

## Potassium channel activators differentially modulate the effect of sodium channel blockade on cardiac conduction

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### Abstract

**Aims:** Diminished repolarization reserve contributes to the arrhythmogenic substrate in many disease states. Pharmacological activation of K<sup>+</sup> channels has been suggested as a potential antiarrhythmic therapy in such conditions. Having previously demonstrated that  $I_{K1}$  and  $I_{Kr}$  can modulate cardiac conduction, we tested here the effects of pharmacological  $I_{KATP}$  and  $I_{Ks}$  activation on cardiac conduction and its dependence on the sodium current ( $I_{Na}$ ).

**Methods and Results:** Bath electrocardiograms (ECGs) recorded from Langendorff-perfused guinea pig ventricles revealed QRS prolongation during  $I_{KATP}$  activation by pinacidil but not during  $I_{Ks}$  activation by R-L3 relative to control. In contrast, when  $I_{Na}$  was partially blocked by flecainide, R-L3 but not pinacidil prolonged the QRS relative to flecainide alone. Conduction velocity ( $\theta$ ) was quantified by optical mapping during epicardial pacing. Both longitudinal ( $\theta_L$ ) and transverse ( $\theta_T$ )  $\theta$  were reduced by pinacidil (by  $10 \pm 1$  and  $9 \pm 3\%$ , respectively) and R-L3 (by  $11 \pm 2\%$  and  $15 \pm 4\%$ , respectively). Flecainide decreased  $\theta_L$  by  $33 \pm 4\%$  and  $\theta_T$  by  $36 \pm 5\%$ . Whereas pinacidil did not further slow  $\theta$  relative to flecainide alone, R-L3 decreased both  $\theta_L$  and  $\theta_T$ .

**Conclusion:** Pharmacological activation of  $I_{KATP}$  and  $I_{Ks}$  slows cardiac conduction; however, they demonstrate diverse effects on  $\theta$  dependence on  $I_{Na}$  blockade. These findings may have significant implications for the use of K<sup>+</sup> channel activators as antiarrhythmic drugs and for patients with Na<sup>+</sup> channel abnormalities or being treated with Na<sup>+</sup> channel blockers.

**Keywords** cardiac conduction,  $I_{KATP}$ ,  $I_{Ks}$ ,  $I_{Na}$ , optical mapping, potassium channels.

Loss of potassium channel function leads to a compromised repolarization reserve and contributes to the arrhythmogenic substrate in several pathophysiological states including the long QT syndromes (LQTS) (Kaufman 2009, Kannankeril *et al.* 2010). The cause for reduced K<sup>+</sup> current can vary between genetic mutations in ion channel proteins (inherited LQTS), pathophysiological remodelling and K<sup>+</sup> channel inhibition by drugs (acquired LQTS); however, it consistently leads to prolongation of APD and increased dispersion of repolarization (Kaufman 2009, Kannankeril *et al.*

2010). These in turn provide a substrate for potentially lethal reentrant arrhythmias (Poelzing & Rosenbaum 2004, Kaufman 2011).

Recent studies have suggested augmenting the repolarization reserve using pharmacological K<sup>+</sup> channel activators as a potential therapy for LQTS. Evidence to support this notion comes primarily from experimental observations that these drugs mitigate APD prolongation and dispersion of repolarization (Grunnet *et al.* 2008, Nissen *et al.* 2009, Radwanski *et al.* 2010). However, these drugs have yet to be

evaluated for other possible pro-arrhythmic effects such as conduction slowing.

We previously demonstrated that pharmacological modulation of the inward rectifier K<sup>+</sup> current ( $I_{K1}$ ) (Veeraraghavan & Poelzing 2008) as well as the rapid delayed rectifier K<sup>+</sup> current ( $I_{Kr}$ ) (Larsen *et al.* 2010) can affect cardiac conduction in a cardiac sodium current ( $I_{Na}$ )-dependent manner. Further, modulation of  $I_{K1}$  only affected conduction when sodium channel availability was not reduced (Veeraraghavan & Poelzing 2008), whereas modulation of  $I_{Kr}$  preferentially affected conduction when conduction was already compromised (Larsen *et al.* 2010). Here, we tested the hypothesis that pharmacological activation of the slow delayed rectifier K<sup>+</sup> current ( $I_{Ks}$ ), a K<sup>+</sup> current carried by voltage-gated K<sup>+</sup> channels (Snyders 1999, Larsen *et al.* 2010), will affect cardiac conduction and its dependence on sodium channel blockade differently from activation of the ATP-sensitive K<sup>+</sup> current ( $I_{KATP}$ ), a K<sup>+</sup> current carried by non-voltage-gated, inwardly rectifying channels (Snyders 1999, Larsen *et al.* 2010).

We demonstrate that augmentation of both  $I_{KATP}$  and  $I_{Ks}$  slows conduction; however, where  $I_{KATP}$  activation only had this effect in the absence of sodium channel blockade,  $I_{Ks}$  activation slowed conduction regardless of sodium channel blockade. These data suggest that K<sup>+</sup> channel activators differently modulate cardiac conduction vis-à-vis its dependence on  $I_{Na}$ . Further, these differences may stem from whether the K<sup>+</sup> current being activated is carried by voltage-gated or non-voltage-gated channels. Therefore, K<sup>+</sup> channel activators must be evaluated for potential effects on cardiac conduction to ensure their safety in a therapeutic context.

## Methods

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Our protocols are in accordance with AVMA guidelines and approved by the University of Utah Animal Welfare Review Board.

### Guinea pig Langendorff preparations

Adult male guinea pig breeders (800–1000 g) were anesthetized (200 mg kg<sup>-1</sup> pentobarbital sodium [Nembutal] IP; a dose sufficient for euthanasia), and their hearts were rapidly excised. Depth of anaesthesia was monitored via corneal reflex and pedal withdrawal reflex. Isolated ventricles were perfused (at 40–50 mm Hg) in Langendorff configuration with oxygenated Tyrode's solution (containing, in mM, CaCl<sub>2</sub>

1.25, NaCl 140, KCl 4.5, dextrose 5.5, MgCl<sub>2</sub> 0.7, HEPES 10; pH 7.4) at 36.5 ± 0.5 °C as previously described (Poelzing & Veeraraghavan 2007, Veeraraghavan & Poelzing 2008). 2,3-butanedione monoxime (7.5 mM; BDM) was added to the perfusate for optical mapping experiments only. Hearts were stimulated via a unipolar silver electrode placed on the anterior epicardial surface at the centre of the mapping field at 1.5 times the stimulation threshold with a basic cycle length (BCL) of 300 ms unless otherwise specified.

### Electrocardiography

A volume-conducted bath ECG was obtained using a silver chloride anode located ~2 cm from lateral wall of the RV and a similar cathode located ~2 cm from the lateral wall of the LV. ECGs were recorded at 1 kHz and filtered to remove 60-Hz noise.

### Optical mapping

Optical voltage mapping was performed using di-4-ANEPPS (15 μM) as a voltage indicator to quantify conduction velocity ( $\theta$ ) and anisotropy ( $AR_{\theta}$ ; defined as the ratio of longitudinal  $\theta$  ( $\theta_L$ ) to transverse  $\theta$  ( $\theta_T$ )) as previously described (Poelzing & Veeraraghavan 2007, Veeraraghavan & Poelzing 2008). Briefly, the preparation was stained with di-4-ANEPPS by direct coronary perfusion for 10 min, then excited by three 60-LED light sources (RL5-A9018, Superbrightleds, St. Louis, MO, USA) fitted with 510 ± 5-nm filters (Chroma, Rockingham, VT, USA). Fluoresced light was passed through a 610-nm LP filter (Newport, Irvine, CA, USA) before being recorded with a SciMedia MiCam02 HS CCD camera (SciMedia, Irvine CA, USA) in a tandem lens configuration capable of resolving membrane potential changes as small as 2 mV with 1-ms temporal resolution from 44 × 30 sites simultaneously.

Motion was reduced by perfusion of 7.5 mM 2,3-butanedione monoxime (BDM). The anterior epicardium was mechanically pressed against the front wall of the perfusion chamber to further stabilize and flatten it.

Activation time was defined as the time of the maximum first derivative of the action potential as described previously (Girouard *et al.* 1996a). The inter-pixel resolution was 0.313 mm in the x-direction (44 pixels) and 0.34 mm in the y-direction (30 pixels).

A parabolic surface was fitted to the activation times as previously described (Bayly *et al.* 1998). The gradient at each point was assigned a conduction velocity vector. The averaged conduction velocity vectors along the slow and fast axes of propagation

(±15°) are reported as they reflect transverse and longitudinal propagation (Girouard *et al.* 1996b).

### Interventions

The class IC sodium channel blocker flecainide was perfused in concentrations ranging from 0 to 1 μM to determine conduction dependence on sodium channel availability. Pinacidil (10 μM; Sigma Aldrich, St. Louis, MO, USA) and R-L3 (10 μM; NeuroSearch A/S, Ballerup, Denmark) were applied to test the effects of increasing  $I_{KATP}$  and  $I_{Ks}$ , respectively, on  $\theta$ . In the dose response experiments, flecainide was applied in increasing doses, first by itself and then, in the presence of a constant concentration of K<sup>+</sup> channel activator.

### Statistical analysis

Statistical analysis of the data was performed using a two-tailed Student's t-test for paired and unpaired data or a single factor ANOVA. The Bonferroni correction was applied to adjust for multiple comparisons. A  $P < 0.05$  was considered statistically significant. All values are reported as mean ± standard error unless otherwise noted.

## Results

### Controls

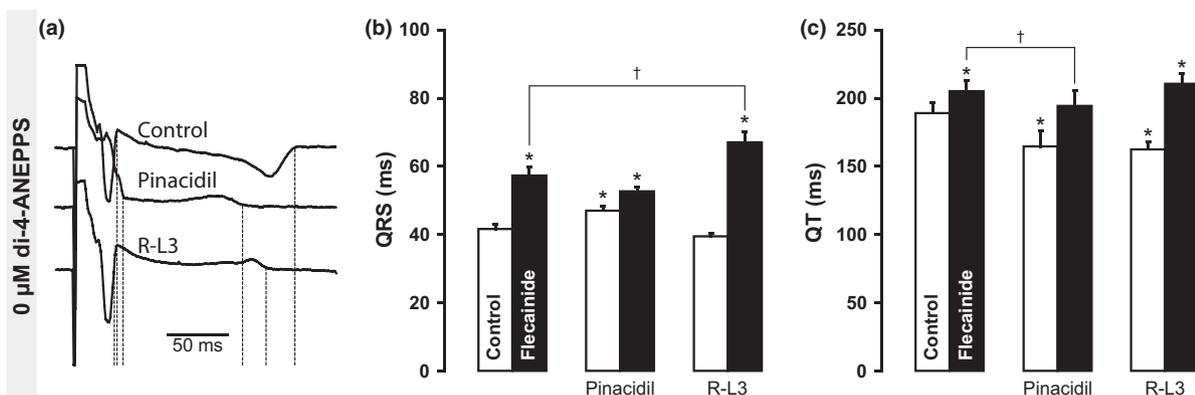
Activating outward potassium currents is expected to shorten APD, which may alter cardiac conduction by altering sodium channel availability (aka refractoriness).

To test this, we globally assessed cardiac conduction (QRS duration) and an index of refractoriness (QT interval). Representative traces of a volume-conducted ECG demonstrate that QRS duration was prolonged during activation of the non-voltage-gated channels that carry the ATP-sensitive potassium current ( $I_{KATP}$ ) by pinacidil relative to control (Fig. 1a). Interestingly, activation of the voltage-gated channels carrying the slow component of the delayed rectifier potassium current ( $I_{Ks}$ ) by R-L3 did not prolong the QRS (Fig. 1a). Summary data in Figure 1b (white bars) demonstrate that  $I_{KATP}$  activation by pinacidil prolonged QRS duration relative to control (\*), while  $I_{Ks}$  activation by R-L3 did not significantly change QRS duration. These data suggest that pinacidil slows cardiac conduction, while R-L3 does not under normal conditions.

Figure 1a also demonstrates that the QT interval, a global metric of APD is shortened during perfusion of pinacidil and R-L3 as expected. Overall, both agents shortened the QT interval relative to control (Fig. 1c, white bars) despite differential effects on conduction.

### Flecainide

To elucidate the mechanism of preferential conduction slowing during pinacidil vs. R-L3 perfusion, conduction velocity was first reduced by perfusion of 1 μM flecainide to partially block sodium channels. Flecainide alone significantly prolonged the QRS duration as well as the QT interval relative to control (Fig. 1b, c, black bars, \*). Interestingly, flecainide + pinacidil did not significantly alter QRS duration (Fig. 1b, black bars), while it shortened the QT interval



**Figure 1** (a) Representative bath electrocardiograms recorded during control (top), pinacidil (middle) and R-L3 (bottom) in the absence of di-4-ANEPPS. (b) QRS duration. Pinacidil ( $n = 4$ ) but not R-L3 ( $n = 3$ ) prolonged the QRS (\*) relative to control (white bars). These measurements were then repeated in the presence of flecainide (black bars). Flecainide by itself prolonged the QRS relative to control and R-L3. Pinacidil + flecainide did not change the QRS relative to flecainide alone, but R-L3 + flecainide did (black bars). (c) QT interval. Both pinacidil and R-L3 abbreviated the QT interval (white bars, \*). These measurements were then repeated in the presence of flecainide (black bars). Flecainide by itself prolonged the QT interval relative to control (\*) and pinacidil + flecainide (†,  $P < 0.05$ ) but not R-L3 + flecainide shortened the QT interval relative to flecainide alone (black bars). In panels b and c: \* –  $P < 0.05$  vs. control, † –  $P < 0.05$  vs. flecainide.

(Fig. 1c, black bars) relative to flecainide alone. In contrast, flecainide + R-L3 prolonged the QRS duration relative to flecainide alone (Fig. 1b, black bars, †). However, flecainide + R-L3 did not shorten the QT interval (Fig. 1c, black bars) relative to flecainide alone.

#### di-4-ANEPPS

Optical mapping was performed with the voltage-sensitive dye, di-4-ANEPPS to directly quantify conduction velocity in the presence of pinacidil or R-L3. However, di-4-ANEPPS was previously demonstrated to prolong the QRS and unmask conduction slowing during  $I_{Kr}$  inhibition (Larsen *et al.* 2010). Indeed, over all experiments, di-4-ANEPPS perfusion prolonged the QRS ( $21 \pm 3\%$ ,  $P < 0.05$ ). Representative ECG traces in Figure 2a demonstrate QRS prolongation and QT interval shortening during  $I_{KATP}$  activation by pinacidil in the presence of di-4-ANEPPS relative to di-4-ANEPPS alone. Summary data demonstrate that pinacidil prolonged the QRS (Fig. 2b, white bars) and abbreviated the QT interval (Fig. 2c, white bars) overall, in the presence of di-4-ANEPPS, as it did in the absence of di-4-ANEPPS. Activation of  $I_{Ks}$  by R-L3 in the presence of di-4-ANEPPS had a similar effect, prolonging the QRS (Fig. 2a,b, white bars) and shortening the QT interval (Fig. 2a,c, white bars) relative to di-4-ANEPPS alone. This is in

contrast to the absence of di-4-ANEPPS, where R-L3 did not significantly alter QRS duration (Fig. 1a,b, white bars).

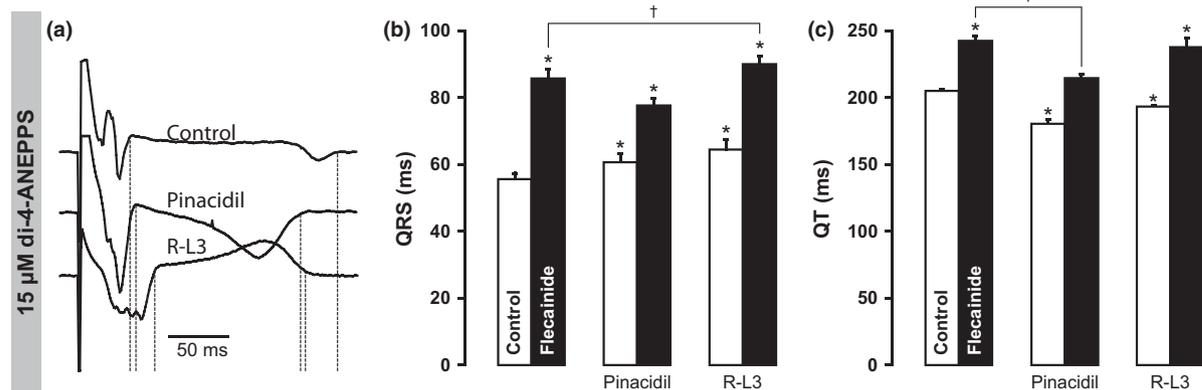
Flecainide, perfused in the presence of di-4-ANEPPS, again significantly prolonged both the QRS duration and the QT interval relative to di-4-ANEPPS alone (Fig. 2b,c). As before, flecainide + pinacidil did not significantly alter QRS duration (Fig. 2b, black bars) but shortened the QT interval (Fig. 2c, black bars) relative to flecainide alone. Lastly, flecainide + R-L3 prolonged the QRS duration (Fig. 2b, black bars) but did not alter the QT interval (Fig. 2c, black bars) relative to flecainide alone.

#### Action potential duration: di-4-ANEPPS

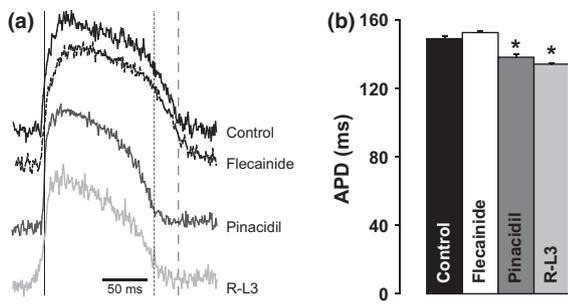
Action potential duration was quantified from optical action potentials. Representative action potentials in Figure 3a demonstrate similar APD between control and flecainide while APD was shorter in the presence of pinacidil and R-L3. Overall, flecainide did not significantly alter APD relative to control while both pinacidil and R-L3 lowered APD relative to control and did so to a similar extent (Fig. 3a,b).

#### Conduction velocity: di-4-ANEPPS

Longitudinal ( $\theta_L$ ) and transverse ( $\theta_T$ ) conduction velocities were subsequently quantified by optical mapping.

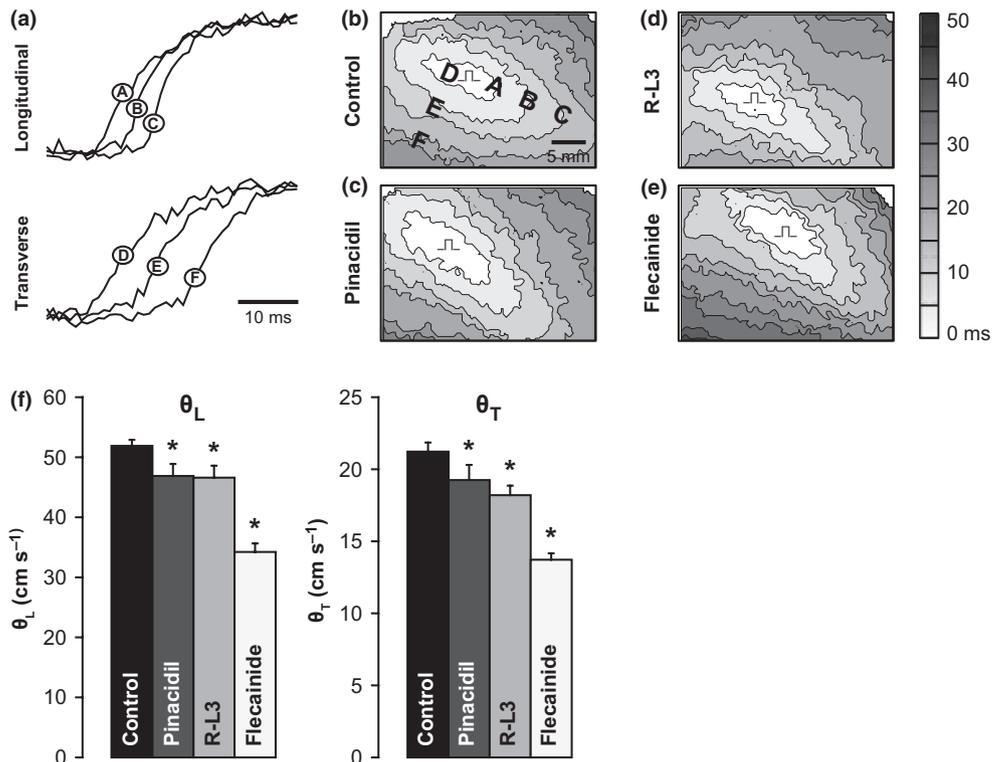


**Figure 2** (a) Representative bath electrocardiograms recorded during control (top), pinacidil (middle) and R-L3 (bottom) recorded in the presence of di-4-ANEPPS. (b) QRS duration. Both pinacidil ( $n = 5$ ) and R-L3 ( $n = 4$ ) prolonged the QRS (\*) relative to control (white bars). These measurements were then repeated in the presence of flecainide (black bars). Flecainide (black bars) by itself prolonged the QRS relative to control (i.e. di-4-ANEPPS alone). Pinacidil + flecainide did not alter the QRS relative to flecainide alone, whereas R-L3 + flecainide prolonged the QRS (†) relative to flecainide alone (black bars). (c) QT interval. Both pinacidil + di-4-ANEPPS and R-L3 + di-4-ANEPPS abbreviated the QT interval (\*, white bars) relative to control (i.e. di-4-ANEPPS alone). These measurements were then repeated in the presence of flecainide (black bars). Flecainide by itself prolonged the QT interval relative to control (i.e. di-4-ANEPPS alone; \*). Pinacidil + flecainide but not R-L3 + flecainide shortened the QT interval (†) relative to flecainide alone (black bars). In panels b and c: \* –  $P < 0.05$  vs. control, † –  $P < 0.05$  vs. flecainide.



**Figure 3** (a) Representative optical action potentials. Solid vertical line marks activation time. Repolarization time is marked by dashed vertical line for control and flecainide and by the dotted vertical line for pinacidil and R-L3. (b) Flecainide ( $n = 9$ ) did not alter action potential duration (APD) relative to control while pinacidil ( $n = 5$ ) and R-L3 ( $n = 4$ ) significantly lowered APD (\*,  $P < 0.05$  vs. control).

Representative action potential (AP) upstrokes recorded during control conditions in Figure 4a demonstrate shorter delays between equally spaced sites along the longitudinal axis (top traces) relative to sites along the transverse axis (bottom traces). This produces the elliptical activation pattern characteristic of cardiac tissue seen in Figure 4b.



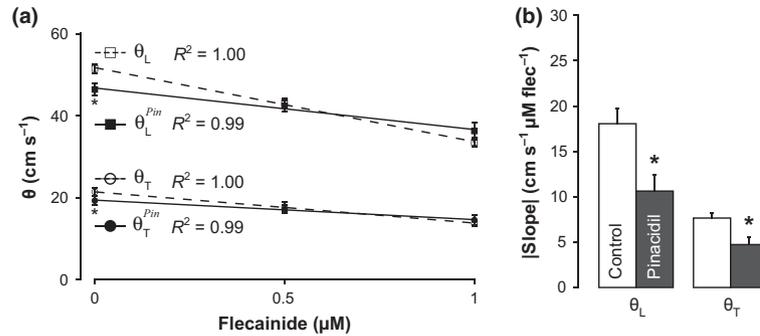
**Figure 4** (a) Action potential upstrokes from equally spaced sites. Representative activation isochrone maps: (b) Control, (c) Pinacidil, (d) R-L3 and (e) Flecainide. (f) Longitudinal ( $\theta_L$ ) and transverse ( $\theta_T$ ) conduction velocity. Overall, pinacidil, R-L3 and flecainide decreased both  $\theta_L$  and  $\theta_T$ .

$I_{KATP}$  activation by pinacidil decreased the spacing between isochrones (Fig. 4c), suggesting mild conduction slowing. Indeed, over all experiments, pinacidil decreased  $\theta_L$  by  $9.1 \pm 2.6\%$  and  $\theta_T$  by  $10.0 \pm 0.6\%$ , respectively ( $P < 0.05$ ; Fig. 4f). Similarly,  $I_{Ks}$  activation by R-L3 also decreased isochrone spacing, suggesting slowed conduction (Fig. 4d). Overall, R-L3 decreased  $\theta_L$  by  $11.7 \pm 2.2\%$  and  $\theta_T$  by  $15.0 \pm 3.8\%$ , respectively ( $P < 0.05$ ; Fig. 4f). No intervention altered anisotropy of conduction ( $AR_\theta$ ;  $p=ns$ ).

#### Conduction velocity: di-4-ANEPPS + flecainide

As reported previously, pinacidil or R-L3 in the presence of flecainide produced diverse conduction changes. Conduction was assessed by optical mapping in the presence of different concentrations of flecainide (0, 0.5, 1  $\mu M$ ) to determine conduction dependence on  $I_{Na}$  during perfusion of pinacidil or R-L3. By itself, flecainide produced a linear decrease in both  $\theta_L$  and  $\theta_T$  with increasing dose (Figs 4, 5 – dashed lines,  $R^2 \geq 0.95$  for a linear fit). Also, flecainide did not alter  $AR_\theta$  relative to control ( $n = 9$ ;  $P = ns$ ).

$I_{KATP}$  activation by pinacidil significantly decreased conduction in the absence (0  $\mu M$ ) but not in the



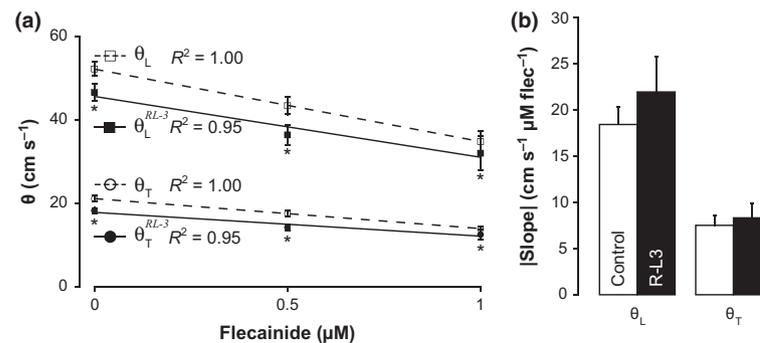
**Figure 5** (a) Plots of  $\theta_L$  and  $\theta_T$  vs. flecainide concentration in the presence (solid lines) and absence (dashed lines) of pinacidil ( $n = 5$ ). Whereas pinacidil decreased both  $\theta_L$  and  $\theta_T$  by itself (0  $\mu$ M flecainide), pinacidil + flecainide did not significantly alter either parameter relative to flecainide alone at any flecainide dose. \* –  $P < 0.05$  vs. 0 pinacidil. (b) Conduction dependence on sodium channel blockade was quantified as the absolute slope of  $\theta$  vs. flecainide dose. Pinacidil (greys bars) significantly decreased conduction dependence on sodium channel blockade (\*) relative to control (white bars).

presence of either 0.5 or 1  $\mu$ M flecainide (Fig. 5 – solid lines). Yet,  $\theta_L$  and  $\theta_T$  decreased linearly with flecainide dose in the presence of pinacidil (Fig. 5 – solid lines,  $R^2 \geq 0.95$ ), and  $AR_\theta$  was not different relative to control or flecainide alone ( $n = 4$ ,  $P = ns$ ). Consequently, pinacidil blunted conduction dependence on pharmacological  $I_{Na}$  inhibition quantified as the absolute slope of  $\theta$  vs. flecainide dose (Fig. 6,  $P < 0.05$ ) as demonstrated by the crossover of the two lines in Figure 5a.

In contrast, R-L3 activation of  $I_{Ks}$  decreased  $\theta_L$  and  $\theta_T$  by itself and also in the presence of all flecainide doses (Fig. 6 – solid lines). Similar to pinacidil,  $\theta_L$  and  $\theta_T$  decreased linearly with flecainide dose in the presence of R-L3 (Fig. 6 – solid lines,  $R^2 \geq 0.95$ ) and  $AR_\theta$  was not altered relative to control or flecainide ( $n = 5$ ,  $P = ns$ ). Consequently, R-L3 did not alter  $\theta_L$  or  $\theta_T$  dependence on pharmacological  $I_{Na}$  inhibition (Fig. 6,  $n = 5$ ,  $P = ns$ ).

## Discussion

It is well established that membrane excitability, in particular the amplitude of the cardiac sodium current ( $I_{Na}$ ), which in turn depends on sodium channel availability, is a key determinant of velocity ( $\theta$ ). It has long been recognized that decreasing  $I_{Na}$  slows conduction (Rohr *et al.* 1998). We recently demonstrated that outward K<sup>+</sup> currents such as the inward rectifier K<sup>+</sup> current ( $I_{K1}$ ) (Veeraraghavan & Poelzing 2008) and the rapid delayed rectifier ( $I_{Kr}$ ) (Larsen *et al.* 2010) can modulate  $\theta$ . Further, we demonstrated that modulation of  $I_{K1}$  and  $I_{Kr}$  altered conduction under different conditions. Therefore, we explored the hypothesis that non-voltage-gated and voltage-gated potassium channels heterogeneously affect conduction. Here we demonstrate that activation of non-voltage-gated  $I_{KATP}$  (Snyders 1999, Flagg & Nichols 2005) slowed conduction by itself but flattened the response of conduction velocity to pharmacological  $I_{Na}$  inhibition.



**Figure 6** (a) Plots of  $\theta_L$  and  $\theta_T$  vs. flecainide concentration in the presence (solid lines) and absence (dashed lines) of R-L3 ( $n = 4$ ). R-L3 by itself (0  $\mu$ M of flecainide) decreased  $\theta_L$  and  $\theta_T$  (\*). R-L3 + flecainide decreased both  $\theta_L$  and  $\theta_T$  (\*) relative to flecainide alone for both 0.5- and 1- $\mu$ M doses of flecainide was present. \* –  $P < 0.05$  vs. 0 R-L3. (b) Conduction dependence on sodium channel blockade was quantified as the absolute slope of  $\theta$  vs. flecainide dose. R-L3 (black bars) did not significantly alter conduction dependence on sodium channel blockade relative to control (white bars).

In contrast, voltage-gated  $I_{Ks}$  (Snyders 1999) activation did not slow conduction by itself, and it did not affect the response of conduction velocity to pharmacological  $I_{Na}$  activation.

#### Pinacidil: effects on conduction

By itself, 10  $\mu M$  pinacidil, an  $I_{KATP}$  agonist, significantly broadened QRS duration. This is in contrast to the study by (Yang *et al.* (1995) which did not report a change in QRS duration up to 50  $\mu M$  of pinacidil. Important differences between the Yang *et al.* study and our study include the sites of pacing (atria and ventricle, respectively), location of ECG electrodes (on the ventricle and bath, respectively), and temporal resolution of ECG quantification (Polaroid photos of the oscilloscope and 1 kHz digital data acquisition, respectively). Further, the QRS complex during atrial pacing is narrower relative to ventricular pacing, and as a result changes in QRS duration in the Yang *et al.* study may have been below the detection limit. Therefore, experimental differences likely explain these discrepant findings. Importantly, the mechanism of pinacidil mediated conduction slowing we observed under normal conditions may be due to pinacidil's effect of hyperpolarizing myocytes (Figure S1) (Baczko *et al.* 2004) and increasing outward current during early depolarization. Hyperpolarization is a well-established mechanism for conduction slowing (Kleber & Rudy 2004), where the amount of charge required to reach the activation threshold for  $I_{Na}$  is increased.

When  $I_{Na}$  was inhibited by flecainide, a drug demonstrated to block sodium channels in their open state (Anno & Hondeghem 1990), the QRS broadened. In the presence of pinacidil + flecainide, QRS did not significantly ( $P = 0.52$ ) prolong more than flecainide alone. Importantly, this is consistent with the lack of QRS change during pinacidil + flecainide in the Yang study, with the same caveats mentioned previously. This result is also consistent with our previous finding that modulation of another inwardly rectifying  $K^+$  current,  $I_{K1}$ , impacted conduction under normal conditions but not when  $I_{Na}$  was reduced (Veeraraghavan & Poelzing 2008). The question remains as to why pinacidil slowed conduction by itself but not in the presence of flecainide. One explanation is that resolution of the measurements may have been insufficient to detect a change in the pinacidil + flecainide groups. Alternatively, the lower resting potential produced by pinacidil (Baczko *et al.* 2004) would decrease random openings of sodium channels during diastole (Fozzard & Hanck 1996), which may mitigate the impact of flecainide, a drug that preferentially binds to sodium channels in the open state (Anno & Hondeghem 1990).

To directly quantify conduction, hearts were optically mapped with the voltage-sensitive dye, di-4-ANEPPS. Pinacidil prolonged the QRS and slowed conduction in the presence of di-4-ANEPPS as it did in the absence of di-4-ANEPPS, suggesting that the effects of pinacidil on conduction were not altered by di-4-ANEPPS. Furthermore,  $AR_{\theta}$  was not significantly different, which we interpret to mean that pinacidil principally affected sarcolemmal currents (Kleber & Rudy 2004). Pinacidil + di-4-ANEPPS + flecainide did not change QRS duration, slow conduction or change  $AR_{\theta}$  relative to flecainide + di-4-ANEPPS. These data suggest that pinacidil has the same effect with and without di-4-ANEPPS.

When flecainide concentration was increased in the presence of pinacidil + di-4-ANEPPS, we observed damping of conduction dependence on pharmacological  $I_{Na}$  inhibition. Specifically, the slope of the conduction velocity–flecainide relationship was flattened by the addition of pinacidil. In our previous study, inhibiting  $I_{K1}$  (which is also carried by channels open during diastole) can increase the steepness of the conduction velocity–flecainide curve (Veeraraghavan & Poelzing 2008). Importantly, these data are consistent in that opening the non-voltage-gated potassium channels flattens the conduction velocity–flecainide relationship, while inhibiting non-voltage-gated potassium channels steepens the same relationship.

Taken together, the pinacidil data suggest that activation of an inwardly rectifying potassium channel may lower resting membrane potential and slow conduction but without exacerbating conduction slowing by sodium channel blockers that bind in the open state.

#### R-L3: effects on conduction

In contrast to pinacidil, R-L3 activates the voltage-activated  $I_{Ks}$ . In addition to differences in voltage-dependent kinetics between  $I_{KATP}$  and  $I_{Ks}$ ,  $I_{Ks}$  activation by R-L3 has not been associated with changes in resting membrane potential (Salata *et al.* 1998, Nissen *et al.* 2009). As might be expected, R-L3 perfusion alone did not significantly change QRS ( $P = 0.95$ ). Thus, it was interesting to note that R-L3 + flecainide significantly broadened QRS more than flecainide alone, whereas we could not measure a difference between pinacidil + flecainide and pinacidil alone. This is consistent with our previous finding that activation of the voltage-activated  $I_{Kr}$  also prolonged the QRS only when conduction was already impaired (Larsen *et al.* 2010). This is different from pinacidil which slowed conduction by itself, but did not cause further slowing in the presence of flecainide. These data suggest that there are mechanistic differences

between the modulation of conduction by K<sup>+</sup> currents like  $I_{Kr}$  and  $I_{Ks}$  which are carried by voltage-gated channels as opposed to K<sup>+</sup> currents like  $I_{K1}$  and  $I_{KATP}$  which are carried by inwardly rectifying, non-voltage-gated channels.

It is interesting to note that flecainide prolongs time to  $dV/dt_{max}$  (i.e. increases latency) and slows conduction by delaying the time to maximal  $I_{Na}$  activation (Anno & Hondeghem 1990). As a voltage-gated potassium channel agonist, R-L3 significantly increases  $I_{Ks}$ . Delaying time to peak  $I_{Na}$  activation may provide sufficient time for  $I_{Ks}$  to provide a significant current opposing depolarization by  $I_{Na}$  and thereby, slow conduction. In isolated guinea pig myocytes, R-L3 concentrations from 1 to 10  $\mu M$  have been demonstrated to increase  $I_{Ks}$  between 750 and 1500% (Salata *et al.* 1998), which could represent a significant repolarizing current during depolarization. This hypothesis requires validation by single cell measurements.

In the presence of di-4-ANEPPS, R-L3 prolonged the QRS whereas it did not do so by itself. This difference is likely related to the impairment of conduction by di-4-ANEPPS: it prolonged the QRS in the current study as well as in our previous study (Larsen *et al.* 2010). This effect of di-4-ANEPPS may be related to its previously suggested interaction with the sodium potassium ATPase (Fedosova *et al.* 1995). Indeed, sodium potassium ATPase inhibition by cardiac glycosides is known to increase intracellular sodium (Demiryurek & Demiryurek 2005) and also broaden the QRS complex (Moe & Mendez 1951, Swain & Weidner 1957). Therefore, di-4-ANEPPS's interaction with the sodium potassium ATPase could underlie the dye's effects on conduction; however, the specific mechanism warrants further study.

Further, the results obtained with R-L3 are consistent with our previous findings that activation of another voltage-gated potassium current ( $I_{Kr}$ ) slows conduction only when conduction is already impaired by di-4-ANEPPS or flecainide (Larsen *et al.* 2010). In contrast, pinacidil prolonged the QRS even in the absence of di-4-ANEPPS. These results further suggest conduction slowing secondary to voltage-gated potassium channel inhibition may require a delay in peak  $I_{Na}$  activation to provide significant opposing current to depolarization. On the other hand the non-voltage-gated, inwardly rectifying channels are active through diastole (Snyders 1999), and affect conduction by decreasing resting membrane potential as well as increasing outward current during early depolarization.

Lastly, when flecainide doses were increased during perfusion of R-L3 + di-4-ANEPPS, conduction was slowed relative to flecainide + di-4-ANEPPS alone. As a result, the slope of the conduction velocity–flecainide

relationship was not significantly altered by R-L3. This is in contrast with pinacidil which did not further decrease  $\theta$  in the presence of flecainide and also decreased conduction dependence on  $I_{Na}$  blockade. These differences further suggest that R-L3 modulates conduction by a different mechanism from pinacidil.

## Conclusions

In summary, we demonstrate here that pharmacological activation of  $I_{KATP}$  and  $I_{Ks}$  both decrease  $\theta$ ; however, they exhibit differential response to  $\theta$  dependence on  $I_{Na}$ . Importantly, modulating K<sup>+</sup> currents that do not display voltage-dependent kinetics may only affect conduction when Na<sup>+</sup> channel availability is not reduced, whereas modulating K<sup>+</sup> currents that display voltage-dependent kinetics may impact conduction when Na<sup>+</sup> channel availability is reduced.

## Clinical relevance

Conduction slowing is an established proarrhythmic factor (Kleber & Rudy 2004). Here we demonstrate conduction slowing secondary to pharmacologic K<sup>+</sup> channel activation. Such effects could be deleterious, particularly in the presence of other pathophysiologic structural/electrophysiological changes. Therefore, pharmacological K<sup>+</sup> channel activators should be evaluated for any possible effects on conduction velocity and safety of conduction before being used as antiarrhythmic therapy.

## Limitations

The drugs used in the study, while relatively selective, do have off-target effects. For instance, pinacidil and flecainide are known to affect the transient outward current ( $I_{to}$ ) (Tseng & Hoffman 1990, Slawsky & Castle 1994). However, guinea pigs do not functionally express  $I_{to}$ ; therefore, these effects are unlikely to affect the results of this study (Litovsky & Antzelevitch 1988). R-L3 has been reported to block the L-type Ca<sup>2+</sup> current ( $I_{Ca,L}$ ) in isolated myocytes as well as  $I_{Kr}$  in mouse atrial tumour (AT-1) cells (Salata *et al.* 1998). However, the QT abbreviation observed with R-L3 in the present study argues against significant  $I_{Kr}$  blockade by R-L3 in our experiments.  $I_{Ca,L}$  is normally active after the action potential upstroke; however, possible  $I_{Ca,L}$  block by R-L3 may contribute to conduction slowing by the drug when  $I_{Na}$  is reduced. BDM has been shown to cause no significant change in conduction velocity at the dose used here; (Liu *et al.* 1993, Baker *et al.* 2004, Kettlewell *et al.* 2004) therefore, BDM is unlikely to have significantly affected the principal findings of this study. This study

does not address any possible effects of K<sup>+</sup> channel activation on regional conduction heterogeneities within the heart secondary to heterogeneous distribution of ion channels. Lastly, the study was conducted in structurally normal hearts with no remodelling; K<sup>+</sup> channel activation may affect conduction differently in diseased hearts.

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## Conflicts of interest

The authors report no conflicts of interest.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Additional methods.

**Figure S1.** (a) Representative action potentials recorded during control (black) and pinacidil (red). (b) Overall, pinacidil significantly lowered resting membrane potential relative to control.

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