Pharmacological Activation of I_{Kr} Impairs Conduction in Guinea Pig Hearts

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Activation of I_{Kr} Impairs Conduction. ***Introduction*: The hERG (Kv11.1) potassium channel underlies cardiac I_{Kr} and is important for cardiac repolarization. Recently, hERG agonists have emerged as potential antiarrhythmic drugs. As modulation of outward potassium currents has been suggested to modulate cardiac conduction, we tested the hypothesis that pharmacological activation of I_{Kr} results in impaired cardiac conduction.**

***Methods and Results:* Cardiac conduction was assessed in Langendorff-perfused guinea pig hearts. Application of the hERG agonist NS3623 (10 μM) prolonged the QRS rate dependently. A significant prolongation (16 ± 6%) was observed at short basic cycle length (BCL 90 ms) but not at longer cycle lengths (BCL 250 ms). The effect could be reversed by the I_{Kr} blocker E4031 (1 μM). While partial I_{Na} inhibition with flecainide (1 μM) alone prolonged the QRS (34 ± 3%, BCL 250 ms), the QRS was further prolonged by 19 ± 2% when NS3623 was added in the presence of flecainide. These data suggest that the effect of NS3623 was dependent on sodium channel availability. Surprisingly, in the presence of the voltage sensitive dye di-4-ANEPPS a similar potentiation of the effect of NS3623 was observed. With di-4-ANEPPS, NS3623 prolonged the QRS significantly (26 ± 4%, BCL 250 ms) compared to control with a corresponding decrease in conduction velocity.**

***Conclusion*: Pharmacological activation of I_{Kr} by the hERG agonist NS3623 impairs cardiac conduction. The effect is dependent on sodium channel availability. These findings suggest a role for I_{Kr} in modulating cardiac conduction and may have implications for the use of hERG agonists as antiarrhythmic drugs.**
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arrhythmias, conduction velocity, I_{Kr}, hERG agonist, NS3623, long QT syndrome

Introduction

The hERG (Kv11.1) potassium channel underlies the rapid delayed rectifier current, I_{Kr}, which is a major determinant of cardiac repolarization.[1,2] Mutations in the *KCNH2* gene encoding the hERG channel have been linked to the inherited long QT syndrome (LQTS) type 2.[3] Additionally, many drugs are known to inhibit the current causing an acquired form of LQTS.[1,4] Both the inherited and acquired forms of LQTS are associated with an increased risk of lethal cardiac arrhythmias such as Torsade de Pointes.

Recent studies have focused on the use of hERG channel agonists as potential drug candidates for the treatment of cardiac arrhythmic disorders, most notably LQTS.[5–8] (See Grunnet *et al.*[9] for review.) In experimental models of cardiac arrhythmia, hERG agonists have been shown to decrease dispersion of repolarization[8] and to depress premature activity.[10,11] However, little is known about the effect of pharmacological activation of I_{Kr} on cardiac conduction.

Cardiac conduction velocity (CV) is in part dependent on membrane excitability.[12] Pharmacological activation of I_{Kr} has been shown to prolong the postrepolarization refractory period (PRRP)[6] presumably by increasing outward potassium conductance. An increase in outward potassium conductance in the diastolic phase is likely to affect membrane excitability and hence cardiac impulse propagation. Slowing of conduction is generally considered to be proarrhythmic. Thus, to test this potentially proarrhythmic effect of hERG channel agonism, we hypothesized that pharmacological activation of I_{Kr} results in impaired cardiac conduction by increasing outward potassium conductance.

In this study, we report that pharmacological activation of I_{Kr} by the hERG agonist NS3623[5] impairs conduction in a rate-dependent manner. We also find that this effect is potentiated in the presence of partial sodium channel block. The results suggest a role for I_{Kr} as a modulator of cardiac conduction and have important implications for safety assessment of hERG channel agonists as potential therapeutic agents.

Methods

Unless otherwise specified all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

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Experimental Preparation

All animal procedures conform 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). Retired male breeder guinea pigs were anesthetized with pentobarbital sodium (30 mg/kg i.p.; Nembutal). The hearts were rapidly excised and perfused as Langendorff preparations with Tyrode solution at 36 ± 1°C. The Tyrode solution was oxygenated with 100% O_2 and consisted of (in mM): $CaCl_2$ 2, NaCl 140, KCl 4.5, dextrose 10, $MgCl_2$ 1, and HEPES 10 (pH = 7.4). Preparations were completely immersed in temperature-controlled (36 ± 1°C) perfusate. Perfusion pressure was 55 mmHg. The preparations were allowed to equilibrate for 30 minutes before interventions. The hearts were stimulated with a unipolar silver wire placed either on the anterior epicardium of the left ventricle (LV) or on the right atrium. Unless otherwise specified the hearts were paced from the LV at a basic cycle length (BCL) of 250 ms.

ECG Recordings

A bath ECG was obtained with a silver chloride anode positioned approximately 2 cm from the midwall of the right ventricle (RV) and a cathode positioned approximately 2 cm from the midwall of the LV. Data were sampled at 1 kHz.

Optical Voltage Mapping

Motion artifacts were minimized by addition of 2,3-butanedione monoxime (BDM, 7.5 mM) to the perfusate. The voltage-sensitive dye di-4-ANEPPS (Invitrogen, Carlsbad, CA, USA) was dissolved in 99% ethanol and diluted in Tyrode solution. The hearts were stained with the dye (15 μM) by direct coronary perfusion for 10 minutes. The final concentration of ethanol during dye perfusion was 0.15 ‰ v/w, which in separate control experiments did not have any effect on the measured parameters (data not shown). The optical voltage mapping system has been described in detail elsewhere.[13] Briefly, after staining with di-4-ANEPPS, the preparation was excited by three 60-LED light sources (RL5-A9018, Superbrightleds, St. Louis, MO, USA) fitted with 510 ± 5 nm filters; the emitted light was filtered through a series of lenses and recorded using 2 CCD cameras (MiCam02 HS, Scimedia, Irvine, CA, USA). The interpixel resolution of the system was 0.184 mm (90 pixels) and 0.199 mm (60 pixels) in the x- and y directions, respectively. Data were sampled at 1 kHz.

Pharmacological Interventions

NS3623 was synthesized at NeuroSearch A/S. Stock solutions of NS3623, flecainide, and E4031 were diluted in Tyrode to obtain the desired working concentrations (10 μM for NS3623, 1 μM for flecainide, and 1 μM for E4031).

Data Analysis

Data were analyzed with custom-made software utilizing the Matlab package (MathWorks Inc., Natick, MA, USA). Activation time was defined as the time corresponding to 50% of the maximum upstroke of the action potential as this gave similar estimations with lower standard deviations as compared to activation time defined as the time of the maximum first derivative. Activation times were fitted to a parabolic surface as previously described.[14] The gradient at each point was assigned a CV vector. The averaged CV vectors in the slowest and fastest axes of propagation are reported as a measure of transverse and longitudinal CV.[15] The anisotropy of conduction was calculated as the ratio between CV in the longitudinal and the transverse direction of propagation.

QRS and QT intervals were determined by manual inspection of the ECG. During epicardial pacing the Q-point was impossible to distinguish from the pacing artifact. Therefore, the QRS was estimated as the duration from the pacing artifact to the end of the QRS complex.

Statistics

Statistical analyses were performed using R version 2.8.1 (http://www.R-project.org). Where appropriate, data were analyzed using a paired *t*-test. Otherwise, data were analyzed using linear mixed-effects models to account for repeated measures. In R the "lme" procedure in the "nlme" package was used for the analyses. These analyses were followed either by Dunnett's or Tukey's test for multiple comparisons (using the "multcomp" package). P-values less than 0.05 were considered statistically significant. Unless otherwise mentioned all data are presented as mean ± SEM.

Results

NS3623 Impairs Conduction in a Rate-Dependent Manner

Pharmacological activation of I_{Kr} was achieved by application of the hERG agonist NS3623 (10 μM). In this concentration NS3623 has been shown to be specific for hERG channels compared to other relevant cardiac ion channels.[5] The effect on conduction was assessed during ventricular epicardial stimulation by measuring the duration of the resulting QRS intervals. Representative QRS complexes measured at 2 different BCL are shown in Figure 1A. At a BCL of 250 ms there was no apparent effect of NS3623 on conduction (ctrl, 39 ± 1 ms; NS3623, 40 ± 1 ms). As BCL was shortened to 90 ms, the QRS intervals were generally prolonged as expected from conduction restitution theory. Importantly, at short BCL of 90 ms, NS3623 prolonged the QRS interval significantly above control (ctrl, 45 ± 1 ms; NS3623, 52 ± 3 ms, $P < 0.05$). To verify that the observed effect on conduction was specific to the activation of I_{Kr}, the specific I_{Kr} blocker E4031 (1 μM) was used. E4031 was able to completely reverse the effect of NS3623 on QRS duration at BCL 90 ms (44 ± 1 ms, ns compared to ctrl). Summary data of the effects on QRS interval are shown in Figure 1B.

The effect of pharmacological activation of I_{Kr} was also evident on the QT interval measured at BCL 250 ms as previously demonstrated.[10] The QT interval was significantly shortened by NS3623 and subsequently restored by application of E4031 (ctrl, 175 ± 4 ms; NS3623, 154 ± 1 ms, $P <$ 0.05 compared to ctrl; NS3623 + E4031, 172 ± 2 ms).

Flecainide Potentiates the Effect of NS3623 on Conduction

Membrane excitability is influenced by the balance between inward and outward conductances. A reduction in inward sodium conductance is therefore likely to potentiate the effect of an increase in outward potassium conductance. In Figure 2A, representative QRS complexes are shown during

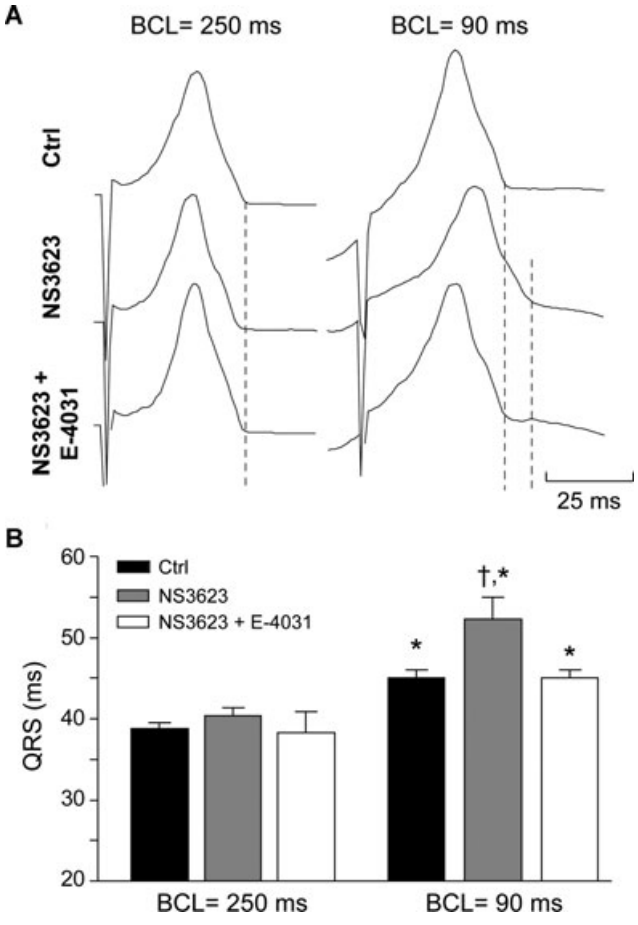

Figure 1. *NS3623 impairs conduction rate dependently. (A) Representative QRS complexes recorded at 2 different basic cycle lengths (BCL 250 and 90 ms) under control conditions, during perfusion of NS3623 (10 μM) with or without E4031 (1 μM) as indicated. NS3623 prolongs the QRS at short (BCL 90 ms) but not at longer (BCL 250 ms) cycle lengths. The effect is reversed by E4031. The recordings are aligned in time using the stimulus artifact. The dotted lines mark the end of the QRS complexes for comparison. (B) Summary of the effect of NS3623 and E4031 QRS intervals. *Indicates statistical significance ($P < 0.05$) compared to BCL 250 ms. †Indicates statistical significance ($P < 0.05$) compared to control.*

both epicardial ventricular and atrial pacing. During ventricular pacing, at BCL 250 ms application of 1 μM of the I_{Na} blocker flecainide prolonged the QRS above control (ctrl, 36 $\pm$ 1 ms; flecainide, 49 $\pm$ 2 ms, $P < 0.05$) as expected. Under these conditions of partial sodium channel block application of NS3623 resulted in a further prolongation of the QRS (flecainide + NS3623, 58 $\pm$ 3, $P < 0.05$ compared to both ctrl and flecainide alone). Similar results were obtained during atrial pacing demonstrating that the effect of NS3623 was indeed potentiated by sodium channel block. The data are summarized in Figure 2B,C.

NS3623 Impairs Conduction in Optical Voltage Mapping Experiments

After assessing the effects of NS3623 and flecainide on an index of ventricular conduction (QRS duration), we attempted to measure CV more directly by optical voltage mapping. Application of the voltage sensitive dye di-4-ANEPPS consistently blocked atrioventricular (AV) conduction and allowed only for ventricular pacing. Optical action potentials were recorded from the anterior epicardium of the LV at BCL 250 ms. Representative upstrokes of epicardial action potentials from equally spaced sites and representative isochrone maps of activation are presented in Figure 3A. The greater spatial distance between upstrokes along the transverse direction of propagation indicates slower conduction in this direction relative to the longitudinal direction. The anisotropy of propagation is also evidenced by the elliptical pattern of the activation isochrones on the maps both under control conditions and in the presence of NS3623. Surprisingly, in this setting NS3623 impaired conduction as demonstrated by closer spacing of the activation isochrones. Quantification of longitudinal and transverse CV in the LV confirmed these observations showing a significant decrease in CV in both directions in the presence of NS3623 (Fig. 3B). The anisotropy of conduction was also quantified but this was not significantly changed by NS3623 (ctrl, 2.2 $\pm$ 0.1; NS3623, 2.4 $\pm$ 0.1). The impaired conduction was also reflected in the duration of the corresponding QRS intervals. Representative QRS complexes before and after application of NS3623 are shown in Figure 3C demonstrating a prolonged QRS in the presence of NS3623. The QRS data from the optical mapping experiments are summarized in Figure 3D.

The optical mapping experiments, demonstrating that NS3623 decreased ventricular CV and prolonged the QRS interval, were inconsistent with the initial experiments where NS3623 only affected conduction during rapid pacing or in the presence of partial sodium channel block by flecainide. These results could thereby indicate that the di-4-ANNEPS dye, applied for the optical mapping studies, by itself has the ability to decrease excitability. The apparent potentiation of NS3623 in the optical mapping experiments prevented us from repeating the initial experiments using this technique.

Interestingly, the QRS intervals during ventricular pacing appeared markedly prolonged in hearts that were stained with di-4-ANEPPS (QRS, 50 $\pm$ 1 ms at BCL 250 ms) versus non-stained hearts (QRS, 37 $\pm$ 1 ms). Subsequent experiments showed that application of di-4-ANEPPS did indeed prolong the QRS interval. In Figure 4, representative QRS complexes together with quantification of QRS intervals measured before ($t = 0$) and at different time points after a bolus of di-4-ANEPPS was added to the perfusate are shown. The data demonstrate that the QRS interval remained prolonged up to 90 minutes after di-4-ANEPPS was added. In the optical mapping experiments, BDM was present to minimize motion artifacts. By comparing the QRS intervals from the optical mapping experiments where the hearts were subjected to both BDM and di-4-ANEPPS (50 $\pm$ 1 ms, BCL 250 ms) with the QRS intervals from hearts subjected only to di-4-ANEPPS (49 $\pm$ 2 ms, 15 minutes, BCL 250 ms) no additional effect of BDM was apparent.

Discussion

Membrane excitability is determined by the balance between inward and outward conductances and is thus a determinant of cardiac conduction. In this study, we tested the hypothesis that pharmacological activation of I_{Kr} would impair conduction by increasing outward potassium conductance.

Pharmacological activation of I_{Kr} by hERG agonists have been shown to shorten action potential duration in native tissue,[6-8,10] suggesting that such compounds increase

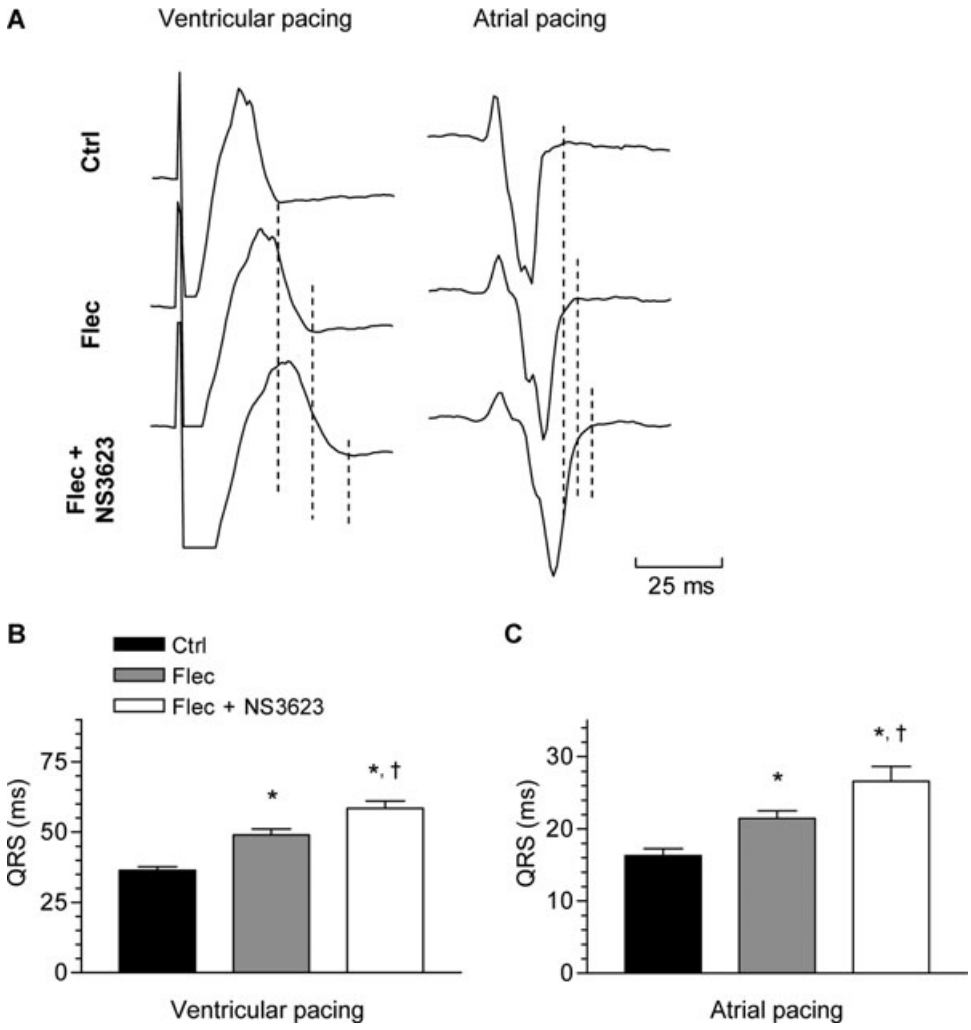

Figure 2. *Flecainide potentiates the effect of NS3623. (A) Representative QRS complexes from hearts paced either from the left ventricle (LV) or from the right atrium as indicated (BCL 250 ms). For both pacing sites flecainide (1 μM) prolongs the QRS above control. Application of NS3623 (10 μM) in the presence of flecainide prolongs the QRS even further. The beginning of the QRS complexes are aligned in time and the end are indicated by dotted lines for comparison. Summary of the effect of flecainide and NS3623 on the QRS intervals during ventricular (B) and atrial (C) pacing are shown. *Indicates statistical significance ($P < 0.05$) compared to control. †Indicates statistical significance ($P < 0.05$) compared to flecainide.*

potassium conductance in phase 3 (repolarization) of the cardiac action potential, thereby promoting repolarization. Furthermore, an increased open probability and thus potassium conductance in phase 4 is also to be expected. Additionally, the structurally related compounds NS1643[6] and NS3623[5] (both diphenyl ureas) have both been shown to reduce premature activity in animal models of LQTS.[10,11] For NS1643, it was shown that while shortening the effective refractory period, this compound in fact prolonged the PRRP.[6] The increase in PRRP is most likely associated with increased outward potassium conductance in the early phase 4 (diastole) and acts to suppress the occurrence of early afterdepolarizations, suggesting a potential mechanism for the observed reduction of premature activity. In theory, increased potassium conductance in early phase 4 is also expected to affect membrane excitability and hence cardiac impulse propagation, especially at fast heart rates. Accordingly, our results show that pharmacological activation of I_{Kr} using the hERG agonist NS3623 does not affect cardiac conduction at relatively normal heart rates (BCL 250 ms), while conduction is significantly impaired at rapid heart rates (BCL 90 ms). Importantly, we could prevent this effect by using the I_{Kr} specific blocker E4031, demonstrating that the effect of NS3623 was due to specific activation of I_{Kr}.

Under normal conditions rapid heart rates result in slower conduction primarily due to a decrease in sodium channel availability.[16] This relationship between pacing rate and conduction was also apparent in our data as the QRS interval was prolonged also under control conditions at fast pacing rates (BCL 90 ms). Therefore, it is likely that the rate-dependent effect of I_{Kr} activation on conduction is dependent on sodium channel availability. Accordingly, at normal heart rates it should be possible to unmask (or potentiate) the effect of I_{Kr} activation on conduction by decreasing sodium channel availability. Indeed, our results show that in the presence of the sodium channel blocker flecainide, activation of I_{Kr} did impair conduction at a relatively normal heart rate (BCL 250 ms). Importantly, without flecainide I_{Kr} activation did not affect conduction at this cycle length. Thus, our results support the hypothesis that pharmacological activation of I_{Kr} impairs conduction. However, this effect is only apparent under conditions of reduced sodium channel availability.

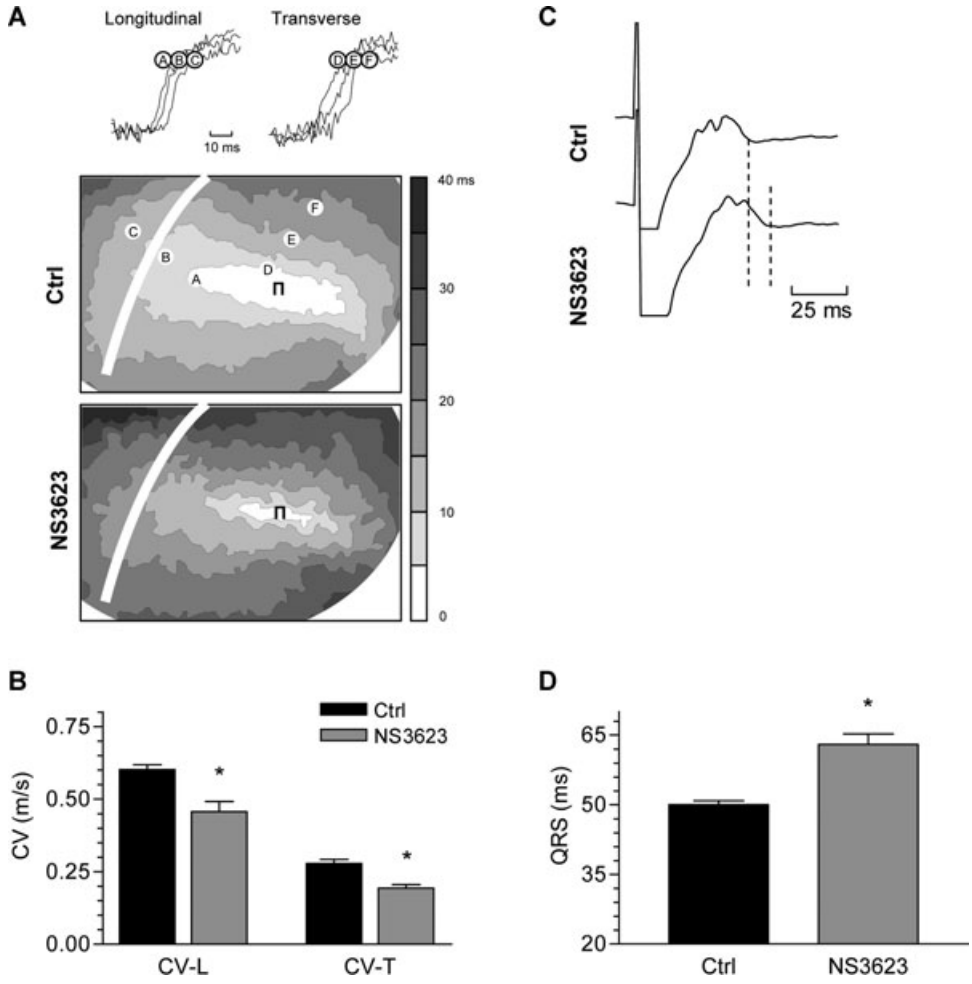

Figure 3. *NS3623 slows conduction in the presence of di-4-ANEPPS. (A) Upstrokes of optically recorded action potentials from equally spaced sites along the 2 axes of propagation are shown. Also shown are representative isochrone maps of activation under control conditions and in the presence of NS3623 (10 μM) in a heart paced from the LV (BCL 250 ms). The white line represents the approximate location of the left anterior descending artery. The closer spacing of the activation isochrones in the presence of NS3623 demonstrates conduction velocity (CV) is decreased in both longitudinal and transverse directions. The square wave (⊓) indicates the pacing site. (B) Quantification of longitudinal (CV-L) and transverse (CV-T) conduction velocities under control conditions and in the presence of NS3623. (C) Representative QRS complexes demonstrating that the decreased CV in the presence of NS3623 is paralleled by a prolongation of the QRS complex. The recordings are aligned in time using the stimulus artifact. The dotted lines mark the end of the QRS complexes for comparison. (D) Summary of the effect of NS3623 on the QRS intervals measured during the optical mapping experiments. *Indicates statistical significance ($P < 0.05$) compared to control.*

Only changes in ionic conductances in phase 4 (diastole) are expected to influence cardiac impulse propagation. Thus, the kinetic profile of a hERG agonist is likely to influence the effect on conduction. Only agonists that result in increased outward potassium conductance in the diastolic phase are expected to affect cardiac impulse propagation.

Recently, Veeraraghavan and Poelzing[17] suggested that differences in the expression of the inward rectifier potassium current (I_{K1}) underlie the inherent difference in CV between the RV and the LV. Specifically, they showed that a greater expression of Kir2.1, the protein underlying I_{K1}, in the LV may underlie slower CV in this ventricle. Similar to the present results, the effect of I_{K1} on conduction could be unmasked by partial sodium channel block. Taken together, these studies suggest that modulating outward potassium currents may influence cardiac conduction significantly by modulating membrane excitability, especially under conditions where sodium channel availability is reduced, such as during treatment with class I antiarrhythmics or during episodes of tachycardia. Pharmacological activation of potassium currents may therefore prove proarrhythmic under such conditions by promoting slow conduction, thereby increasing the risk of reentry.

The cardiac effect of NS3623 has previously been studied in guinea pigs, including measurements in both living animals and in Langendorff perfused isolated hearts.[10,18] These studies showed that NS3623 shortened both QT interval and action potential duration demonstrating the effect of I_{Kr} activation on repolarization parameters while no significant effect on conduction parameters were reported. However, none of these studies examined the effect of NS3623 at fast pacing rates or during partial sodium channel block. Our findings of the effect of NS3623 at normal heart rates, in the absence of flecainide, demonstrating only effect on the QT interval, are thus comparable to the previous reports. The proarrhythmic potential of NS3623 that we have uncovered in Langendorff-perfused hearts needs to be verified under comparable conditions *in vivo*.

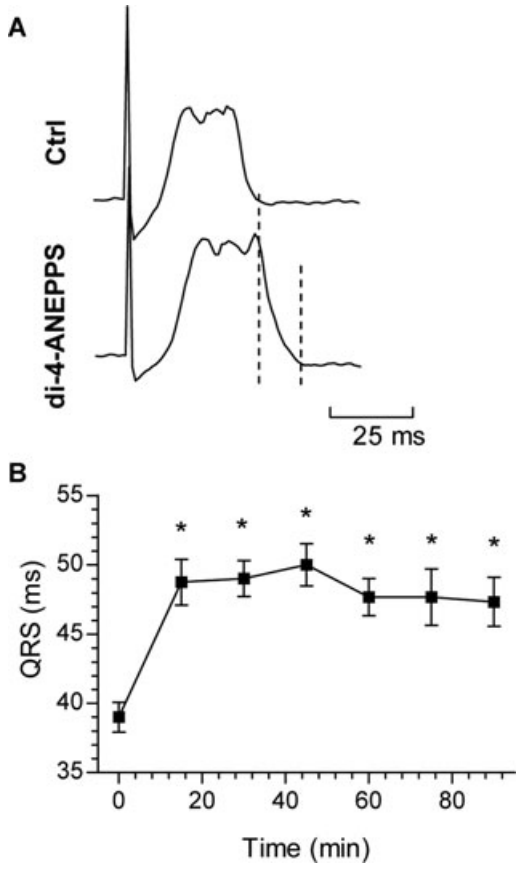

Figure 4. *di-4-ANEPPS prolongs the QRS interval. QRS intervals measured at various time points after a bolus of di-4-ANEPPS was added to the perfusate. The data demonstrate that di-4-ANEPPS significantly prolonged the QRS up to 90 minutes after application. *Indicates statistical significance compared to control ($t = 0$ minute, $P < 0.05$).*

Surprisingly, NS3623 slowed conduction in optical mapping experiments compared to control (in the presence of the voltage-sensitive dye di-4-ANEPPS). In these experiments, NS3623 prolonged the QRS interval significantly with a corresponding decrease in CV already at a cycle length of 250 ms. Specifically, the optical mapping data were inconsistent with the initial experiments where NS3623 only affected conduction at short cycle lengths or in the presence of partial sodium channel block. Interestingly, in a series of control experiments we found that application of di-4-ANEPPS prolonged the QRS interval, indicating that the dye itself may have affected cardiac conduction. There was no observable dependence on the frequency of illumination suggesting that the effect of di-4-ANEPPS on conduction was not due to a phototoxic effect. Unfortunately, this apparent confounding effect of di-4-ANEPPS prevented us from using this technique to quantify changes in CV induced by NS3623 alone and in combination with flecainide. We did not pursue the mechanism behind the potentiation of NS3623 in the optical mapping experiments further as this was outside the scope of the present study. However, future studies looking into the effects of voltage-sensitive dyes on membrane excitability may shed light on these findings. It is important to realize that most studies using di-4-ANEPPS compare effects before and after a given intervention (both carried out in the presence of the dye) and that dye-negative control experiments (i.e., the effect of the intervention with and without the dye) are rarely described. Our results suggest that results obtained using di-4-ANEPPS be confirmed using another experimental approach. However, the effect that we have observed in our experiments may in part be dependent on dye concentration and/or staining procedure and does therefore not necessarily detract from or contradict the findings of previous studies using this dye.

Conclusion

In conclusion, we have shown that pharmacological activation of I_{Kr} may modulate cardiac impulse propagation. The effect is dependent on reduced sodium channel availability and is unmasked by rapid pacing or pharmacological block of sodium channels. These results suggest a role for I_{Kr} as a modulator of cardiac impulse propagation and may have implications for the use of hERG agonists as antiarrhythmic drugs.

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