were similar in Xenopus oocytes and mammalian cells, N629D and WT were co-expressed in L-cells. Panels C and D in Fig. 3 show a representative example of the N629D/WT co-expression current before and after transient exposure to a [K\(^+\)]\(_o\) of 40 mM/l in L-cells. Similar to the results in Xenopus oocytes, mean tail currents of N629D/WT at baseline were dominantly inward, and the time-dependent current showed no apparent inward rectification. After transient exposure to a [K\(^+\)]\(_o\) of 40 mM/l, the tail currents of N629D/WT became outward, and the inward rectification of the time-dependent current became manifest. These data indicate that the baseline electrophysiologic characteristics of WT, N629D and N629D/WT are similar when heterologously expressed in either Xenopus oocytes or L-cells. Moreover, transient elevation of [K\(^+\)]\(_o\) results in partial restoration of the outward tail currents and inward rectification when N629D/WT is expressed in either Xenopus oocytes or L-cells.

3.2. Transient exposure to increases in [K\(^+\)]\(_o\) alters the reversal potential of N629D/WT

To ascertain whether transient elevation of [K\(^+\)]\(_o\) restored reversal potentials towards those observed in WT, the protocol shown in the inset of Fig. 4 was applied. All measurements were made at a constant [K\(^+\)]\(_o\) of 2.5 mM. Representative reversal potential measurements of N629D/WT currents, obtained before \((-53\) mV) and after \((-83\) mV) transient exposure to a [K\(^+\)]\(_o\) of 40 mM/l, are shown in Fig. 4A–C when expressed in Xenopus oocytes. Panel D shows the mean reversal potential responses to transient exposure to variable concentrations of [K\(^+\)]\(_o\). At baseline, the mean reversal potential of N629D/WT \((\bullet)\) was \(-49\pm3\) mV (panel D). Transient exposure to 5, 10 and 40 mM/l [K\(^+\)]\(_o\) shifted the reversal potential leftward to \(-72\pm4\), \(-76\pm2\) and \(-80\pm3\) mV, respectively \((n=5, P<0.001;\ ANOVA); all treatments were significantly different but there was no difference between 5, 10 and 40 mM [K\(^+\)]\(_o\)). Importantly, this change in the reversal potential...
Fig. 4. Transient exposure to elevated $[K^+]_o$ partially normalizes reversal potentials in N629D/WT channels expressed in *Xenopus* oocytes. Representative examples of reversal potentials measured at a $[K^+]_o$ of 2.5 mM at control (A) and after transient exposure to a $[K^+]_o$ of 40 mM (B). The tail current–voltage relationship for the representative example from A and B are shown in panel C. Panel D relates the mean reversal potential to the concentration of $[K^+]_o$, to which the oocytes were exposed in WT (△) and in N629D/WT (●). All measurements were made at a constant $[K^+]_o$ of 2.5 mM/l ($n=5$). * Designates $P<0.05$ compared to control.

3.3. Restoration of baseline N629D/WT phenotype by transient exposure to $\mu M$ $[K^+]_o$.

To discover whether a more profound lowering of the $[K^+]_o$ to $\mu M$ concentrations could result in a return to the control phenotype, the N629D/WT channels were first transiently exposed to 40 mM/l $[K^+]_o$ and then returned to 2.5 mM. Thereafter, the oocyte was transiently exposed to much lower ($\mu M$) concentrations of $K^+$ and then returned to a $[K^+]_o$ of 2.5 mM/l. Fig. 5A–D shows representative examples of currents expressed in *Xenopus* oocytes recorded at control, after transiently higher and then after transiently lower $[K^+]_o$. All measurements were made at a constant $[K^+]_o$ of 2.5 mM. At control (A), there was little or no outward tail current whereas transient exposure to an elevated $[K^+]_o$ of 40 mM/l resulted in an outward tail current (B). After exposure to 100 $\mu M$ (C) and 30 $\mu M$ $[K^+]_o$ (D), the inward tail current phenotype was restored to control. Panel E shows the mean reversal potential observed at control and after transient exposure to higher and then lower $\mu M$ $[K^+]_o$.

3.4. Absence of response to transient elevation of $[K^+]_o$ during Na$_o$ replacement.

If Na$^+$ and K$^+$ ions compete for binding sites in the outer vestibule of N629D/WT, it might be expected that replacing Na$^+$ with NMG$_o$ would mimic the effect of transiently increasing $[K^+]_o$. Additionally, in the setting of NMG$_o$, the response to transiently increasing $[K^+]_o$ would be diminished. Fig. 6 shows the mean reversal potential observed at baseline, after replacing the Na$^+$ with NMG$_o$, and then after transient exposure to a $[K^+]_o$ of 40 mM/l. We observed: (1) replacing Na$^+$ with NMG$_o$ mimics the effect of transiently increasing $[K^+]_o$; (2) the reversal potential is significantly shifted to the left (more hy-
perpolarized) and (3) the response to transient increases in $[K^+]_o$ was abrogated ($n=5$).

**3.5. Exposure of N629D/WT to $[K^+]_o$ of 40 mM/l does not alter onset of inactivation**

Since transiently increasing $[K^+]_o$ restored inward rectification and since inward rectification of HERG is due to C-type inactivation [16,17], we assessed the characteristics of onset and recovery from inactivation at control and after transient increases in $[K^+]_o$. Representative examples of onset of inactivation elicited by a triple pulse protocol [16,17] are shown in Fig. 7A before (control) and after transient exposure to a $[K^+]_o$ of 40 mM/l. Surprisingly, the onset of inactivation both in terms of tau values and voltage-dependence was similar before and after an increase of $[K^+]_o$ to 40 mM/l. However, the magnitude of the tail current (panel C) was significantly increased after exposure to a $[K^+]_o$ of 40 mM/l secondary to the leftward shift in the reversal potential (panel D and Fig. 4). Panel E shows representative examples of recovery from inactivation before (control) and after exposure to a $[K^+]_o$ of 40 mM/l using a protocol similar to that of Spector et al. [17]. A qualitative difference in the character of recovery from inactivation was observed. At baseline, little, if any, time-dependent recovery from inactivation (virtually instantaneous) was observed. After exposure to a $[K^+]_o$ of 40 mM/l, a distinct time-dependent recovery from inactivation was manifested, which is characteristic of the WT channel. The mean magnitude of the tail currents expressed in *Xenopus* oocytes is plotted against voltage in panel D. These data indicate that transient exposure to elevated $[K^+]_o$ alters net $K^+$ permeation during the tail.

**3.6. Pharmacologic significance of exposure of N629D/WT to 40 mM/l $[K^+]_o$**

Since dofetilide binds to amino acids near the intracellular interface of the HERG channel [22], we addressed
Fig. 7. Transient exposure to a [K+]o of 40 mM does not alter onset of inactivation but restores recovery from inactivation in N629D/WT channels expressed in *Xenopus* oocytes. Panel A shows representative examples of the onset of inactivation at control and after a [K+]o of 40 mM/l was assessed using a triple pulse protocol as shown in the inset. The −110 mV interpulse interval was 25 ms in duration. Recovery from inactivation at −110 mV proceeds with a time constant of 4 ms for both WT and N629D/WT. The duration of the interpulse interval of 25 ms was sufficient to allow approximately 90% of the channels to recover from inactivation but was too short for substantial deactivation. Panel B shows representative examples of recovery from inactivation. At control, there was little, if any, time dependence to recovery from inactivation (virtually instantaneous, see arrow) whereas after exposure to a [K+]o of 40 mM/l, a distinct time-dependent recovery from inactivation was manifest (see arrow). Panel C shows the mean magnitude of the tail current at control (○) and after exposure to elevated [K+]o (●). Panel D shows the mean current–voltage relationship of the tail currents (n=5). * Designates *P*<0.05 vs. control.

whether changes in the conformation of the N629D/WT channel, when transiently exposed to [K+]o of 40 mM/l, would also change the conformation of these sites within the S6 domain. Fig. 8 shows the extent of block of tail-currents in N629D/WT expressed in *Xenopus* oocytes before (control) and after transient exposure to elevated [K+]o. All measurements were made at a constant [K+]o of 2.5 mM. The IC50 for N629D/WT was 8.3±2 μM/l before (control) and 1.8±1 μM/l after exposure to a [K+]o of 40 mM/l (*P*<0.05). The IC50 for WT is 0.125±0.003 μM for WT and its concentration response is also shown in Fig. 8. These data indicate that transient exposure to a [K+]o of 40 mM/l results in an increased affinity of the N629D/WT channel for dofetilide.

4. Discussion

4.1. Potential physiologic relevance

The dominant in vitro phenotype of N629D/WT is the loss of the outward repolarizing current during phase 3 repolarization due to an impaired K⁺ selectivity of the pore. We propose that transient exposure to a higher [K+]o results in K⁺ occupancy of critical K⁺ binding sites near the selectivity filter, which results in [K+]o-dependent normalization of the folding of the outer vestibule of the mutant channel thus altering selectivity and recovery from C-type inactivation. We provide evidence that Na⁺ and K⁺ ions compete for sites within the selectivity filter and
4.2. Mechanism of partial restoration of the WT phenotype: potassium selectivity

Our findings, that a change in the selectivity signature sequence from GFGN to GFGD mediates a change in ion selectivity, are in keeping with previous studies by Heginbotham [18]. Zhou et al. [19] have reported that the carbonyl oxygen atoms within the selectivity signature sequence of KeSA crystal form an antiprism around each K⁺ binding site. Moreover, the selectivity filter changes its ion coordination structure at low [K⁺]₀ solutions [19]. Thus, our findings that [K⁺]₀ can change the co-ordinate binding structure of the selectivity filter is in keeping with previous studies. In addition, our findings are in keeping with those of Korn’s laboratory who reported [K⁺]₀-dependent changes in occupancy of binding sites near the selectivity filter in the outer vestibule of the Kv2.1 channel modulating its folding, K⁺ selectivity and sensitivity to TEA [12–14].

Evidence that Na⁺ and K⁺ ion compete for binding sites near the selectivity filter in the N629D/WT channel includes: (1) replacing extracellular Na⁺ with NMG, mimics the effects of increasing extracellular [K⁺]₀ and (2) transiently increasing [K⁺]₀ has no effect when extracellular Na⁺ had been replaced with NMG. These data are in keeping with those of Luzhkov et al. [20] who reported that Na⁺ occupancy, at even one of the binding sites within the selectivity filter of a K⁺ channel, can inhibit outward currents and predispose to inward currents, as is observed herein with N629D/WT.

4.3. Mechanism of partial restoration of inward rectification: recovery from inactivation

In the N629D/WT channel, [K⁺]₀-dependent changes in the conformation of the outer vestibule partially restores the characteristic of inward rectification. In a previous study, we had considered that the loss of inward rectification of N629D related to a loss of inactivation [10]. While the N629D/WT channel can inactivate, it recovers from inactivation virtually instantaneously, thus limiting the accumulation of channels in the inactivated state and eliminating inward rectification. Moreover, the biophysical mechanism of the [K⁺]₀-dependent recovery of the inward rectification of this mutant channel relates to a slowing of the recovery of inactivation. The slowing of recovery from inactivation would allow the accumulation of channels in the inactivated state and thus manifest inward rectification. Similarly, Gomez-Lagunas et al. [21] reported that the permeant ion is a determinant of recovery from N-type inactivation in the Shaker B K⁺ channel. In their study, the rapid phase of recovery from inactivation is enhanced by permeation with K⁺ ions but not by Na⁺ ions. Thus, the permeant ion is a determinant of recovery from inactivation. Additional studies by Kiss et al. [23] provide evidence that occupancy of binding sites by permeant ions simultaneously modulate the selectivity filter and the process of inactivation by a common allosteric mechanism.

4.4. Other effects of changes in folding: dofetilide binding

Previous studies indicate that changes in K⁺ occupancy of binding sites in the external vestibule of the Kv2.1 channel change the affinity for its blocker, TEA [12–14]. Similarly, in this study, we report that transient exposure of the channel to higher [K⁺]₀ can significantly increase the affinity for dofetilide. The four-fold change in affinity is relatively modest. Since dofetilide binds to amino acids in the S6 [22,24] near the intracellular interface, our data suggest [K⁺]₀-dependent conformational change in the channel as a whole, or that the process of inactivation governs dofetilide binding, as has been previously proposed [22].

4.5. Potential clinical relevance

This in vitro experimental finding is in keeping with the findings of Compton et al. [25] who reported partial correction of repolarization abnormalities by increases in serum potassium in patients with HERG LQTS mutations. The present study provides the molecular characterization of the mechanism for the correction of the phenotype in one specific HERG LQTS mutation.

In summary, transient exposure of the N629D/WT channel to a [K⁺]₀ of as little as 5 mM/l replaces an inward depolarizing Na⁺ current during phase 3 with an
outward repolarizing $K^+$ current. If these in vitro data can be extrapolated to humans with the N629D mutation, an increase in $[K^+]_o$ might be expected to partially normalize the long QT interval and oppose arrhythmogenesis.

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