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ORIGINAL RESEARCH

Basal late sodium current is a significant contributor to the duration of action potential of guinea pig ventricular myocytes

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Abstract

In cardiac myocytes, an enhancement of late sodium current (I_{NaL}) under pathological conditions is known to cause prolongation of action potential duration (APD). This study investigated the contribution of I_{NaL} under basal, physiological conditions to the APD. Whole-cell I_{NaL} and the APD of ventricular myocytes isolated from healthy adult guinea pigs were measured at 36°C. The I_{NaL} inhibitor GS967 or TTX was applied to block I_{NaL}. The amplitude of basal I_{NaL} and the APD at 50% repolarization in myocytes stimulated at a frequency of 0.17 Hz were -0.24 ± 0.02 pA/pF and 229 ± 6 msec, respectively. GS967 (0.01–1 μmol/L) concentration dependently reduced the basal I_{NaL} by 18 ± 3–82 ± 4%. At the same concentrations, GS967 shortened the APD by 9 ± 2 to 25 ± 1%. Similarly, TTX at 0.1–10 μmol/L decreased the basal I_{NaL} by 13 ± 1–94 ± 1% and APD by 8 ± 1–31 ± 2%. There was a close correlation ($R^2 = 0.958$) between the percentage inhibition of I_{NaL} and the percentage shortening of APD caused by either GS967 or TTX. MTSEA (methanethiosulfonate ethylammonium, 2 mmol/L), a $Na_V1.5$ channel blocker, reduced the I_{NaL} by 90 ± 5%, suggesting that the $Na_V1.5$ channel isoform is the major contributor to the basal I_{NaL}. KN-93 (10 μmol/L) and AIP (2 μmol/L), blockers of CaMKII, moderately reduced the basal I_{NaL}. Thus, this study provides strong evidence that basal endogenous I_{NaL} is a significant contributor to the APD of cardiac myocytes. In addition, the basal I_{NaL} of guinea pig ventricular myocytes is mainly generated from $Na_V1.5$ channel isoform and is regulated by CaMKII.

Introduction

The late Na^+ current (I_{NaL}) is a component of the fast inward I_{Na}, which remains activated during the plateau and the repolarization of a cardiac action potential (Noble and Noble 2006; Antzelevitch et al. 2014). I_{NaL} is increased in congenital and acquired pathological conditions, such as long QT syndrome type 3, cardiac hypertrophy, heart failure, and myocardial ischemia (Belardinelli et al. 2015; Makielski 2016). An enhancement of I_{NaL} under these pathological conditions may cause a prolongation of the action potential duration (APD) and is considered potentially arrhythmogenic (Belardinelli et al. 2015; Makielski 2016). Inhibition of I_{NaL} by I_{NaL} blockers, such as ranolazine, has shown promising antiarrhythmic value (Belardinelli et al. 2015; Makielski 2016). However, because the amplitude of I_{NaL} under physiological conditions is relatively small, its role (i.e., the I_{NaL} in the absence of drug or pathological modification) in cardiac repolarization has not been fully recognized.

Several lines of evidence suggest that the inward I_{NaL} may play a significant role in maintaining cardiac depolarization under physiological conditions. (1) I_{NaL} can remain activated throughout the action potential plateau, where the membrane resistance is high (Weidmann 1951). Therefore, even a small net inward current may cause a significant lengthening of the plateau and thus the APD. (2) The APD is shortened in the presence of TTX, an inhibitor of I_{NaL} (Coraboeuf et al. 1979; Kiyosue and Arita 1989). (3) In canine ventricular myocytes, the density of I_{NaL} is greater in the mid-myocardium, compared with that in the epi- and endomyocardium (Zygmunt et al. 2001). In keeping with that, TTX-induced APD shortening is greater in the mid-myocardium than in the epi- and endomyocardium (Zygmunt et al. 2001). (4) In failing hearts of both human and canine models, inactivation of I_{NaL} of ventricular myocytes is further slowed, compared with that in myocytes of normal hearts (Maltsev et al. 2007). The enhanced I_{NaL} contributes to the prolongation of APD (Maltsev et al. 2007), and conversely, inhibition of I_{NaL} by ranolazine shortens the APD of ventricular myocytes isolated from a canine heart failure model (Undrovinas et al. 2006).

The goal of this study was to determine the contribution of basal I_{NaL} to the APD of ventricular myocytes of healthy guinea pigs. In the past, a precise evaluation of the contribution of basal I_{NaL} to the APD has been hindered by the small amplitude of the current and the lack of a selective inhibitor. Most studies of I_{NaL} have been conducted in the presence of I_{NaL} enhancers, such as anemone toxin II (ATX-II) (Isenberg and Ravens 1984; Song et al. 2004). In this study, the selective I_{NaL} inhibitor GS967 (Belardinelli et al. 2013) and low concentrations of TTX were applied to selectively block I_{NaL}. The amplitude of I_{NaL} in this study was not preenhanced by drugs or special experimental conditions, except for one series of experiments in which the I_{NaL} enhancer ATX-II was applied to verify the specificity of the action of GS967. Thus, the subject of this study was cardiac I_{NaL} under basal conditions. The role of basal I_{NaL} in maintaining the depolarization of the ventricular action potential was assessed by comparing the percentage inhibition of I_{NaL} with the percentage shortening of APD. In addition, we examined whether the basal I_{NaL} is generated from $Na_V1.5$ channels, and whether the basal I_{NaL} is regulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), respectively, by applying the selective $Na_V1.5$ channel blocker MTSEA (methanethiosulfonate ethylammonium) (Haufe et al. 2005; O'Reilly and Shockett 2012) and the CaMKII inhibitors KN-93 and AIP (autocamtide-2-related inhibitory peptide).

Materials and Methods

Animal use was approved by the Institutional Animal Care and Use Committee, and conformed to the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). Hearts of guinea pigs of either sex were isolated and perfused via the aorta with warm (35°C) and oxygenated solutions in the following order: (1) Tyrode solution containing (in mmol/L) 135 NaCl, 4.6 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.4, for 5 min; (2) Ca^{2+}-free solution containing (in mmol/L) 100 NaCl, 30 KCl, 2 $MgCl_2$, 10 glucose, 10 HEPES, 15 taurine, and 5 pyruvate, pH 7.4, for 5 min; and (3) Ca^{2+}-free solution containing collagenase (120 units/mL) and albumin (2 mg/mL), for 20 min. At the end of the perfusion, the ventricles were minced and gently shaken for 10 min in the collagenase solution to release single cells. Only the quiescent myocytes with clear striations were used for this study.

Transmembrane voltages and currents were recorded using the whole-cell patch-clamp technique. Data were acquired and analyzed with an Axopatch-200 amplifier, a Digidata-1440A digitizer, and pCLAMP-10 software. All experiments were performed at 36°C.

For measurements of action potentials, cells were incubated in the Tyrode solution (bath solution). The recording pipettes were filled with a solution containing (in mmol/L) 120 K-aspartate, 20 KCl, 1 $MgSO_4$, 4 Na_2ATP, 0.1 Na_3GTP, and 10 HEPES, pH 7.3. A depolarizing pulse was applied every 6 sec to elicit action potentials. The APD was determined from the beginning of depolarization to the time when 30% (APD_{30}), 50% (APD_{50}), and 90% (APD_{90}) of repolarization were completed.

For measurements of I_{NaL}, myocytes were superfused with a bath solution containing (in mmol/L) 135 NaCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, 4.6 CsCl, 0.05 $NiCl_2$, and 0.01 nitrendipine, pH 7.4. The recording pipettes were filled with a solution containing (in mmol/L) 120 Cs-aspartate, 20 CsCl, 1 $MgSO_4$, 4 Na_2ATP, 0.1 Na_3GTP, and 10 HEPES, pH 7.2. Sodium current was activated by 200–250 msec long voltage-clamp pulses applied every 10 sec, from a holding potential of −90 mV to a test potential of −30 or −50 mV. The amplitude of I_{NaL} was calculated as the average amplitude of current during the last 100 msec of a depolarizing pulse.

GS967 was synthesized by Gilead Sciences. MTSEA was purchased from Toronto Research Chemicals, KN-93 and KN-92 from Calbiochem, AIP from Tocris, and ATX-II from Sigma. KN-93, KN-92, and AIP were applied through the recording pipette solution; other drugs were added to the bath solutions. The duration of each drug treatment was 3 min before recording.

Data are expressed as mean ± SEM. Sample size (n) is shown as number of cells/from number of hearts. Statistical analyses were conducted using SigmaPlot software. Concentration–response relationship and EC_{50} for GS967 inhibition of I_{NaL} were calculated from a standard four-parameter logistic curve fitted with the following equation:

$$y = \min + \frac{\max - \min}{1 + \left(\frac{x}{EC50}\right)^{-\text{Hillslope}}}$$

Coefficient of determination (R^2) was calculated from a standard linear regression curve fitted with the following model:

$$f = y^0 + a^* x$$

The *t*-test or one-way ANOVA followed by Holm–Sidak method was applied for statistical analysis. A $P < 0.05$ was considered statistically significant.

Results

Contribution of basal I_{NaL} to APD

To verify the action of GS967 as an I_{NaL} blocker, the effect GS967 on I_{NaL} induced by the I_{NaL} enhancer ATX-II was examined. In this series of experiments, I_{NaL} was activated by voltage-clamp pulses from −90 to −50 mV. ATX-II (5 nmol/L) increased the amplitude of I_{NaL} at −50 mV from −0.12 ± 0.01 to −0.47 ± 0.03 pA/pF (n = 24/9, $P < 0.001$). GS967 reversibly and concentration dependently inhibited the I_{NaL} in the presence of ATX-II. GS967 at concentrations of 0.1, and 0.3 μmol/L significantly ($P < 0.001$, n = 12/5) reduced the amplitude of ATX-II-stimulated I_{NaL} by 41 ± 2% and 93 ± 5%, respectively (Fig. 1). In another group of myocytes (n = 12/4), the ATX-II-stimulated I_{NaL} was inhibited by 0.03 and 1 μmol/L GS967 by 24 ± 3% and 100%, respectively ($P < 0.001$, not shown).

To estimate the amplitude of basal I_{NaL}, voltage-clamp pulses from −90 to −30 mV were applied to activate inward I_{Na}. The average amplitude of I_{NaL} at −30 mV was −0.24 ± 0.02 pA/pF (n = 40/17). GS967 at concentrations of 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μmol/L, respectively, concentration dependently reduced the amplitude of basal I_{NaL} by 18 ± 3, 28 ± 3, 38 ± 2, 46 ± 2, 82 ± 4, 91 ± 4, and 100% ($P < 0.05$, n = 10/3–5 for each concentration; Each myocyte was treated with 2–3 concentrations of GS967), with an IC_{50} of 0.46 μmol/L (Fig. 2, A and B). TTX at concentrations of 0.1, 1, and 10 μmol/L, respectively, significantly ($P < 0.001$) decreased the amplitude of I_{NaL} by 16 ± 2% (n = 13/4), 52 ± 4% (n = 13/4), and 94 ± 1% (n = 18/6; Fig. 2C and D), further confirming that the I_{NaL} was indeed an inward sodium current.

The baseline APD_{30}, APD_{50}, and APD_{90} measured from the myocytes were 198 ± 5 msec, 229 ± 6 msec, and 248 ± 6 msec, respectively (n = 43/14). GS967 at concentrations of 0.01 (n = 10/4), 0.1 (n = 25/10), and 1 (n = 19/7) μmol/L, respectively, significantly ($P < 0.003$) shortened the APD_{30} by 11 ± 2, 17 ± 1, and 27 ± 2%, APD_{50} by 9 ± 2, 16 ± 1, and 25 ± 1%, and APD_{90} by 8 ± 2, 14 ± 1, and 22 ± 1% (Fig. 3, A and B). TTX at concentrations of 0.1(n = 9/2), 1 (n = 15/4), and 10 (n = 15/3) μmol/L significantly ($P < 0.05$) decreased the APD_{30} by 8 ± 1, 24 ± 2, and 33 ± 2%, APD_{50} by 8 ± 1, 23 ± 2, and 31 ± 2%, and APD_{90} by 7 ± 1, 21 ± 2, and 29 ± 2%, respectively (Fig. 3, C and D).

There was a close correlation (R^2 = 0.958) between the percentage inhibition of basal I_{NaL} and the percentage shortening of APD caused by either GS967 or TTX (Fig. 4), indicating that basal I_{NaL} significantly contributes to the APD.

Inhibition of basal I_{NaL} by $Na_V1.5$ channel blocker

MTSEA is a selective blocker of $Na_V1.5$ channels (Haufe et al. 2005; O'Reilly and Shockett 2012). In this study, MTSEA (2 mmol/L) was added to the bath solution to

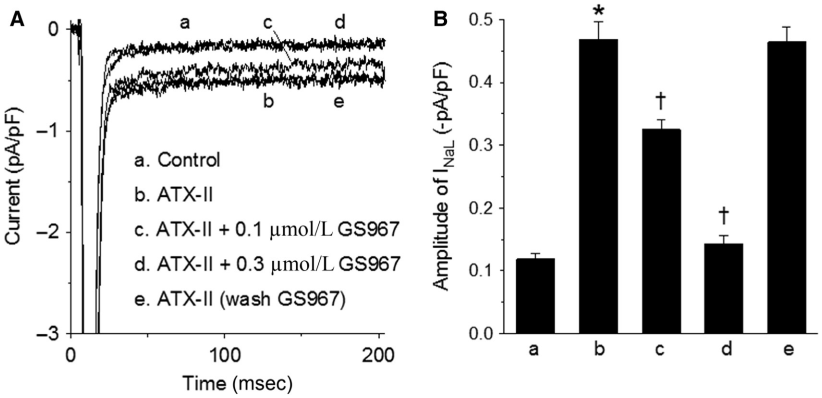

Figure 1. Concentration-dependent inhibition by GS967 of ATX-II (5 nmol/L)-induced I_{NaL}. Inward currents were activated by depolarizing pulses from −90 to −50 mV. Panel A, superimposed currents recorded in the order of a–e from a single myocyte before (control) and after drug treatments. Panel B, summary of the average amplitude of I_{NaL} recorded before (A) and after (B–E) drug treatments, as shown in panel A (n = 12/5). *$P < 0.001$ versus control; $^{\dagger}P < 0.001$ versus ATX-II alone.

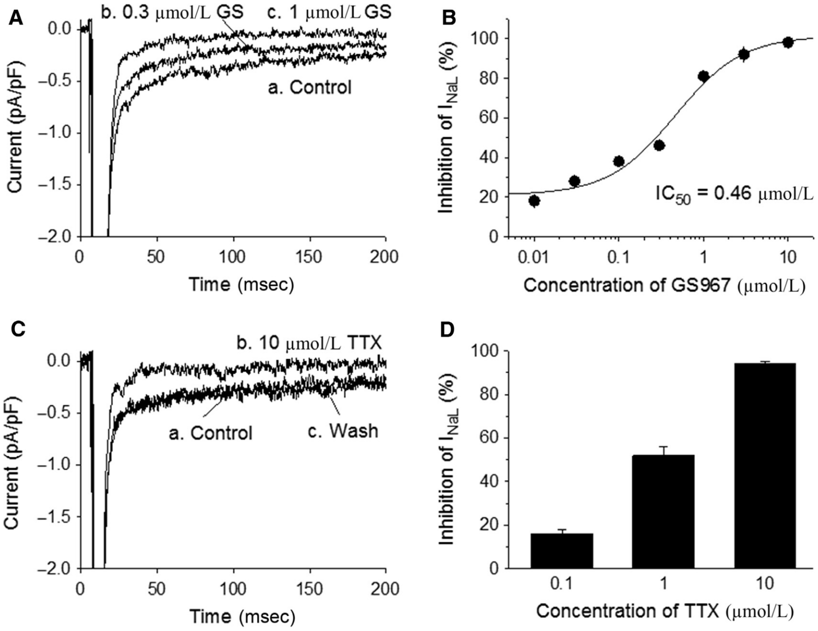

Figure 2. Concentration-dependent inhibition by GS967 or TTX of basal I_{NaL}. I_{NaL} was elicited by voltage-clamp pulses from −90 to −30 mV. Panel A, example of current traces recorded from a single myocyte in the absence of drugs (control) and in the presence of 0.3 and 1 µmol/L GS967 (GS). Panel B, concentration–response relationship of the inhibitory effect of GS967 on I_{NaL}. Each data point represents an average inhibition observed from 10 myocytes isolated from 3 to 5 hearts. Data points are fitted with a four-parameter logistic curve. Panel C, current traces recorded before (A) and after (B) application of TTX, and after washing out TTX (C). Panel D, bars show an average inhibition of I_{NaL} by 0.1 (*n* = 13/4), 1 (*n* = 13/4), and 10 (*n* = 18/6) µmol/L TTX, respectively.

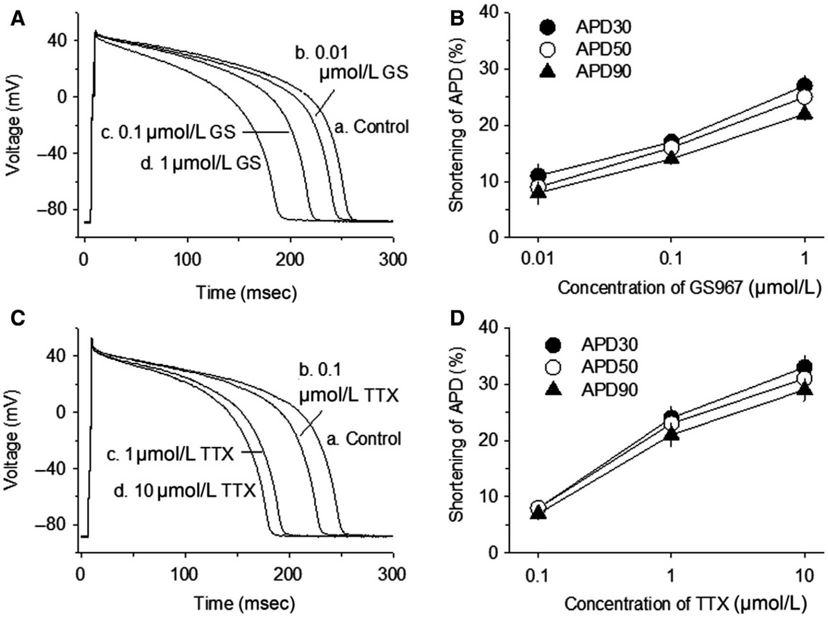

Figure 3. Concentration-dependent shortening by GS967 or TTX of the action potential duration (APD). Panel A, example of action potential traces recorded from a single myocyte before (control) and after applications of 0.01, 0.1, and 1 µmol/L GS967 (GS). Panel B, summary of APD shortening caused by GS967 at concentrations of 0.01 (*n* = 10/4), 0.1 (*n* = 25/10), and 1 (*n* = 19/7) µmol/L, respectively. Panel C, action potentials recorded from a myocyte in the absence of drug (control) and in the presence of 0.1, 1, and 10 µmol/L TTX. Panel D, average shortening of APD caused by TTX at concentrations of 0.1 (*n* = 9/2), 1 (*n* = 15/4), and 10 (*n* = 15/3) µmol/L.

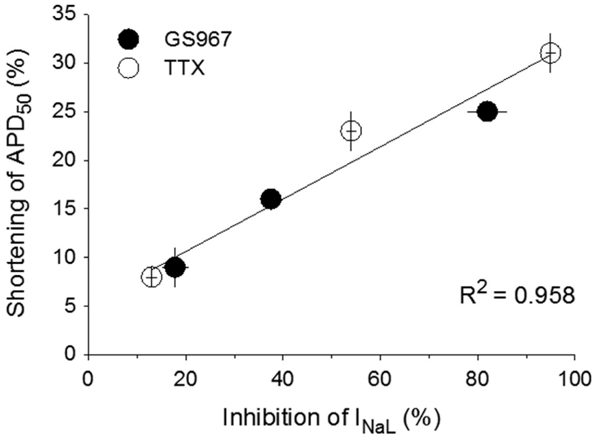

Figure 4. Correlation of APD shortening and I_{NaL} inhibition in the presence of GS967 (●) and TTX (○). The percentage shortenings of APD are plotted against the percentage inhibition of I_{NaL} caused by GS967 and TTX at the same concentrations. Coefficient of determination (R^2) is calculated using SigmaPlot linear regression curve analysis.

determine whether the basal I_{NaL} of myocytes was generated from the $Na_V1.5$ channels. I_{NaL} was activated by depolarizing pulses from −90 to −30 mV. In this series of experiments, MTSEA decreased the amplitude of I_{NaL} by 90 ± 5%, from −0.20 ± 0.03 to 0.03 ± 0.01 pA/pF ($n = 12/6$, $P < 0.001$; Fig. 5). The result suggests that under the experimental conditions, the $Na_V1.5$ channel is the major contributor to the I_{NaL} of guinea pig ventricular myocytes.

Decrease in basal I_{NaL} by CaMKII inhibitors

Activation of CaMKII was reported to slow sodium channel inactivation. We used the CaMKII inhibitors KN-93 and AIP, and an inactive analog of KN-93 and KN-92, as a negative control, to determine whether CaMKII plays a significant role in maintaining basal I_{NaL}. The three drugs were applied through the recording pipette solution to three separate groups of myocytes, respectively.

I_{NaL} was activated by voltage-clamp pulses from −90 to −30 mV. The amplitude of I_{NaL} measured in the absence of drugs was −0.24 ± 0.02 pA/pF. KN-93 (10 μmol/L) and AIP (2 μmol/L) reduced the I_{NaL} to −0.17 ± 0.02 pA/pF ($n = 11/3$, $P < 0.05$) and −0.17 ± 0.02 pA/pF ($n = 11/5$, $P < 0.05$), respectively (Fig. 6), whereas KN-92 (10 μmol/L) had no effect on I_{NaL} (−0.24 ± 0.02 pA/pF, $n = 12/4$; Fig. 6).

Discussion

This study revealed that the basal I_{NaL} is of sufficient magnitude to affect the duration of the action potential of ventricular myocytes isolated from healthy guinea pigs. In the presence of the I_{NaL} blocker GS967 or low concentrations of TTX, the reduction in I_{NaL} was closely correlated with the shortening of APD (Figs. 2–4). Furthermore, the study showed that the basal I_{NaL} of guinea pig ventricular myocytes was mainly generated from the $Na_V1.5$ channels (Fig. 5) and was regulated by CaMKII (Fig. 6). Thus, the results of the present study suggest that the basal, CaMKII-mediated $Na_V1.5$ I_{NaL} is a significant and physiological contributor to the action potential duration of guinea pig ventricular myocytes.

The action potentials of cardiac ventricular myocytes are characterized by a prominent plateau phase (phase 2) (Draper and Weidmann 1951). Repolarization is delayed during the plateau phase, and thus the duration of a myocardial action potential is largely determined by the length of the plateau phase. The action potential plateau is caused by a

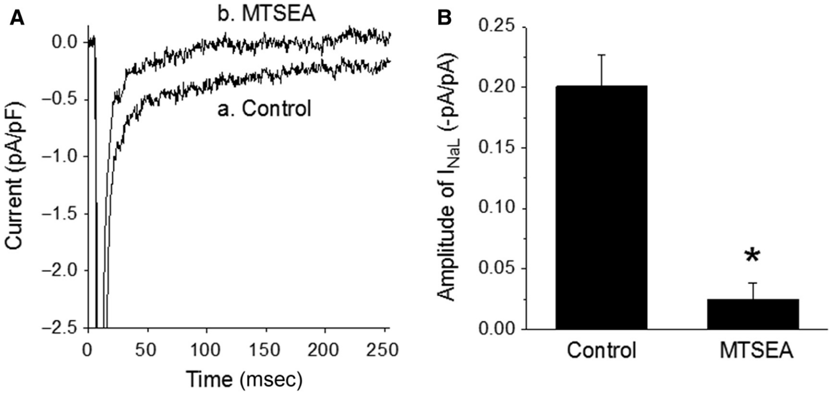

Figure 5. Inhibition of I_{NaL} by the selective $Na_V1.5$ channel blocker MTSEA (2 mmol/L). Panel A, currents recorded from a myocyte before (control) and after application of MTSEA. Panel B, summary of the results obtained from experiments shown in panel A. Bars represent the average amplitude of I_{NaL} determined from 12 myocytes isolated from six hearts. *$P < 0.001$ versus control.

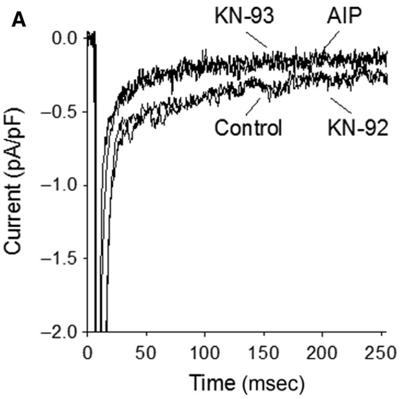

Figure 6. Decrease in I_{NaL} in the presence of CaMKII inhibitors. Panel A, representative current traces recorded from four myocytes treated with no drug (control), KN-93 (10 μmol/L), KN-92 (10 μmol/L), and AIP (2 μmol/L), respectively. Panel B, summary of the results obtained from experiments shown in panel A. Bars represent the average amplitude of I_{NaL} in control (n = 40/17) and in the presence of KN-93 (n = 11/3), KN-92 (n = 12/4), and AIP (n = 11/5). *$P < 0.05$ versus control.

balance between depolarizing currents, including the inward L-type (I_{CaL}) and T-type (I_{CaT}) Ca^{2+} current, Na^+–Ca^{2+} exchange current (I_{NCX}) and I_{NaL}, and repolarizing currents, such as the outward delayed rectifier K^+ current (I_K) (Ten Eick et al. 1992). The amplitude of I_{NaL} under physiological conditions is relatively small. In this study, the average amplitude of I_{NaL} was -0.24 ± 0.02 pA/pF. However, because I_{NaL} remains activated throughout the plateau phase and the membrane resistance is known to be high at the plateau (Weidmann 1951), even a small inward current such as I_{NaL} could play a significant role in maintaining cardiac depolarization, and thereby the duration of action potential. Myocytes used in this study were isolated from the whole ventricles; therefore, the present results represented the average effect of basal I_{NaL} on the duration of ventricular action potentials. As it has been reported that the density of I_{NaL} is greater in the mid-myocardium than that in the epi- and endomyocardium (Zygmunt et al. 2001), it will be interesting to know if there is a regional difference in the contribution of I_{NaL} to the APD among different parts of ventricular myocardium.

Assessment of the contribution of basal I_{NaL} to APD requires the use of an inhibitor that, at least at certain concentrations, selectively and concentration dependently reduces I_{NaL}, and has no effect on other ion currents that can modulate the APD. We used the I_{NaL} inhibitor GS967 at concentrations and conditions in which its inhibition was selective for the I_{NaL} (Belardinelli et al. 2013). For comparison, the Na^+ channel blocker TTX applied at low concentrations was used to confirm that the inward current recorded was a Na^+-channel current. The selectivity of GS967 to inhibit I_{NaL} has been studied using rabbit ventricular myocytes (Belardinelli et al. 2013). The results of that study showed that, at a holding potential of −120 mV and a stimulation frequency of 0.1–3 Hz, GS967 (0.1–5 μmol/L) concentration dependently blocked ATX-II-stimulated I_{NaL} without reducing the peak I_{Na}. In addition, GS967 (1–3 μmol/L) had no significant effect on I_{CaL}, I_{CaT}, and ATP-sensitive K^+ current, although GS967 at a high concentration of 10 μmol/L caused a small (17%) inhibition of the rapid component of I_K. In this study of guinea pig ventricular myocytes, GS967 concentration dependently inhibited ATX-II-induced I_{NaL} (Fig. 1), further confirming that this compound is a suitable pharmacological tool to investigate the role of I_{NaL} in cardiac repolarization. GS967 at 1 μmol/L blocked the ATX-II stimulated and the basal I_{NaL} by 100% and 82 ± 4%, respectively. Thus, it appears that the potency of GS967 to inhibit I_{NaL} is greater in the presence, than in the absence, of ATX-II. This could be due to a sensitization by ATX-II of sodium channels to the inhibitory action of GS967, as it has been found that sodium channel site-3 toxins (such as ATX-II) can enhance the binding and action of site-1 toxin (such as TTX) and local anesthetics on this channel (Nishio et al. 1991).

A contribution of basal I_{NaL} to the APD was suggested by a previous study (Kiyosue and Arita 1989). In that study, TTX at a concentration of 60 μmol/L caused a decrease in APD of ventricular myocytes isolated from healthy guinea pigs. However, TTX at such a high concentration could block not only the peak I_{Na}, but also the L- and T-type Ca^{2+} channels (Sun et al. 2008; Hegyi et al. 2012), which would also lead to a shortening of the APD. To verify the role of I_{NaL} in modulation of APD, we used the selective I_{NaL} blocker GS967 and low concentrations of TTX to determine the effect of an inhibition of basal I_{NaL} on the APD. Our results showed that GS967 and TTX at a concentration as low as 0.01 μmol/L and 0.1 μmol/L, respectively, could cause a significant shortening of the APD (Fig. 3). Furthermore, a quantitative analysis indicated that the inhibition of basal I_{NaL} and the shortening of APD caused by GS967 and TTX were closely correlated (Fig. 4).

$Na_V1.5$ channel has been recognized as the dominant sodium channel of ventricular myocytes (Gellens et al. 1992; Maltsev et al. 2008; Veerman et al. 2015). In addition to the $Na_V1.5$ channel, other sodium channel isoforms may also contribute to the cardiac sodium current. One study reported that A-803467, a $Na_V1.8$ channel blocker, blocked I_{NaL} of mouse and rabbit ventricular myocytes, suggesting that $Na_V1.8$ channel contributes to cardiac I_{NaL} ((Yang et al. 2012). In contrast, another study found that A-803467 had no effect on sodium current of mouse ventricular myocytes (Verkerk et al. 2012). In this study, the selective $Na_V1.5$ channel blocker MTSEA (Haufe et al. 2005; O'Reilly and Shockett 2012) decreased the amplitude of basal I_{NaL} by 90 ± 5%, indicating that under the conditions of our experiments, the $Na_V1.5$ channel isoform is a major contributor to basal I_{NaL} of guinea pig ventricular myocytes.

Cardiac myocytes overexpressing CaMKII showed an enhanced I_{NaL} (Wagner et al. 2006). In this study, we investigated the role of CaMKII in regulating basal I_{NaL} by comparing the amplitude of I_{NaL} in the absence and presence of the CaMKII inhibitor KN-93 or AIP. Because KN-93 and its inactive analog KN-92 may have CaMKII-independent effects on ion channels if applied extracellularly (Rezazadeh et al. 2006), these drugs and AIP were applied intracellularly through the pipette solution. Our results showed that the amplitude of basal I_{NaL} was decreased by either KN-93 or AIP, but not by KN-92, suggesting a significant role of CaMKII in regulating cardiac I_{NaL} under basal conditions (Fig. 6). However, CaMKII phosphorylation may not be the only mechanism to maintain basal I_{NaL}. Other mechanisms, such as protein kinase C (Ma et al. 2012), may also be involved in the regulation of basal I_{NaL}.

In summary, in this study we investigated the role of basal I_{NaL} in modulating the cardiac APD, by quantitatively determining the relationship between the amplitude of I_{NaL} and the duration of action potential. The results showed a close correlation between a decrease in I_{NaL} and a shortening of the APD, and thus provide strong evidence that basal endogenous I_{NaL} is a significant contributor to the APD of cardiac myocytes. The present results also demonstrated that the basal I_{NaL} of guinea pig ventricular myocytes is mainly generated from $Na_V1.5$ channel isoform and is regulated by CaMKII.

Conflict of Interest

Y. Song received a research grant from Gilead Sciences; L. Belardinelli was an employee of Gilead Sciences at the time of this study.

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