Prolonged repolarization and triggered activity induced by adenoviral expression of HERG N629D in cardiomyocytes derived from stem cells

GuoQi Tenga, Xiang Zhaoa, James C. Crossb, Pin Lia, James P. Lees-Millera, Jiqing Guoa, Jason R.B. Dyckc,d, Henry J. Duffa,*

aDepartment of Medicine, University of Calgary, Calgary, Alberta, Canada
bDepartment of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada
cDepartment of Paediatrics, University of Alberta, Edmonton, Alberta, Canada
dDepartment of Pharmacology, University of Alberta, Edmonton, Alberta, Canada

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Abstract

Objective: The long QT syndrome, N629D HERG mutation, alters the pore selectivity signature sequence, GFGN to GFGD. Heterologous co-expression of N629D and the wildtype HERG resulted in a relative loss of the selectivity of K+ over Na+, but its physiologic relevance has not been assessed in cardiac myocytes. Methods and results: Accordingly, N629D was overexpressed, via adenoviral gene transfer, in cardiomyocytes derived from mouse stem cells. Three IKr phenotypes were observed: (1) the wildtype-like IKr showed inward rectification and a positive tail current; (2) the N629D-like IKr showed outward rectification and an inward tail current; and (3) intermediate IKr showed a small outward tail current. Action potentials (AP) were paired with the IKr measurements in each cell. Resting membrane potential (RMP) was critically dependent on the IKr phenotype. The resting membrane potential of the cells was −61 ± 5 mV (n = 40) in wildtype, −63 ± 3 mV (n = 18) in wildtype-like IKr phenotype, −30 ± 2 mV (n = 12) in N629D-like and −47 ± 2 mV (n = 24) in intermediate phenotype (p < 0.00001). Triggered action potential durations (APD) were: 62 ± 12 ms (n = 6) in wildtype, 65 ± 11 ms (n = 6) in wildtype-like IKr phenotypes and 106 ± 10 ms (n = 6) (p < 0.01) in intermediate IKr phenotypes. Lowering [K+]o hyperpolarized wildtype cells and cells with a wildtype-like IKr phenotype, but depolarized those with intermediate phenotype (from −45 ± 1 to −35 ± 0.5 mV (n = 12), p < 0.01). In 6 of 12 cells, with intermediate phenotype, the hypokalemia-induced depolarization resulted in triggered activity. TTX suppressed this triggered activity. Conclusion: Overexpression of N629D in cardiomyocytes derived from stem cells results in phenotypic variability in IKr, which was the critical determinant of the resting membrane potential, action potential duration and arrhythmogenic response to low [K+]o.

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1. Introduction

The heartbeat depends on a coordinated wave of depolarization followed by repolarization. Potassium channels play an important role in repolarization. More particularly, the HERG potassium channel plays an important role in phase 3 of repolarization in humans. Mutations of the human ether-a-go-go-related gene, HERG1 cause forms of the long QT syndrome (LQTS) [1–4] in humans. Patients with the LQTS2 have a delayed repolarization phase, which predisposes to fatal arrhythmias. The N629D HERG mutation [5–7] is of particular interest as it alters the pore selectivity signature sequence GFGN (glycine–phenylalanine–glycine–asparagine) to GFGD (glycine–phenylalanine–glycine–aspartate). In contrast to wildtype (WT) HERG currents, homotetrameric N629D channels manifest

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* Corresponding author. Department of Medicine, University of Calgary, 3330 Hospital Drive, N.W., Calgary, Alberta, Canada, T2N 4N1. Tel.: +1-403-220-6841; fax: +1-403-270-0313.
E-mail address: hduff@ucalgary.ca (H.J. Duff).
outward rather than inward rectification and inward (Na⁺) rather than outward (K⁺) tail currents [6,7]. Since the LQTS is manifest in patients heterozygous for this trait, N629D and the wildtype constructs have been co-expressed at equimolar concentrations in Xenopus oocytes and in mammalian cells [7]. Such co-expression resulted in cells manifesting three different mutant I\(_{Kr}\) phenotypes: (1) a WT-like phenotype (outward tail current and inward rectification), (2) a N629D-like phenotype (inward tail current and outward rectification) and (3) an intermediate phenotype (small outward tail current and minimal inward rectification [6]). A common consequence was a variable loss of selectivity of the channel for K⁺ over Na⁺, resulting in a positive shift in the reversal potential. Limitations of the previous studies [6,7] included the inability to assess whether such complex phenotypic variability was also manifest in cardiac myocytes and the inability to establish the physiologic relevance of the various phenotypes in cardiac myocytes. Accordingly, using adeno viral gene transfer, we overexpressed N629D in spontaneously beating cardiomyocytes derived from stem cells, which expressed endogenous HERG currents. We assessed the character of the I\(_{Kr}\) and its associated changes in action potential (AP) features.

2. Methods

This 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. Construction of recombinant adenoviruses

A replication-deficient recombinant adenovirus containing cDNA for the mutated form of HERG1 (N629D) and the green fluorescence protein (Ad. N629D/GFP) was generated as described by He et al. [8]. A recombinant adenovirus containing the cDNA for GFP alone (Ad.GFP) was also

![Fig. 1. Overexpression of N629D results in cells manifesting three phenotypes. Representative examples of the I\(_{Kr}\) phenotypes observed in wildtype (WT) (Panel A) cardiac myocytes and in those overexpressing N629D (Panels B–D). Mean current voltage relationships of the time-dependent currents (E) and the tail currents (F) are shown.](image-url)
generated via the same method and was used as a control. The viruses were isolated and purified by discontinuous CsCl gradient. The final viral titer was $1.5 \times 10^{11}$ pfu/ml for Ad.GFP/N629D and $2.4 \times 10^{11}$ pfu/ml for Ad.GFP. Cardiomyocytes were infected at the m.o.i. of 10 pfu/cell to overexpress either the green fluorescence protein (WT control) alone or in combination with the point mutation N629D.

2.2. Cell culture and differentiation

The undifferentiated R1 ES cells were cultivated on a feeder layer of mouse embryonic fibroblasts [9]. The ES cells were then cultivated on gelatin (0.1%)-coated tissue culture dishes and grown to confluency. To allow differentiation into cardiomyocytes, the ES cells were harvested with trypsin-EDTA and transferred to Petri dishes for suspension culture with differentiation medium (ES medium without leukemia inhibitory factor and grown to a density of $1.5\times10^6$ cells/dish [9]). During suspension culture, cells aggregated to form embryonic bodies. Generally, by the 8–10th days in suspension culture at 37 °C, beating cell clusters were observed. The embryonic bodies were then plated on gelatin-coated tissue culture dishes. One to 2 days after plating, ES cell-derived cardiac myocytes were isolated [10]. Approximately 50 spontaneously contracting embryonic bodies were collected and washed in the low-Ca$^{2+}$ medium for 30 min at room temperature. The bodies were then incubated in the enzyme medium (Yakult, Japan) for 30 min at 37 °C. The dissociated cells were resuspended in KB solution at room temperature for 1 h and then plated on gelatin (0.1%)-coated glass cover slips in 24-well culture plates with differentiation medium, and cultured at 37 °C for 24 h. Cells were infected with the Ad.GFP or the Ad.GFP/N629D and myocytes were examined 16–50 h after infection. For electrophysiological recording, the glass cover slips were transferred to a temperature-controlled recording chamber (36 ± 1 °C) and superfused with extracellular solution.

2.3. Electrophysiological recording

$I_{Kr}$ was recorded as the dofetilide-sensitive current. $I_{Kr}$ was recorded using whole-cell standard patch-clamp methods and AXOPATCH 200B amplifier (Axon Instruments). Micropipettes were pulled from borosilicate glass capillary tubes on a programmable horizontal puller (Sutter Instruments, Novato, CA). When filled with the intracellular solution, the pipette tip resistance was 2–3 MΩ. After gigaohm seal formation, the membrane patch under the micropipette was disrupted by suction to establish whole cell-recording configuration. The series resistance was less then 5 MΩ. Data were sampled at 1 kHz. From a holding potential of $-70$ mV, the currents were elicited by 4 s depolarizing voltage steps to test potentials ranging from $-60$ to $+50$ mV in 10 mV increments, tail current was measured upon repolarization to the $-60$ mV. The current densities were expressed as current normalized to capacitance. After recording $I_{Kr}$, the paired spontaneous AP were recorded using the current clamp mode in the same cell, and using the same extracellular and internal solutions used for recording $I_{Kr}$.

Cardiac myocytes were recognized by their typical shapes [11–13]. In order to avoid bias, both spontaneously beating and non-beating myocytes were randomly selected from the myocytes on the cover slips. If no spontaneous AP was observed, the resting membrane potential (RMP) was recorded.

2.4. Solutions

The low Ca$^{2+}$ medium, for isolating single cardiomyocytes, contained in mM: NaCl 120, KCl 5.4, MgCO$_4$ 5, Na pyruvate 5, glucose 20, taurine 20, HEPES 10 with NaOH to adjust pH 7.2 at 24 °C. The enzyme solution consisted
of the low Ca\textsuperscript{2+} medium supplemented with 1 mg/ml collagenase (Yakult) and 30 μM CaCl\textsubscript{2}. The KB medium contained in mM: KCl 85, K\textsubscript{2}HPO\textsubscript{4} 30, MgSO\textsubscript{4} 5, EGTA 1, Na\textsubscript{2}ATP 2, Na pyruvate 5, creatine 5, taurine 20, glucose 20, pH 7.2 at 24 °C. For patch-clamp recording, the internal solution contained in mM: KCl 10, K-aspartate 110, MgCl\textsubscript{2} 5, Na\textsubscript{2}ATP 5, EGTA 10, HEPES 5, CaCl\textsubscript{2} 1, corrected to pH 7.2 with KOH. Unless specifically noted, the regular extracellular solution contained NaCl 140, KCl 5.4, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2} 1, HEPES 10, glucose 10, pH 7.4 with NaOH. For patch-clamp recording, the internal solution contained in mM: KCl 10, K-aspartate 110, MgCl\textsubscript{2} 5, Na\textsubscript{2}ATP 5, EGTA 10, HEPES 5, CaCl\textsubscript{2} 1, corrected to pH 7.2 with KOH. Unless specifically noted, the regular extracellular solution contained NaCl 140, KCl 5.4, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2} 1, HEPES 10, glucose 10, pH 7.4 with NaOH. KB-R 7943 was applied at a concentration of 3 μM/l [14], ryanodine at 20 μM/l, nicardipine at 0.2 μM/l and TTX at 0.1 μM/l.

2.5. Statistics

The average data are expressed as mean ± S.E.M. Statistical analysis was performed using the paired and unpaired Student’s t-test, p-values of <0.01 were considered as significant. When more than two groups were analyzed, one-way ANOVA and Dunnett’s multiple range test was employed.

3. Results

3.1. Paired AP and dofetilide-sensitive currents

Cardiomyocytes fluorescing green (as a result of expressing GFP) were selected for electrophysiological recordings. Fig. 1 shows illustrative examples of the dofetilide-sensitive I\textsubscript{Ks} currents in WT cardiac myocytes (Panel A) and in cells after adenoviral-N629D infection (Panels B–D). The WT I\textsubscript{Ks} currents show inward rectification, a negative slope conductance of the time-dependent current at voltages positive to 0 mV and a tail current that is as large as the time-dependent current. The characteristics of the endogenous I\textsubscript{Ks} currents in cardiac myocytes
derived from stem cells are similar to that reported in freshly isolated fetal cardiac myocytes [15]. In adenoviral-N629D infected cardiac myocytes, three phenotypes similar to that reported during heterologous equimolar expression in Xenopus oocytes and mammalian cells [6,7] were observed. The mean current–voltage relationships for the time-dependent and the tail currents for the three current phenotypes are shown in Panels E and F (both \( n = 6 \)), respectively. Both WT and WT-like \( I_{Kr} \) show inward rectification and a positive tail current; the N629D-like \( I_{Kr} \) shows an outward rectification, inward time-dependent current between \(-40 \) and \(-60 \) mV, and an inward tail current, whereas the intermediate \( I_{Kr} \) shows a small outward tail current.

Fig. 2 (Panel A) shows the frequency distribution of the various phenotypes observed after adenoviral-N629D infection. The intermediate phenotype was the most common manifestation. Panel B shows the natural variation in the current density of endogenous \( I_{Kr} \) in a population of control (GFP) myocytes. All control (WT) myocytes manifest \( I_{Kr} \) and its density was normally distributed.

Myocytes were studied at the intermediate stage of development, as previously reported [10]. While the mean RMP (mV) of WT cells was \(-61 \pm 5 \) \((n = 40)\), the WT cells segregate themselves into two groups based on RMP. The majority of cells (38 of 40 cells) had a RMP in the range of \(-60 \) mV. A typical AP is shown in Fig. 3 (Panel A). This AP feature is consistent that previously reported [10] for cells at the intermediate stage of development. A minority of cells (2 of 40) had a RMP of \(-40 \) mV and AP features consistent with a sinus node-like cell. The mean data is always calculated using data from both groups.

The spontaneous AP features of each cell were related to their paired \( I_{Kr} \) characteristics. Fig. 3 compares representative examples of spontaneous AP recorded from WT myocytes (Panel A) and myocytes manifesting the WT-like (Panel B), the intermediate (Panel C), and the N629D-like \( I_{Kr} \) phenotype (Panel D). Spontaneous APs were observed in only 1/12 myocytes that manifest a N629D-like \( I_{Kr} \) phenotype, but were always observed in myocytes with a WT-like or intermediate phenotype. The mean AP characteristics are shown in Panels E and F. The RMP was critically dependent on the underlying \( I_{Kr} \) phenotype. In cells with a WT-like \( I_{Kr} \) phenotype, the RMP (mV) was relatively hyperpolarized \((-63 \pm 3, n = 18)\); in cells with a N629D-like \( I_{Kr} \) current, their RMP was depolarized \((-30 \pm 2, n = 12)\); and in myocytes manifesting the intermediate phenotype, the RMP was modestly depolarized \((-47 \pm 2, n = 24, p < 0.00001 \) by ANOVA, compared to WT-like). Most myocytes \((11/12)\) with the N629D-like \( I_{Kr} \) phenotype did not manifest spontaneous AP. The beating rate of spontaneous AP was more rapid in WT myocytes and myocytes with a WT-like \( I_{Kr} \) phenotype. The mean data is always calculated using data from both groups.

3.2. Response to lowering \([K^+]_o\)

Previous studies have reported that lowering \([K^+]_o\) can trigger arrhythmias in patients with HERG LQTS [16]. Accordingly, paired \( I_{Kr} \) and AP were recorded at a \([K^+]_o\) of 5.4 and then at 2 mM/l. Fig. 5 shows that the response to
lowering \([K^+]_o\) was dependant on the initial \(I_{Kr}\) phenotype. In WT cells and those manifesting a WT-like \(I_{Kr}\) phenotype, lowering the \([K^+]_o\) resulted in a decrease in the time-dependent current and a relative increase in the tail current. In cells manifesting the intermediate \(I_{Kr}\) phenotype, lowering the \([K^+]_o\) resulted in abrogation of the outward \(I_{Kr}\) tail current. In cells manifesting a N629D-like phenotype, the tail current remained inward at both levels of \([K^+]_o\).

Changes in the \([K^+]_o\) would be expected to change the paired spontaneous AP shape as well as the RMP. Fig. 6 shows the response of the AP to lowering the \([K^+]_o\). In WT cells (Panel A) and those manifesting a WT-like \(I_{Kr}\) phenotype (Panel B), lowering the \([K^+]_o\) resulted in the expected hyperpolarization of the RMP as predicted by the Nernst equation for a highly selective potassium channel [6]. In contrast, in cells manifesting the intermediate phenotype, lowering the \([K^+]_o\) resulted in significant depolarization, as would be expected if the resting potential was dependent on conductance of a nonselective cation channel (Panel C). Panel D shows the mean RMP values before and after lowering the \([K^+]_o\) in cells. In WT, the RMP hyperpolarized after lowering the \([K^+]_o\) from \(-64 \pm 2\) to \(-71 \pm 1\) mV, \(n = 8\), \(p < 0.01\) by paired Student’s \(t\)-test, in those with a WT-like \(I_{Kr}\) phenotype from \(-66 \pm 1\) to \(-72 \pm 2\) mV, \(n = 12\), \(p < 0.01\), and cells with an intermediate \(I_{Kr}\) phenotype depolarizing from \(-43 \pm 1\) to \(-35 \pm 0.5\) mV, \(n = 12\), \(p < 0.01\) by paired Student’s \(t\)-test.

### 3.3. Development of depolarization-induced triggered activity only in cells manifesting the intermediate phenotype

Fig. 7 (Panels A and B) shows that lowering the \([K^+]_o\) in WT and WT-like cells never resulted in depolarization-induced triggered activity. In contrast, 1 of 12 cells with the intermediate phenotype manifest depolarization-induced triggered activity at a \([K^+]_o\) of 5.4 mM/l. All cells with the intermediate phenotype showed depolarization at a \([K^+]_o\) of 2.0 mM/l. In 5 of 12 cells, lowering the \([K^+]_o\) resulted only in depolarization without the development of triggered activity whereas in 6 of 12 cells, the cells manifested...
oscillatory triggered activity at a \([K^+]_o\) of 2.0 mM/l (Panel C). The depolarization of the RMP and triggered activity consistently resolved after returning the \([K^+]_o\) to 5.4 mM/l (Panel C).

A number of potential mechanisms could underlie the development of the triggered activity seen at a \([K^+]_o\) of 2.0 mM/l in cells manifesting the intermediate \(I_{Kr}\) phenotype. The N629D mutation results in an alteration of channel selectivity allowing an inward N629D current carried by Na⁺ [6]. Previous studies have indicated that increases in intracellular Na⁺ could increase the propensity to the reverse-mode Na⁺/Ca²⁺ exchange resulting in Ca²⁺ overload [17,18]. In order to address this mechanism, myocytes manifesting the intermediate \(I_{Kr}\) phenotype were pretreated with the Na⁺/Ca²⁺ exchange blocker KB-R 7943 [14] and the \([K^+]_o\) was then reduced to 2.0 mM/l. All six of these cells continued to show depolarization-induced triggered activity even after treatment with KB-R 7943 (Fig. 8, Panel A). To provide further evidence that SR-Ca²⁺ overload are likely candidates as a cause for the triggered activity.

An alternative hypothesis is that depolarization exhibited at a \([K^+]_o\) of 2.0 mM/l results in activation of the steady-state window currents of either the \(Ca^{2+}\) or the Na⁺ channels. Accordingly, myocytes were pretreated with the \(Ca^{2+}\) channel blocker nicardipine (Fig. 8, Panel C) or the specific Na⁺ channel blocker, tetrodotoxin (TTX) (Panel D). TTX consistently suppressed depolarization-induced triggered activity, whereas nicardipine did not. These data indicate that depolarization-induced activation of the window component of the Na⁺ current is likely responsible for this triggered activity.

### 4. Discussion

To our knowledge, this is the first study to express a human long QT mutation in cardiac myocytes derived from stem cells. This model allows an assessment of the relationship between the character of the mutant HERG channels, their associated spontaneous AP features and propensity to arrhythmias in cardiac myocytes. The novel information provided by this study includes: (1) phenotypic variability in the character of \(I_{Kr}\) was observed at the cellular level when overexpressing N629D in cardiac myocytes and...
this phenotypic variability is the critical determinant of RMP, APD and propensity to triggered activity at low [K⁺]₀; and (2) cardiac myocytes manifesting the intermediate phenotype respond to lowering the [K⁺]₀ with significant depolarization resulting in triggered activity. Treatment with TTX but not KB-R 7943, ryanodine and nicardipine suppressed this triggered activity. These data indicate that hypokalemia causes depolarization into the range of voltages required to activate the steady-state window Na⁺ current, which contributes to the triggered activity. Previous studies have confirmed that the Na⁺ window current is maximal at voltages in the range of –40 to –35 mV [20] the exact RMP observed in myocytes manifesting an intermediate phenotype exposed to low [K⁺]₀. This response represents a novel mechanism by which a human LQTS mutation can result in arrhythmias.

4.1. Response to lowering [K⁺]₀: clinical relevance

The finding that lowering the [K⁺]₀ provokes further depolarization, failure to repolarize and the development of triggered activity is of considerable clinical relevance. Increasing the [K⁺]₀ to 5.4 mM/l (Fig. 8) partially resolves the depolarization and the triggered activity. This finding is in keeping with previous studies that indicate that increasing the [K⁺]₀ has a protective effect in patients manifesting the LQT2 phenotype [16]. This study provides insights into the mechanisms for the antiarrhythmic activity produced by increasing the [K⁺]₀.

The N629D mutation alters the selectivity filter resulting in a change in the channel properties from a highly selective potassium channel to a non-selective cation channel allowing an inward Na⁺ current during repolarization. Moreover, this Na⁺ current is exacerbated by lowering the [K⁺]₀. Such an inward Na⁺ current might be expected to increase [Na⁺], increasing the propensity for reverse mode Na⁺/Ca²⁺ exchange which could result in SR-Ca²⁺ overload [21]. To address the potential contribution of Na⁺/Ca²⁺ exchange to the development of triggered activity at low [K⁺]₀, we assessed whether KB-R 7943 would protect myocytes from these events. KB-R 7943 did not protect. To further confirm that SR-Ca²⁺ overload did not contribute to the pathogenesis of the triggered activity, we assessed the potential protective effect of ryanodine. Ryanodine did not prevent the development of triggered activity.

Previous studies have confirmed that the steady-state Na⁺ window current, which is maximally activated at depolarized potentials of –40 to –37 mV [14,18] can modulate
AP duration in a number of species [19]. Herein, we provide evidence that the TTX-sensitive Na⁺ window current contributes to the pathogenesis of the triggered activity seen when hypokalemia causes depolarization of cells with an intermediate phenotype. Myocytes with the N629D-like phenotype (RMP/C0 = 30 mV) would not be expected to activate the window current since the Na⁺ window current becomes exceedingly small at potentials in the range of −30 mV [20].

4.2. Adenovirus overexpression of LQTS gene products in cardiac myocytes derived from stem cells: strengths and limitations of this model system

Heterologous equimolar co-expression of HERG LQTS monomers with WT monomers has been previously studied in both Xenopus oocytes and in mammalian cells [6,7]. The limitation of these previous studies was the inability to address the physiologic impact of this mutation on RMP, automaticity, spontaneous AP shape/duration and arrhythmogenesis in spontaneously beating cardiac myocytes. It seemed important to study these LQTS constructs in cardiac myocytes since other ion channels endogenous to cardiac myocytes contribute to the substrate for the generation of afterdepolarizations. Viswanathan and Rudy [22] reported the contribution of the Na⁺-Ca²⁺ exchange and time-dependent reactivation of the L-type Ca²⁺ channel in the generation of the depolarizing currents that leads to afterdepolarizations. To our knowledge, no previous study has used this model system, which provides the possibility of evaluating the impact of the HERG channel mutations on spontaneously beating AP characteristics and conditions for arrhythmogenesis.

This cardiac myocyte model system transfected with adenoviral vectors also has limitations. One limitation is the ability to discriminate the differences between the phenotype related to the mutation versus the natural cell-to-cell variability in WT AP characteristics. Three experimental approaches were employed in tandem in this study to allow such discrimination. First, we studied a gain of function mutation, which resulted in quite a substantial change in the selectivity filter properties; thus the phenotype was not subtle. Second, after recording the I_Kr phenotype, the spontaneous APs were recorded from the same cell (paired information for each cell). Since the intermediate and N629D-like I_Kr phenotypes are distinctly different than WT-I_Kr, it was possible to relate AP characteristics to the I_Kr phenotypes. Finally, the responses of the RMP and triggered activity to lowering the [K⁺]o to 2 mM/L were evaluated. WT cells always hyperpolarized, never depolarized and never developed triggered activity in response to lowering [K⁺]o, whereas these events were common in myocytes manifesting the intermediate phenotype. While natural cell-to-cell variability in AP characteristics is a concern for this model system, the three experimental approaches, applied in tandem, effectively allow us to discern the mutant phenotypes and to discriminate it from cell-to-cell variability in WT. If these experimental approaches had not been applied, then the discrimination might have been impossible. Moreover, if a subtle “loss of function” trafficking mutation was examined in this same model system, it might be quite difficult to discriminate the difference between natural cell-to-cell variability in AP characteristics in WT cells and the mutant phenotypes.

4.3. Phenotypic variations in the physiologic consequences of overexpressing N629D in cardiac myocytes

Phenotypic variability, even within a single family, is characteristic of individuals who carry the same mutation in the HERG gene [23]. Phenotypic variability in the character of I_Kr has been reported during heterologous expression of equimolar transfections of N629D and WT constructs in Xenopus oocytes and mammalian cells. Similarly, in the current study, we observe phenotypic variability at the cellular level in cardiac myocytes following adenoviral overexpression of N629D in cardiac myocytes. The phenotypic variability at the cellular level likely relates to cell-to-cell variability in the synthesis and/or processing of WT and

![Fig. 8. Depolarization-induced triggered activity is suppressed by TTX. Myocytes manifesting the intermediate I_Kr phenotype are treated with the Na⁺/Ca²⁺ exchange blocker KB-R 7943, ryanodine, nicardipine and tetrodotoxin and then exposed to a [K⁺]o of 2.0 mM/L.](image-url)
N629D. While all WT myocytes manifest Ik,, its current density is normally distributed. This cell-to-cell variability likely reflects variability in the synthesis or turnover of the endogenous Ik, channel. Overexpression of N629D resulted in cell-to-cell phenotypic variability. This variability in phenotype may, in part, reflect the intrinsic variability in the synthesis or turnover of endogenous Ik,. A cell manifesting the highest levels of endogenous Ik, might be expected to manifest a WT-like phenotype, whereas cells synthesizing or processing the lowest amounts of endogenous Ik, may manifest a N629D-like phenotype. Since most cells synthesize or process a median amount of endogenous Ik, they might be expected to produce an intermediate phenotype, which was observed in this study. Alternatively, the variability may relate to the number of viruses infecting each myocyte. This seems unlikely since the same three phenotypes were also observed when heterologously co-expressing equimolar ERG1 WT and N629D in Xenopus oocytes and in mammalian cells [6,7]. Other important biologic variables include tetramerization, trafficking, insertion, and sarcosom al residence time. In addition, WT cardiac myocytes derived from stem cells have a natural variability in AP characteristics. The variability is largely dependent on the presence and magnitudes of other ionic currents such as If and ICa-L. Cell-to-cell variability in the magnitudes and function of these other currents will modulate the phenotype. The present study cannot control the cell-to-cell variability that exists in other endogenous currents before viral infection. This is a limitation of this model system. However, the N629D Ik, phenotype is the critical determinant of the RMP in these cells. Since RMP is the critical regulator of most ion currents, its dominant role appears to overshadow the cell-to-cell variability in other currents.

In conclusion, overexpression, via adenoviral transfer, of the human LQTS mutation N629D in cardiomyocytes derived from mouse stem cells results in prolonged APD and depolarization of the RMP. Low [K+]o, triggers further depolarization of the RMP and the development of triggered activity in cells with the intermediate phenotype. Depolarization of the RMP represents a novel mechanism by which this human LQTS mutation generates arrhythmogenesis.

References


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