Calcium-activated chloride current contributes to action potential alternations in left ventricular hypertrophy rabbit

Donglin Guo,1 Lindon Young,2 Chinmay Patel,1 Zhen Jiao,1 Ying Wu,1 Tengxian Liu,1 Peter R. Kowey,1,3 and Gan-Xin Yan1,3

1Main Line Health Heart Center, Wynnewood, 2Department of Pathology, Microbiology and Immunology, Philadelphia College of Osteopathic Medicine, Philadelphia, and 3Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania

Submitted 7 September 2007; accepted in final form 25 April 2008

Am J Physiol Heart Circ Physiol 295: H97–H104, 2008. First published April 25, 2008; doi:10.1152/ajpheart.01032.2007.—T-wave alternans, characterized by a beat-to-beat change in T-wave morphology, amplitude, and/or polarity on the ECG, often heralds the development of lethal ventricular arrhythmias in patients with left ventricular hypertrophy (LVH). The aim of our study was to examine the ionic basis for a beat-to-beat change in ventricular repolarization in the setting of LVH. Transmembrane action potentials (APs) from epicardial and endocardial were recorded simultaneously, together with transmural ECG and contraction force, in arterially perfused rabbit left ventricular wedge preparations. APs and Ca2+-activated chloride current (I_{Cl, Ca}) were recorded from left ventricular myocytes isolated from normal rabbits and those with renovascular LVH using the standard microelectrode and whole cell patch-clamping techniques, respectively. In the LVH rabbits, a significant beat-to-beat change in endocardial AP duration (APD) was also observed in both left ventricular endocardial and epicardial myocytes at various pacing rates. APD alternans was suppressed by adding 1 μM ryanodine, 100 μM 4,4’-diisothiocyanostilbene-2,2’-disulfonic acid (DIDS), and 100 μM 4-acetamido-4’-isothiocyanostilbene-2,2’-disulfonic acid (SITS). The density of the Ca2+-activated chloride currents (I_{Cl, Ca}) in left ventricular myocytes was significantly greater in the LVH rabbits than in the normal group. Our data indicate that abnormal intracellular Ca2+ was significantly greater in the LVH rabbits than in the normal group. Clearly, T-wave alternans is the consequence of an alternation of ventricular action potential (AP) duration (APD) at the cellular level (29, 42, 45). Multiple studies have suggested that APD alternation is related to intracellular Ca2+ overload and fluctuation, in which the sarcoplasmic reticulum (SR) function plays an important role (11, 21, 36). Direct evidence has shown that an alternation of the intracellular Ca2+ transit can modulate electrical activation and induce APD alternans (20, 28). Recent studies have shown that Ca2+/calmodulin-dependent protein kinase II, an enzyme that phosphorylates several Ca2+ transport proteins, can initiate intracellular Ca2+ alternation that induces APD alternans (5, 22). All of this suggests that intracellular Ca2+ oscillation plays a critical role in the genesis of APD alternans.

Several hypotheses have been proposed to explain the mechanism of Ca2+ oscillations and its coupling to APD alternans, including a beat-to-beat change in the refractoriness of SR Ca2+ release channel, L-type Ca2+ channel, and Na+/Ca2+ exchange current (I_{Na/Ca}) (33, 35, 36). However, the precise ionic mechanism for T-wave alternans or APD alternations is still poorly understood. The prominent feature of myocytes from a failing heart is the alternation of intracellular Ca2+ handling (2). We hypothesize that abnormal intracellular Ca2+ oscillation in a failing heart may cause a strong augmentation of Ca2+-activated membrane currents, such as K+ current (I_K), I_{Na/Ca}, and Ca2+-activated chloride currents (I_{Cl, Ca}). A recent study from our laboratory has shown that LVH is associated with a decrease in slow delayed rectifier K+ current (I_{K,S}) (44), which excludes the possibility that Ca2+-activated I_{K,S} is responsible for APD alternans. LVH-induced remodeling of I_{Na/Ca} in myocytes of various animals has been studied in detail (8, 30, 37); however, little is known about I_{Cl, Ca} channel remodeling and its role in the genesis of T-wave alternans in LVH and heart failure. The present study was designed to evaluate LVH-induced changes in I_{Cl, Ca} and to delineate the underlying ionic mechanisms responsible for APD alternans.

METHODS

Experimental animals and LVH model. Adult New Zealand rabbits (1.4–1.8 kg) underwent unilateral nephrectomy with contralateral renal artery banding to produce LVH using techniques reported previously (32). Rabbits with unilateral renal artery banded in this way uniformly develop LVH within 3 mo. Control rabbits were matched for age in the experiment. Data were collected from 16 LVH rabbits.
rabbits (8 of either sex) and 16 control rabbits (8 of each sex). Animal care and protocols were approved by the Institutional Animal Care and Use Committee.

**Heart weight measurement and wall thickness.** Rabbis were harapanized (800 U/kg iv) and then anesthetized with ketamine 40 mg/kg iv. When deep anesthesia was achieved, the heart was excised. All hearts were washed in cold bicanonate-based Ca$^{2+}$-free solution, containing (in mmol/l) 125 NaCl, 3.5 KCl, 1.5 KH$_2$PO$_4$, 1 MgCl$_2$, 20 NaHCO$_3$, and 20 glucose, saturated with 95% O$_2$-5% CO$_2$ to clear the chambers of blood. After a quick blotting, the heart was weighed. Hearts from rabbits used in the wedge preparations were used to measure the wall thickness. The LV posterior wall thickness was measured with calipers at the level of the papillary muscles.

**Arterially perfused rabbit ventricular wedge preparation.** Surgical preparation of the rabbit LV wedge has been described in detail in previous publications (46). Briefly, rabbits weighing 3–3.5 kg were harapanized (800 U/kg iv) and then anesthetized with ketamine (40 mg/kg iv). When deep anesthesia was achieved, the heart was excised. After the ratio of heart weight to body weight was calculated, the heart was placed in a cardioplegic solution consisting of cold (4°C) Tyrode solution containing (in mM) 129 NaCl, 3.5 KCl, 1.5 KH$_2$PO$_4$, 1 MgCl$_2$, 20 NaHCO$_3$, and 20 glucose, saturated with 95% O$_2$-5% CO$_2$ to clear the chambers of blood. A quick blotting, the heart was weighed. Hearts from rabbits used in the wedge preparations were used to measure the wall thickness. The LV posterior wall thickness was measured with calipers at the level of the papillary muscles.

**Recording of the transmural ECG, transmembrane APs, and isometric contractile force.** The ventricular wedges were allowed to equilibrate in the tissue bath until electrically stable, usually 1 h. The preparation was then placed in a small tissue bath and arterially perfused with Tyrode solution of the following composition: (in mM) 129 NaCl, 4 KCl, 0.9 NaH$_2$PO$_4$, 20 NaHCO$_3$, 1.8 CaCl$_2$, 0.5 MgSO$_4$, and 5.5 glucose and 1 U/l insulin, buffered with 95% O$_2$-5% CO$_2$ at 36°C. The preparation was placed to equilibrate in the tissue bath for 1 h before electrical recordings.

**Data analysis.** Data are expressed as means ± SE. Student’s t-test was used to determine the statistical significance of differences between the control and test conditions. Significance was defined as a value of P < 0.05.

**RESULTS**

**Alteration of T wave and QT interval in a LVH rabbit LV wedge.** After 3 mo of surgery (unilateral nephrectomy with contralateral renal artery banding), rabbits developed significant LVH. LVH manifested as an increased LV wall thickness and heart weight. The wall thickness was 0.49 ± 0.02 cm in the LVH group versus 0.34 ± 0.01 cm in the control group (means ± SE, n = 10 hearts, P < 0.01) at 3 mo. The ratio of heart weight to body weight was 2.9 ± 0.01 g/kg in the LVH group versus 2.2 ± 0.01 g/kg in the control group (means ± SE, n = 10 hearts, P < 0.01) at 3 mo.

As seen in Fig. 1, more significant beat-to-beat changes in endocardial APD than in epicardial APD resulted in a beat-to-beat alteration in transmural voltage gradient that manifested as T-wave alternans on the ECG. T-wave alternans was associated with an increased susceptibility to early afterdepolarization in LVH myocytes (Fig. 2B). APD alternations were associated with an increased susceptibility to early afterdepolarization in LVH myocytes (Fig. 2C). At a basic cycle of length of 2,000 ms, the phenomenon as shown in Fig. 2 was observed more frequently in single myocytes of LVH than in those of the normal rabbits. However, significant APD alternans did occur in single myocytes of the normal rabbits at an extremely
high-stimulating frequency (cycle lengths = 150 ms, Fig. 4). The incidence of APD alternans observed in LVH myocytes increased significantly with increasing pacing rates. The incidence of APD alternans in 10 LVH rabbits at pacing cycle lengths of 2,000, 1,000, 500, 250, 200, and 150 ms was 40%, 40%, 50%, 100%, 100%, and 100%, respectively.

**Characteristics of APD alternations in LVH epicardial and endocardial myocytes.** To better understand the mechanism underlying APD alternans in LVH myocytes, we compared APD alternations and their characteristics in epicardial and endocardial myocytes of the LVH rabbits at a cycle length of 250 ms (Fig. 3). Figure 3, A and B, shows the steady-state APs recorded in a single endocardial and epicardial myocyte of LV in control rabbit. AP alternations were not observed in all tested myocytes from control rabbits. However, significant beat-to-beat AP alternations could be induced in all tested endocardial and epicardial myocytes of LVH rabbits (Fig. 3, C and D). Interestingly, beat-to-beat AP alternations were more pronounced in endocardial than in epicardial myocytes (Fig. 3, C and D). Figure 3, E and F, shows the beat-to-beat changes in APD in three consecutive beats in endocardial and epicardial myocytes at the cycle length of 250 ms. The APD in the first beat (N) and the second beat (N + 1) in endocardial myocytes were 171.4 ± 6.6 and 133.6 ± 5.1 ms (n = 10 cells from 10 rabbits, P < 0.01), respectively. The APD values of the first beat (N) and the second beat (N + 1) in epicardial myocytes were 160.6 ± 5.1 and 145.6 ± 4.9 ms (n = 10 cells from 10 rabbits, P < 0.05), respectively.

Figure 4 summarizes the mean of beat-to-beat alternations in APD in three consecutive beats in endocardial and epicardial myocytes (Fig. 3, A and B). However, extremely fast pacing rates (such as at cycle lengths of 200 and 150 ms) slightly increased the mean alternans of APD both in endocardial and epicardial myocytes. In LVH myocytes, however, the mean alternans of APD were significantly increased at all tested cycle lengths in both endocardial and epicardial myocytes. It is noteworthy that beat-to-beat alternations of the APD were more profound in endocardial myocytes than in epicardial myocytes under LVH conditions.

To test whether intracellular Ca2+ overload plays a critical role in the genesis of APD alternations, we added ryanodine (a specific inhibitor for SR Ca2+ uptake) to the perfusate. Interestingly, ryanodine at the concentration of 1 μM completely suppressed the APD alternations in endocardial myocytes of LVH rabbits at a cycle length of 250 ms (Fig. 5, A and B). To our surprise, DIDS (100 μM), a specific ICa blocker, completely suppressed the APD alternations of endocardial myocytes in LVH rabbits (Fig. 5, C and D). Similarly, SITS at 100 μM, another anion channel blocker, also suppressed the APD alternations (Fig. 5, E and F). In our wedge preparation experiment, DIDS at a concentration of 100 μM did not significantly affect the isometric contractile force (5.45 ± 0.48 g in control vs. 5.70 ± 0.49 g at 100 μM, n = 4 hearts, P > 0.05). It is also noteworthy that DIDS caused a small but statistically significant increase in APD (data not shown).

**Characteristics of ICa in LVH endocardial and epicardial myocytes.** ICa is defined as DIDS-sensitive current and could be recorded in the single ventricular myocytes of rabbit. Figure 6 shows typical ICa values recorded in endocardial and epicardial myocytes in control rabbits. The ICa trace demonstrated a transient outward direction and rapidly declined to a zero level within 25–50 ms, which is consistent with previous reports in rabbit ventricular myocytes by other authors (40, 47).

Figure 7 shows superimposed traces of ICa from endocardial myocytes of control and LVH rabbits. ICa values were elicited by depolarizing test pulses from −40 to −60 mV in 10-mV increments with a holding potential of −50 mV. There were no
significant differences of $I_{\text{Cl,CA}}$ densities between endocardial and epicardial myocytes in control rabbits (Fig. 7C). Interestingly, the density of the $I_{\text{Cl,CA}}$ was significantly greater in myocytes of the LVH rabbits than in normal rabbits at voltages above 10 mV. Also, there was no significant difference in $I_{\text{Cl,CA}}$ density between endocardial and epicardial myocytes of LVH rabbits.

**DISCUSSION**

The present study shows that a significant beat-to-beat change in endocardial APD created a beat-to-beat alteration in transmural voltage gradient that manifested as T-wave alternans on the ECG in the intact LV wall of LVH rabbits. In the single myocytes study, our results show sustained APD alternations can be induced in LVH myocytes at various pacing cycle lengths, and the endocardial myocytes are more susceptible to alternation than epicardial myocytes. Our data demonstrate that ryanodine, DIDS, and SITS suppressed the APD alternations in LVH myocytes, indicating that intracellular Ca$^{2+}$ loading and $I_{\text{Cl,CA}}$ contribute importantly to the APD alternations in LVH myocytes. Moreover, a significantly larger $I_{\text{Cl,CA}}$ density in LVH myocytes suggests that abnormal intracellular Ca$^{2+}$ fluctuation may influence the membrane $I_{\text{Cl,CA}}$, leading to beat-to-beat APD alternations in LVH rabbits.

T-wave alternans, defined as a beat-to-beat variation of T-wave morphology, amplitude, and/or polarity is commonly observed in patients with LVH and heart failure. It is a well-established predictor of susceptibility to develop polymorphic ventricular tachycardia and sudden cardiac death in this group of patients (9, 18, 23, 33). Many studies have shown that T-wave alternans in most circumstances results from an alternation of ventricular repolarization (29, 31, 34). Using a canine LV wedge preparation, Shimizu and Antzelevitch (36) suggested that T-wave alternans observed at rapid rates under long QT conditions is largely the result of the M-cell APD alternations, leading to the exaggeration of transmural disper-

---

Fig. 3. Beat-to-beat APD alternans in LVH myocytes. A and B: steady-state AP recorded from endocardial and epicardial myocytes from left ventricle of a control rabbit at BCL of 250 ms. C and D: steady-state AP recorded from endocardial and epicardial myocytes of LVH rabbit. E and F: beat-to-beat change in APD at 90% polarization ($\text{APD}_{90}$) of 3 successive beats in endocardial and epicardial myocytes of control and LVH rabbits. Data are presented as means ± SE; $n = 10$ cells from 10 rabbits. *$P < 0.05$, **$P < 0.01$ vs. first beat ($N$) or third beat ($N + 2$).

Fig. 4. Summaries of mean alternans of beat-to-beat change of $\text{APD}_{90}$ in endocardial (A) and epicardial (B) myocytes of control and LVH rabbits at various cycle lengths. The mean alternans data obtained from the difference of $\text{APD}_{90}$ between 2 successive beats under steady-state condition. Data are presented as means ± SE; $n = 10$ cells from 10 rabbits. **$P < 0.01$ vs. control.
sion of repolarization during alternate beats. In the present study, we also found that beat-to-beat APD alternations were more pronounced in the endocardium than in the epicardium in LVH rabbits. The dispersion of repolarization between epicardial and endocardial cells results in a transmural voltage gradient that leads to the inscription of T wave. A more significant beat-to-beat change in endocardial APD than in epicardium APD results in a beat-to-beat change in transmural voltage gradient, which manifests as T-wave alternans on the ECG.

There is growing evidence that Ca^{2+}/H^{+} release from SR plays a critical role in the genesis of APD alternans (20, 21, 36). Hirayama et al. (11) reported that ryanodine and caffeine, which prevent the release of Ca^{2+} from SR, abolished the APD and mechanical alternans in canine heart. They concluded that delayed intracellular Ca^{2+} cycling plays an important role in the development of APD alternans (11). In the guinea pig model of T-wave alternans, Pruvot et al. (31) demonstrated that the mechanism underlying T-wave alternans is more closely associated with intracellular Ca^{2+} cycling. In a hypertrophied and failing heart, the characteristic slow decay of the Ca^{2+} transient and increased dia-stolic intracellular Ca^{2+} may predispose the cell to oscillatory release of Ca^{2+} from the SR (15, 17). This hypothesis is supported by the observation that APD alternans in LVH was eliminated after the blockade of the SR Ca^{2+} release by ryanodine in the endocardial myocytes of LVH rabbits in our study. Our results support the notion that intracellular Ca^{2+} oscillation contributes to the APD alternans in LVH. It is noteworthy that APD alternans in LVH myocytes were seen not only at higher stimulating rates but also at lower

Fig. 5. Effects of ryanodine (A and B), DIDS (C and D), and SITS (E and F) on APD alternans in the single endocardial myocyte of LVH rabbit. V, voltage. G and H: APD_{90} of 3 successive beats in the absence and presence of DIDS and SITS. Data are presented as means ± SE; n = 10 cells from 10 rabbits. **P < 0.01 vs. N or N + 2.
stimulating rates (Fig. 2), indicating that APD alternans is an important abnormal electrophysiological feature in hypertrophied hearts.

A single myocyte from LVH and a failing heart have been shown to have an altered intracellular Ca\(^{2+}\) handling (2). The impaired intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_i\)]) handling may potentially augment the membrane Ca\(^{2+}\)-modulated cell surface ion channels and transporters, such as \(I_{Ks}\), \(I_{Na/Ca}\), and \(I_{Cl/Ca}\), leading to alternations in cardiac repolarization. A number of studies have investigated the changes in \(I_{Cl/Ca}\) in various animal models of LVH and heart failure. However, the results have always been inconsistent and remained controversial (8, 12, 13, 30, 37). A recent study from our laboratory has shown that LVH is associated with a decrease in \(I_{Ks}\) (44). The functional downregulation of K\(^+\) channels is the most consistent phenomenon and contributes to the APD prolongation in hypertrophied and failing myocytes (13, 39). Therefore, changes in \(I_{Ks}\) and \(I_{Na/Ca}\) are not sufficient to explain the APD alternans secondary to intracellular Ca\(^{2+}\) fluctuation under the conditions of LVH and heart failure. At present, little is known about \(I_{Cl/Ca}\) remodeling and its role in the APD alternans in myocytes of the hypertrophied and failing heart. The presence of \(I_{Cl/Ca}\) has been demonstrated in ventricular myocytes of rabbits (16, 47). It is activated by an increase in the [Ca\(^{2+}\)\(_i\)] associated with Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR and plays an important role in AP repolarization in rabbit ventricular myocytes. In our experiment, DIDS at a concentration of 100 \(\mu\)M completely suppressed the induction of APD alternans in myocytes of LVH rabbits (Fig. 5), indicating that \(I_{Cl/Ca}\) may be an important candidate current involved in APD alternans under the conditions of LVH. Some reports have suggested that DIDS can affect SR Ca\(^{2+}\) handling (10, 24, 27). To rule out this possibility, we tested SITS, another anion channel blocker that has shown no effect on intracellular Ca\(^{2+}\) handling (24), on the effect of APD alternans. Similarly, SITS at the concentration of 100 \(\mu\)M also inhibited the APD alternans (Fig. 5, E and F). Furthermore, DIDS at the concentration of 100 \(\mu\)M showed no significant changes in the contraction force in our wedge preparation. It is also noteworthy that there were significantly different effects on endocardial APD between DIDS and ryanodine. For example, DIDS had an APD-prolonging effect compared with ryanodine (Fig. 5). The direct blocking effect of DIDS on \(I_{Cl/Ca}\) may contribute to the observed APD prolongation.

Verkerk et al. (41) recorded a significantly larger outward \(I_{Cl/Ca}\) in ventricular myocytes from the failing rabbit heart; however, the density of \(I_{Cl/Ca}\) in failing rabbit cells did not differ significantly from the control rabbit myocytes due to that the cell capacitances found threefolds larger in the failing heart cells. Benitah et al. (1) also reported that a significant chloride current component was induced in hypertrophied cardiac myocytes. They suggested that the outward chloride current could prevent the excessive AP prolongation in hypertrophied and failing heart. Kameyama et al. (15) reported that DIDS attenuated the monophasic AP alternans that was induced with an abrupt shortening of the cycle length from 1,000 to 350 ms in the canine beating heart. They suggested that \(I_{Cl/Ca}\) might contribute to the appearance of the electrical alternans. Interestingly, we found that the density of \(I_{Cl/Ca}\) was significantly increased in both endocardial and epicardial myocytes of the LVH rabbits compared with those of the control rabbits (Fig. 7). Our data suggest that impaired intracellular Ca\(^{2+}\) handling of LVH myocytes may directly regulate the cell membrane \(I_{Cl/Ca}\). Under conditions of LVH and failure, elevated [Ca\(^{2+}\)\(_i\)], may exert a strong feedback effect on \(I_{Cl/Ca}\) that shortens APD. APD shortening will in turn limit Ca\(^{2+}\) influx through cell membrane and cause less Ca\(^{2+}\) release from SR in the subsequent cardiac cycle (4). A smaller intracellular Ca\(^{2+}\) transient and delayed \(I_{Cl/Ca}\) recovery will result in a weaker \(I_{Cl/Ca}\) that prolongs APD. In an isolated cardiac muscle preparation, an opposite relationship between alter-
nation of APD and alternation of the strength of contraction is frequently observed (35, 38, 43). APD alternans secondary to $I_{\text{Cl,Ca}}$ fluctuation may provide an excellent explanation for those “out of phase” T-wave alternans with a longer AP associated with a smaller contractility and a shorter APD corresponding to a larger contractility.

Limitations of the study. To date, $I_{\text{Cl,Ca}}$ has been reported to be present in isolated myocytes from various species, such as rat (14) and rabbit (47) ventricle. However, there is no conclusive evidence that $I_{\text{Cl,Ca}}$ is also present in human ventricular myocytes (6, 19, 41). Therefore, the role of $I_{\text{Cl,Ca}}$ in the genesis of cardiac arrhythmias associated with T-wave alternans should be interpreted with caution in the failing human heart. Also, our interpretation of the data is based on the assumption that intracellular Ca$^{2+}$/H$^{+}$ undergoes a fluctuating change under conditions of LVH since many studies have demonstrated that impaired intracellular Ca$^{2+}$ handling is in LVH and heart failure. Therefore, further studies are required to elucidate the relationship between APD alternans and intracellular Ca$^{2+}$ transient. In addition, studies from hypertrophied and failing heart have demonstrated an increase in both $I_{\text{Na,Ca}}$ mRNA and protein, suggesting that enhanced $I_{\text{Na,Ca}}$ function may also be involved in the genesis of APD alternans in LVH rabbit myocytes.

The duration and shape of the AP is the result of a delicate balance between the depolarizing and repolarizing currents that are active during the plateau phase. T-wave alternans, a cellular electrophysiological abnormality associated with LVH, is also the result of the summation of changes in the membrane currents. Therefore, multiple ion current abnormalities (remodeling) should contribute to the genesis of T-wave alternans in LVH rabbits. Our current results only suggest an association between $I_{\text{Cl,Ca}}$, hypertrophy and alternans but in no way proves a causal relationship with T-wave alternans.

Some studies have suggested that DIDS could affect SR Ca$^{2+}$ handling (10, 24, 27) and, hence, it is possible that the inhibition of APD alternans by DIDS may be secondary to its effect on intracellular Ca$^{2+}$ and not completely dependent on the inhibition of $I_{\text{Cl,Ca}}$. In our present study, the perfusion of DIDS at 100 µM in the arterially perfused LVH rabbit wedge preparation did not result in any significantly change in the isometric contractile force, indicating that DIDS unlikely influences [Ca$^{2+}$]. However, the possibility that DIDS may directly influence intracellular Ca$^{2+}$ handling cannot be completely ruled out due to our inability to directly measure [Ca$^{2+}$].

GRANTS

This study was supported by an American Heart Association Scientist Developmental Grant 0530160N (to D. Guo), the Albert M. Greenfield Foundation (to P. R. Kowey and G.-X. Yan), the Sharpe-Strumia Research Foundation (to G.-X. Yan), and W. W. Smith Charitable Trust (to D. Guo and P. R. Kowey).
REFERENCES


H104 AP ALTERNATIONS AND LVH