Effect of Wenxin Keli and Quinidine to Suppress Arrhythmogenesis in an Experimental Model of Brugada Syndrome

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Abstract

Background—Wenxin Keli (WK), a Chinese herb extract, is reported to be effective in the treatment of atrial and ventricular cardiac arrhythmias. Recent studies suggest that WK inhibits the transient outward current (I_{to}).

Objective—The present study examines the effectiveness of WK, alone and in combination with quinidine, to suppress arrhythmogenesis in an experimental model of Brugada syndrome (BrS).

Methods and Results—Action potential and ECG recordings were obtained from epicardial and endocardial sites of coronary-perfused canine right ventricular wedge preparations. The I_{to} agonist NS5806 (10–15 μM) was used to pharmacologically mimic a genetic predisposition to BrS. The Ito agonist induced Phase 2 reentry (P2R) in 13/19 preparations and polymorphic ventricular tachycardia (pVT) in 11/19 wedge preparations. WK (10g/L) suppressed P2R and pVT in 100% (3/3) of preparations. A lower concentration of WK (5g/L) suppressed P2R in 60% (3/5) and pVT in 50% (2/4), but in combination with a low concentration of quinidine (5 μM) was 100% effective in suppressing P2R and pVT. Quinidine alone suppressed P2R and pVT in 60% (3/5) and 50% (2/4), respectively and in combination with WK (5g/L) suppressed P2R and pVT by 80% (4/5) and 75% (3/4), respectively. WK reduced I_{to}, I_{Ca} and contractility in single cardiomyocytes, but dose-dependently increased contractility in intact wedge preparations, an effect mimicked by tyramine.

Conclusions—Our data provide support for the hypothesis that Wenxin Keli, particularly in combination with quinidine, effectively suppresses arrhythmogenesis in an experimental model of BrS via inhibition of I_{to} and indirect adrenergic sympathomimetic effects.

Keywords

Transient outward potassium channel current; positive inotropic effect; cardiac arrhythmias; sudden cardiac death

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Introduction

Wenxin Keli (WK), a Chinese herb extract comprised of 5 components (Nardostachys chinensis batal extract (NcBe), Codonopsis, Notoginseng, Amber, and Rhizoma polygonati), is reported to be effective for the treatment of cardiac arrhythmias and heart failure.[1, 2] WK has been reported to block the transient outward potassium channel current (I_{to}), sodium current (I_{Na}) and L-type calcium current (I_{Ca}) in rat and rabbit ventricular cardiomyocytes.[3–5] The mechanism by which WK improves cardiac function is not well established.

Brugada Syndrome (BrS) is an inherited cardiac disorder associated with a high incidence of sudden death due to the development of life-threatening polymorphic ventricular tachycardia (VT) and ventricular fibrillation (VF). Previous studies performed in the canine ventricular wedge preparation have demonstrated that the Brugada ECG-phenotype can be induced by an outward shift in the balance of currents active at the end of phase 1 of the ventricular epicardial (Epi) action potential (AP) via inhibition of I_{Na}, I_{Ca} or augmentation of I_{to} or I_{K-ATP}.[6–11] Inhibition of the transient outward current (I_{to}) was first identified by our group as a therapy for BrS over 13 years ago.[6, 12]

The present study evaluates the potency with which WK inhibits I_{to} as well as other currents contributing to the balance of current during the early phases of the cardiac action potential and test the hypothesis that WK can suppress the electrocardiographic and arrhythmic manifestations of BrS in an experimental model of BrS.

Methods

Wedge Preparations

Detailed methods for isolation, perfusion, and recording of transmembrane activity from coronary-perfused canine right ventricular wedge preparations have been reported previously[7, 13] and are briefly describe in the Online Supplement.

Voltage-clamp studies using canine cardiomyocytes

Cardiomyocytes were isolated as previously described.[14] I_{to} was measured in isolated left ventricular epicardial and M cells at 36.5 °C using whole-cell patch clamp techniques.[15] Details of the protocol are presented in the Online Supplement.

Unloaded cell shortening in isolated myocytes

Myocytes were isolated from canine mid-myocardium as described above and unloaded cell shortening was recorded using a CCD camera and video-based edge detector as detailed in the Online Supplement.

Simulation of Ventricular Epicardial Action Potentials

Right ventricular (RV) epicardial action potentials were simulated using a LRII AP model, modified to include the I_{to} as previously described.[16, 17] Details are provided in the Online Supplement.

Statistical approaches and drugs used are described in the Online Supplement.

Results

Figure 1 depicts APs and ECG recordings obtained from a RV coronary-perfused wedge preparation under control conditions (Fig. 1A), following addition of 15 μM NS5806 (Fig.

1B), and addition of 10 g/L WK to the perfusate (Fig. 1C). The I_{to} agonist NS5806 was used to mimic a presumed genetic predisposition to BrS. NS5806 caused loss of the AP dome in Epi2 but not Epi1. Phase 2 Reentry (P2R) developed as the Epi AP dome propagated from Epi1 to Epi2, leading to development of pVT. Addition of 10 g/L WK to the perfusate reduced the AP notch throughout the preparation, leading to recovery of the AP dome at Epi1, normalization of the ST segment and suppression of pVT in 3/3 preparations (Fig. 4A). Online Table 1 shows composite data of 5 RV wedge preparations. Neither NS5806 nor the combination of NS5806 plus WK affected transmural conduction time, as estimated by width of the R wave. NS5806 (10–15 μM) significantly prolonged Epi action potential duration (APD) measured at 90% repolarization (APD_{90}) secondary to a dramatic increase in the I_{to}-mediated AP notch. Online Table 1 also shows that WK (10g/L) significantly shortened APD_{90} due to normalization of the Epi AP notch.

Figure 2 shows AP and ECG recordings obtained from a RV wedge preparation under control conditions (Fig. 2A), following perfusion with 12 μM NS5806 (Fig. 2B) and after addition of WK (5 g/L) (Fig. 2C). Figure 2D illustrates the effect of WK (5 g/L) in the presence of a relatively low concentration of quinidine (5 μM). In this example, WK (5 g/L) alone did not prevent P2R (Fig. 2C), although this arrhythmia mechanism was effectively suppressed when quinidine (5 μM) was added to the perfusate. The lower concentration of WK effectively suppressed P2R and pVT in 60% (3/5) and 50% (2/4) of preparations tested, respectively. The combination of a low concentrations of WK and quinidine suppressed P2R and pVT in 100% of cases (i.e. 5/5 and 4/4, respectively) as illustrated in Figure 4B. Online Table 1 shows composite data of RV wedge preparations following the protocol illustrated in Figure 2.

Figure 3 displays AP and ECG traces recorded from a RV preparation under control conditions (Fig. 3A), following perfusion with 14 μM NS5806 (Fig. 3B) and addition of quinidine (5 μM) (Fig. 3C). Figure 3D, illustrates the effect of quinidine in the presence of low concentrations of WK (5 g/L). In this experiment, quinidine alone prevented pVT, but not P2R. Addition of WK (5 g/L) suppressed all arrhythmic activity. A low concentration of quinidine effectively suppressed P2R and pVT in 60% (3/5) and 50% (2/4) of the preparations, respectively. The combination of 5 μM quinidine and 5 g/L WK suppressed P2R and pVT in 80% (4/5) and 75%, respectively (Fig. 4C). Online Table 1 shows composite data of RV wedge preparations based on the experimental protocol illustrated in Figure 3.

Figure 5 presents summary data of the effect of WK and quinidine to reduce the epicardial action potential notch magnitude and J wave area in the ECG. Notch magnitude index, an estimate of the epicardial action potential notch area and J wave area were significantly increased by NS5806 and dramatically reduced following exposure to WK (10g/L). WK (5 g/L) and quinidine (5 μM) produced more modest reductions in notch index and J wave area, but in combination produced a synergistic effect to dramatically reduce these electrophysiologic parameters.

We next examined the effect of WK with and without quinidine on the two major currents contributing to the early phases of the epicardial AP, I_{to} and I_{Ca}. We evaluated these effects in canine left ventricular myocytes using patch clamp techniques (Fig. 6A and B). WK (10g/L) significantly inhibited I_{to} in a concentration-dependent manner (n=5, p<0.0001). In concentrations of 5g/L and 10g/L, WK caused a shift in steady-state voltage dependence of inactivation of I_{to} towards negative potentials (Fig. 6D). Quinidine (5 μM) reduced the total charge carried by I_{to} by 21 (Fig. 6E) (p<0.05), and the combination of WK (5g/L) and quinidine (5 μM) further reduced total charge by a total of 41% at a cycle lengths of 1 sec (Fig. 6E) (p<0.05). To better evaluate the combined effect of I_{to} block by quinidine and WK

on the AP, we simulated APs from the RV using LRII model (see Methods) and incorporated our experimental findings. Figure 6F shows the effect of NS5806-induced augmentation to cause loss of the epicardial action potential dome and the I_{to} blocking effects of quinidine alone and together with WK to restore the action potential dome, thus normalizing the AP.

We also evaluated the effects WK on calcium currents. Interestingly, in concentrations of 5 and 10 g/L, WK reduced I_{Ca} by 40 % and 60 %, respectively (Fig. 7) and 10g/L WK caused a shift of ICa activation towards negative potentials (Fig. 7C). Consistent with this observation, WK also exhibited a concentration-dependent inhibition of unloaded cell shortening of myocytes. Cell shortening was inhibited by 20%, 35% and 50% in the presence of 1 g/L, 5 g/L and 10 g/L respectively (Fig. 7D).

The potent effects of WK to inhibit ICa and to induce a negative inotropic effect in isolated myocytes are at odds with its beneficial effect in the treatment of heart failure. To assess its inotropic effect in the intact ventricular wall, we evaluated the effects of WK in coronary-perfused right ventricular wedge preparations. In sharp contrast to its negative inotropic effect in single myocytes, WK produced a concentration-dependent increase in contractile force in the wedge preparation. Figure 8 illustrates the effect of 2, 5 and 10 g/L of WK on isometric tension development. WK exerted an enduring positive inotropic effect that peaked within 5–10 min at all the concentrations tested. Similar responses to WK were observed in 12/12 preparations. WK produced an average increase in peak-contractile force of 34±29% at 2 g/L (n=3), 71±34% at 5 g/L (n=5) and 230±59% at 10 g/L (n=4; Fig. 8D).

To explain the disparity between myocyte and wedge responses to WK, we considered the hypothesis that WK's positive inotropic effect is due to its ability to release catecholamines from sympathetic nerve ending. As a test of this hypothesis, we exposed the perfused wedge preparations to tyramine, a compound known to induce release of catecholamines from the adrenergic nerve terminals. Tyramine (1.0–1.5 μM) produced a positive inotropic effect recapitulating the action of WK (Figs. 8E and F). The addition of 10 g/L WK during tyramine exposure failed to produce a further increase in contractile force (Fig. 8E; n= 3). Moreover, β adrenergic blockade using propranolol inhibited the positive inotropic effect of tyramine and WK (n=3).

Discussion

An outward shift of current active during the early phases of the AP is known to facilitate the development of the BrS phenotype and is the basis for the genetic predisposition to BrS. We have previously reported that an outward shift in the balance of current active during the early phases of the right ventricular Epi AP by the I_{to} agonist NS5806[11] can recapitulate the BrS phenotype in the canine right ventricular wedge preparation.[6–8]

The present study demonstrates the effect of WK to inhibit or totally suppress the electrocardiographic and arrhythmic manifestations of BrS in a coronary-perfused canine RV wedge model of the syndrome. Our findings suggest that WK's actions to suppress Phase 2 Reentry and VT/VF involve inhibition of I_{to} as well as an indirect adrenergic stimulation via a tyramine-like effect. These ameliorative actions of WK are accompanied by normalization of the repolarization defects in the epicardial action potential as well as normalization of the ECG by a diminution of the accentuated J waves and ST segment elevation.

WK is comprised of 5 components including NcBe, Codonopsis, Notoginseng, Amber, and Rhizoma polygonati. Little data are available relative to the effect of the individual components on cardiac ion channel currents. Codonopsis has been reported to cause the

inhibition of phosphodiesterase activity, leading to an increase in cAMP in rat myocytes.[18] Notoginseng is reported to inhibit Na+/K+-ATPase activity.[19] These effects may contribute to the positive inotropic effect of WK. Nordostachys has been reported to protect cardiomyocytes against oxidative injury.[20] NcBe is reported to block I_{to} by 57.9%[3] or 69.8%[4] in rabbit ventricular myocytes at a concentration of 10 g/L. In our study involving canine ventricular myocytes, 10g/L WK inhibited peak I_{to} by 40%. Moreover, effect of WK (5 g/L) and quinidine (5 μM) to decrease the total charge carried by I_{to} was greater than that of quinidine alone (Fig. 6). This is consistent with the enhanced ability of these two drugs when combined to diminish the notch amplitude of the AP, as well as to suppress P2R and pVT. These experimental findings were corroborated by computer simulations of APs from the RV using the LRII model, showing that the combined effect of quinidine and WK is more effective in reducing the AP notch than quinidine alone (Fig. 6E).

In addition we also demonstrate an effect of WK to block I_{Ca} by 40 and 60 % at concentrations of 5 and 10 g/L, respectively. The potent effect of WK to block I_{Ca} is expected to contribute to an outward shift in the balance of currents active during the early phases of the action potential and thus to accentuate the BrS phenotype.[21, 22] Moreover, this action of the drug, which is associated with a negative inotropic effect in isolated myocytes (Fig. 7), is at odds with the ameliorative effects of WK in the management of heart failure.[2] Interestingly, we found that WK induces a concentration-dependent *positive* inotropic effect in the RV wedge preparation and provide evidence that this action of the drug is due to its tyramine-like effect. Tyramine is a naturally occurring monoamine compound that acts as a catecholamine releasing agent. It is taken up by the catecholamine reuptake transporter and thus leads to release of norepinephrine from sympathetic nerve endings. Thus, the indirect adrenergic effect of WK, together with the positive inotropic effects of the other components of WK, totally counters the negative inotropic effect of the drug and converts it into a positive inotropic effect. The positive inotropic effect is likely due principally to an increase in I_{Ca} secondary to release of catecholamines. The boost in I_{Ca} also contributes to restoration of the action potential dome and suppression of phase 2 reentry and pVT. We first reported the ability of tyramine to restore the AP dome in RV Epi tissue slices in 1990.[23]

Pharmacologic therapy for BrS is aimed at rebalancing the currents active during the early phases of the epicardial AP, either by increasing inward currents such as I_{Ca} or reducing outward currents such as I_{to}. WK exerts its ameliorative effects in the setting of BrS by reducing I_{to} as well as increasing I_{Ca} via indirect adrenergic stimulation.

Because of its action to inhibit I_{to}, quinidine was proposed for the management of BrS in 1999.[24] Experimental studies have shown quinidine to be effective in restoring the Epi AP dome, thus normalizing the ST segment and preventing P2R and pVT in experimental models of the Brugada syndrome.[6] Clinical evidence of the effectiveness of quinidine in normalizing ST segment elevation in patients with BrS has been reported.[25–30] At the doses required to treat BrS (900–1500 mg/day), quinidine produces diarrhea in 40–50 % of patients.[31]

In another series of experiments, we tested the hypothesis that the combination of quinidine and WK, at relatively low concentrations at which adverse effects could be minimized, are effective in suppressing the electrocardiographic and arrhythmic manifestations of BrS. Either drug alone was effective in preventing the development of pVT, but not P2R. The combination of the two drugs effectively suppressed all arrhythmic activity and normalized the ECG.

Study Limitations

As with all data derived from experimental animal models, extrapolation to the clinic must be done with great caution. It is noteworthy that coronary-perfused models of BrS have been well characterized and have consistently been shown to recapitulate the clinical ECG and arrhythmic manifestations of BrS as well as the response to drugs. Extrapolation of the data from these *in vitro* models to the clinic must be done with care since a number of assumptions must be considered. Among these is the supposition that pretreatment with NS5806 is a reasonable surrogate for elevated levels of I_{to} that may be encountered physiologically or pathophysiologically in association with a genetic predisposition to BrS. Mutations in three genes associated with BrS have been shown to cause a gain of function in I_{to} and two others have been described in preliminary reports, suggesting that this mechanism of BrS is not a very rare one.

It is also important to point out that the degree to which the effectiveness of the combination of WK and quinidine depends on a BrS mechanism involving a gain of function of I_{to} is not known. However, it is noteworthy that in preliminary experiments, we have obtained qualitatively similar results with other models of BrS, including those involving inhibition of I_{Ca} (unpublished observation).

Finally, WK is a complex mixture of plant extracts and little is known about the effects of the individual components and their interaction with other medications. The precise concentration of active ingredients in solution is difficult to gauge since only a fraction of the herbal mixture makes it into solution with much of it filtered away. Consequently, it is difficult to reconcile the dose to which the experimental preparations are exposed with clinical dose or with plasma levels that are achieved clinically.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

APD	action potential duration
APD$_{90}$	action potential duration at 90% duration
AP	action potential
BrS	Brugada syndrome
Epi	epicardial
I$_{Ca}$	L-type calcium current
I$_{Na}$	transient outward sodium current
I$_{to}$	transient outward potassium current
LRII	Luo-Rudy II

NcBe	nardostachy chinenis batal extract
P2R	phase 2 reentry
pVT	polymorphic ventricular tachycardia
RV	right ventricle
VF	ventricular fibrillation
VT	ventricular tachycardia
WK	Wenxin Keli

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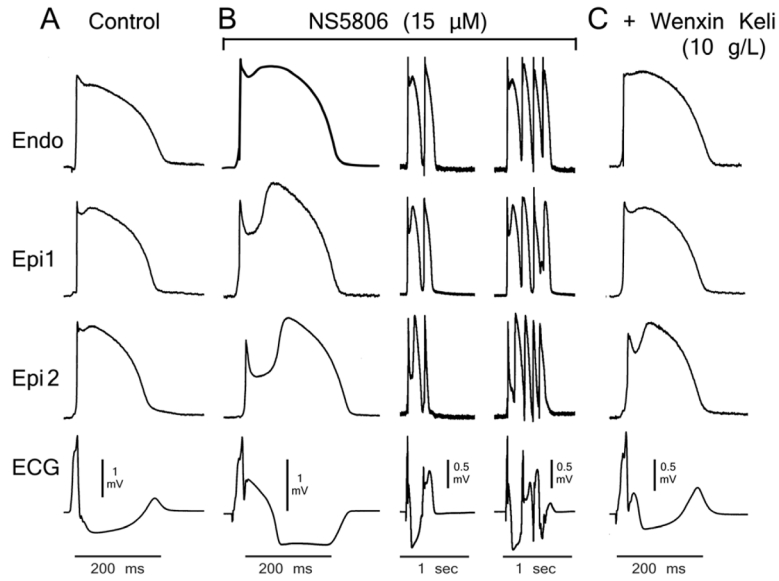
Figure 1.
Wenxin Keli (10g/L) suppression of NS5806-induced Brugada syndrome phenotype. Shown are transmembrane action potentials (APs) simultaneously recorded from two sites on the epicardial (Epi) surface and one site on the endocardial (Endo) surface of a coronary-perfused right ventricular wedge preparation. The bottom trace is the pseudo-electrocardiogram (ECG) recorded across the bath. **A:** Control. **B:** The transient outward potassium channel current agonist NS5806 (15 μM) accentuates the Epi AP notch and electrocardiographic J wave, leading to loss of the AP dome at Epi2 but not Epi1. Heterogeneous loss of the dome leads to the development of a closely–coupled phase 2 reentrant extrasystole which precipitates a polymorphic ventricular tachycardia. **C:** Wenxin Keli (10g/L) suppresses NS5806-induced phase 2 reentry and ventricular tachycardia/ventricular fibrillation and restores the AP dome, thus restoring homogeneity and aborting all arrhythmic activity. Basic cycle length=2000 ms.
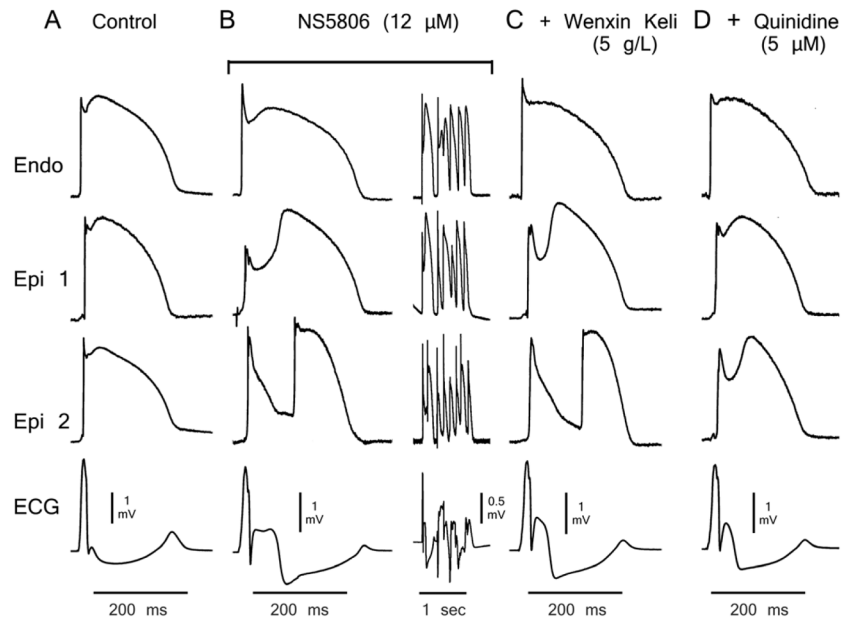
Figure 2.
Wenxin Keli (5g/L) and quinidine (5 µM) suppression of NS5806-induced Brugada syndrome phenotype. Shown are transmembrane action potentials (APs) simultaneously recorded from two sites on the epicardial (Epi) surface and one site on the endocardial (Endo) surface of a coronary-perfused right ventricular wedge preparation. The bottom trace is the pseudo-electrocardiogram (ECG) recorded across the bath. **A:** Control. **B:** The transient outward potassium channel current agonist NS5806 (12 µM) accentuates the Epi AP notch and electrocardiographic J wave, leading to loss of the AP dome at Epi1 but not Epi2. Heterogeneous loss of the dome leads to the development of a closely–coupled phase 2 reentrant extrasystole which precipitates a polymorphic ventricular tachycardia. **C:** Wenxin Keli (5g/L) suppressed ventricular fibrillation but did not prevent phase 2 reentry. **D:** The combination of Wenxin Keli (5g/L) and quinidine restored homogeneity and aborted all arrhythmic activity. Basic cycle length=2000 ms.
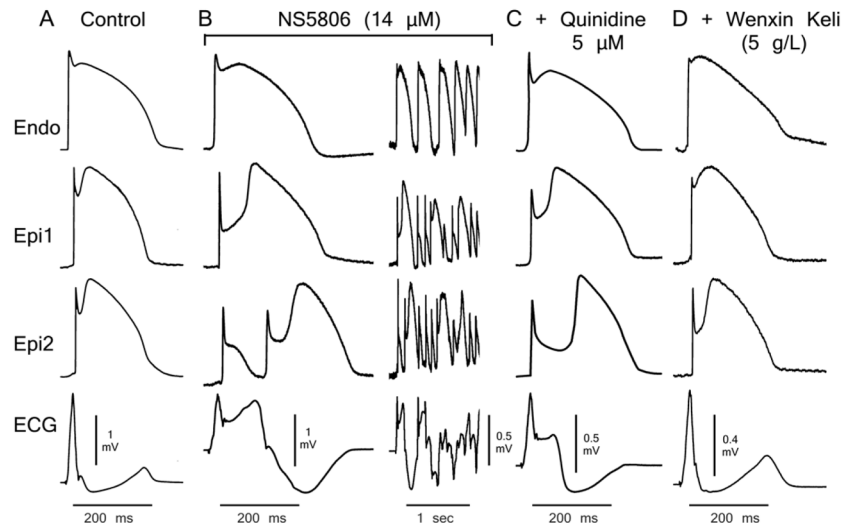
Figure 3.
Quinidine (5 µM) and Wenxin Keli (WK 5g/L) suppression of NS5806-induced Brugada syndrome phenotype. Shown are transmembrane action potentials (APs) simultaneously recorded from two sites on the epicardial (Epi) surface and one site on the endocardial (Endo) surface of a coronary-perfused right ventricular wedge preparation. The bottom trace is the pseudo-electrocardiogram (ECG) recorded across the bath. **A:** Control. **B:** The transient outward potassium channel current agonist NS5806 (14 µM) accentuates the Epi AP notch and electrocardiographic J wave, leading to loss of the AP dome at Epi1 but not Epi2. Heterogeneous loss of the dome leads to the development of a closely–coupled phase 2 reentrant extrasystole which precipitates a polymorphic ventricular tachycardia. **C:** Quinidine (5 µM) inhibited ventricular fibrillation but couldn't inhibit phase 2 reentry. **D:** The combination of quinidine and WK (5g/L) restored homogeneity and aborted all arrhythmic activity. Basic cycle length=2000 ms.
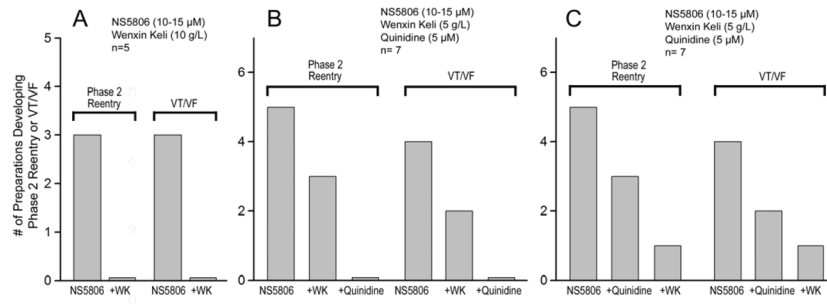
Figure 4.
Incidence of Phase 2 Reentry or ventricular tachycardia/ventricular fibrillation (VF/VT) in response to the indicated treatments. WK=Wenxin Keli.
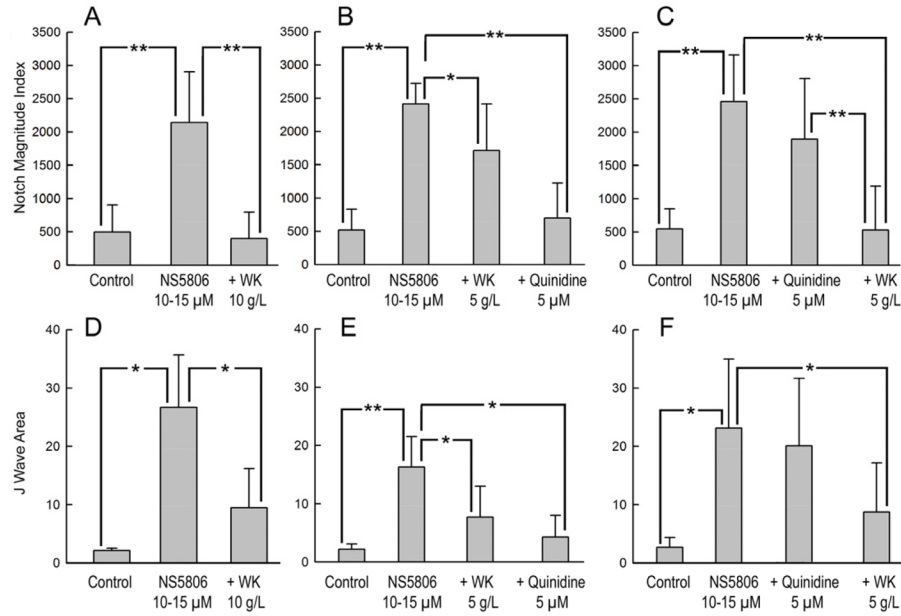
Figure 5.
Effect of Wenxin Keli (WK) and quinidine on epicardial action potential notch magnitude and J wave area in the electrocardiogram. **A and D:** Notch magnitude index (NMI) and J wave area are significantly increased by NS5806 and reduced following addition of WK (10g/L) to the coronary perfusate (n=3–4). **B and E:** NMI and J wave area are significantly increased by NS5806 and significantly reduced by WK (5g/L) and further reduced by the combination of quinidine (5 μM) and WK (5 g/L).(n=5–6). **C and F:** The NS5806-induced increases in NMI and J wave area are significantly reduced by quinidine (5 μM) and further reduced by WK (5g/L). n=5–6. Values are mean±SD. * P<0.05, **P<0.001.
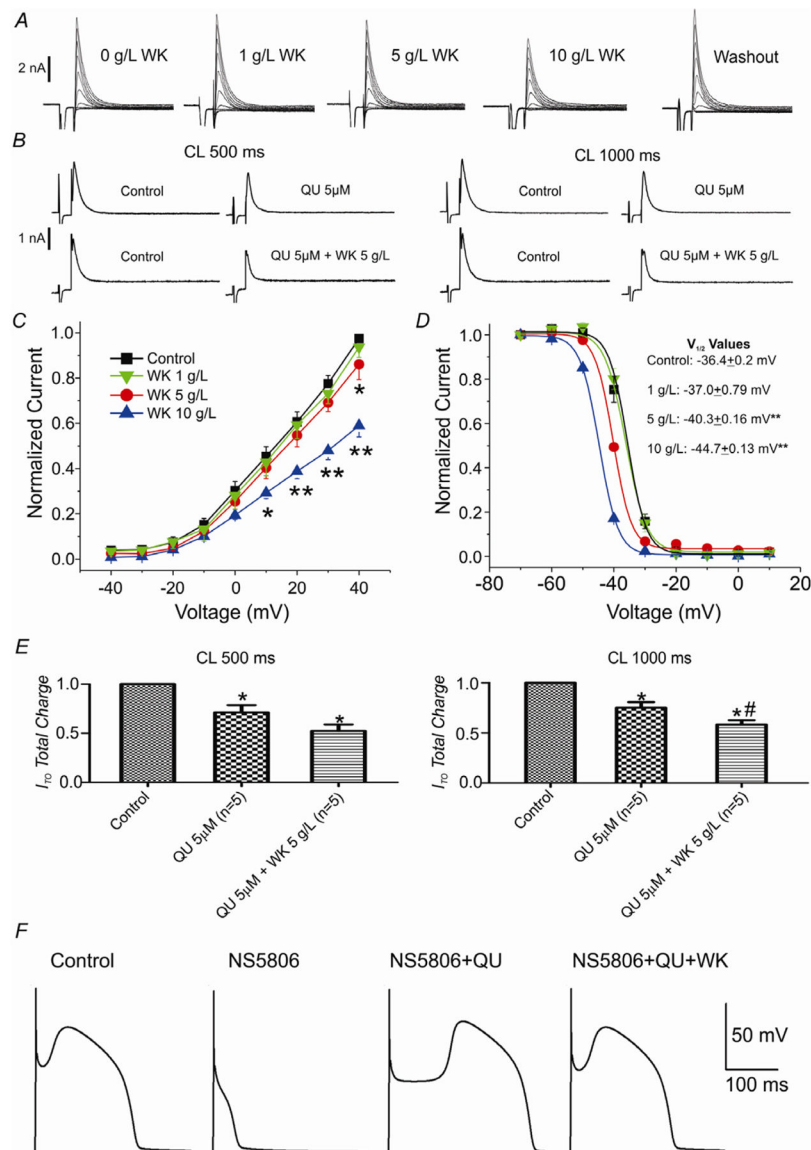
Figure 6.
Effect of Wenxin Keli (WK) and quinidine on the transient outward potassium current (I_{to}).
A: Representative current traces recorded from ventricular cardiomyocytes under control conditions, following increasing concentrations of WK, and after washout. Also shown are I_{to} traces recorded after exposure to WK (5 g/L) as well as a combination of WK (5 g/L) and quinidine (5 μM) at CLs of 500 and 1000 ms. **B:** Representative current traces at cycle lengths (CL) of 500 and 1000 ms of I_{to} comparing effects of 5μM quinidine (QU) and QU +WK with controls.: **C:** Normalized averaged I-V plot for I_{to} at different concentration of WK (n=6–10). **D:** Effect of different concentrations of WK on average steady-state inactivation of I_{to}. Data where fit with a single Boltzmann function (n=6, 2, 3 and 4 for control, 1g/L, 5g/L and 10 g/L WK, respectively. **E:** The total charge carried by I_{to} (relative to control) following exposure to QU (5uM) alone and the combination of QU and WK (5 g/L) at cycle lengths (CL) of 500 and 1000 ms. p<0.05 vs. control. **, p<0.01 vs. control. #, p<0.05 vs. QU (5μM) alone.

F: Right ventricular action potentials simulated using Luo-Rudy II model incorporating normal I_{to} (1.2 mS/µF) representing baseline "control" conditions, enhanced I_{to} (2.2 mS/µF) simulating the effect of NS5806, partial block of enhanced I_{to} (1.6 mS/µF) simulating effect of 5 µM of QU, and further block of I_{to} (1.3 mS/µF) simulating the combined effect of 5 µM of QU and 5 g/L of WK.
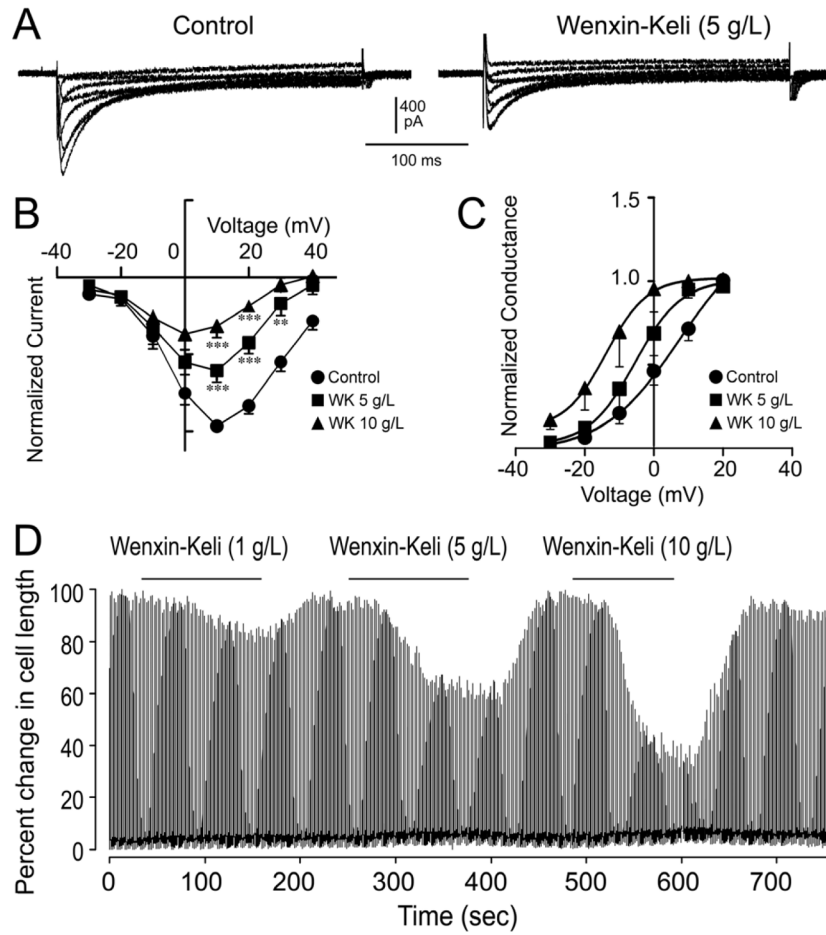
Figure 7.
Effects of Wenxin Keli (WK) on L-type calcium channel current (I_{Ca}). **A:** Representative current traces in the presence and the absence of 5g/L WK. **B:** Normalized averaged I-V relationship for I_{Ca} at different concentrations of WK (n=4–9). **C:** Averaged steady-state activation plot for I_{Ca} at different concentrations of WK. The normalized conductance vs. voltage plots where fit with a single Boltzmann function (n=9, 5 and 4 for control, 5g/L and 10 g/L WK, respectively). **D:** Representative example of the inhibitory effects of WK on unloaded cell shortening in field stimulated isolated myocytes. WK (1 g/L, 5g/L, 10g/L) was added sequentially to the bath solution for 120 seconds each during continuous field stimulation at 0.5 Hz.
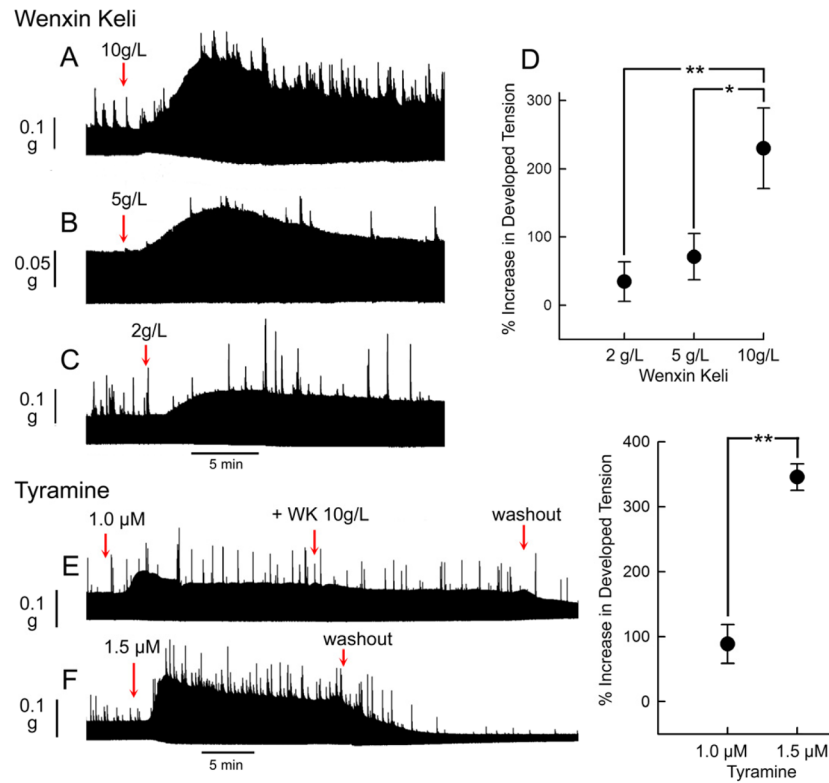
Figure 8.
Effect of Wenxin Keli (WK) and tyramine on isometric tension development in a coronary-perfused right ventricular wedge preparation. **A, B and C:** WK caused a progressively greater positive inotropic effect at concentrations of 2, 5 and 10 g/L. **D:** Composite data of effect of 2, 5, and 10 g/L WK to increase developed tension. **E and F:** Concentration-dependent effect of tyramine (1 and 1.5 μM) to cause a positive inotropic effect. Addition of WK during exposure to tyramine did not produce a further increase in contractile force. * P<0.05, **P<0.001.