Swelling-activated chloride current is activated in guinea pig cardiomyocytes from endotoxic shock

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Abstract

Objective: Myocardial swelling occurs during endotoxic shock. The hypothesis that swelling-activated Cl\textsuperscript{−} current (\(I_{\text{Cl,swell}}\)) activates during endotoxic shock was tested.

Methods: Endotoxic shock was induced by intravenous lipopolysaccharides (10 mg/kg) in guinea pigs. The effects of \(I_{\text{Cl,swell}}\) blockers on the cardiac action potentials in papillary muscles and on the \(I_{\text{Cl,swell}}\) in single ventricular myocytes were tested.

Results: Action potential duration (APD) at 90% of repolarization (APD\textsubscript{90}) was significantly shortened after 5-h endotoxic shock in guinea pig papillary muscles. \(I_{\text{Cl,swell}}\) blockers, 9-anthracene carboxylic acid (9-AC) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), dose-dependently prolonged the shortened APD\textsubscript{90}. Inducible nitric oxide synthase (iNOS) inhibitors, L-N\textsubscript{6}-(1-iminoethyl) lysine (L-NIL) and N-[3-(aminomethyl)phenyl][methyl]-ethanimidamide (1400 W), also prolonged the APD\textsubscript{90}. Protein kinase C (PKC) activators, 4\textsuperscript{6}-phorbol 12-myristate 13-acetate (PMA) and phorbol 12,13-didecanoate (PDD), also prolonged the APD. The addition of glibenclamide (an ATP-sensitive K\textsuperscript{+} channel blocker) on top of these \(I_{\text{Cl,swell}}\) blockers hastened the recovery of APD\textsubscript{90} compared to the use of \(I_{\text{Cl,swell}}\) blockers alone. Whole-cell voltage-clamp study in single ventricular myocytes from endotoxic shock heart disclosed activation of a DIDS- and 9-AC-sensitive current. These currents displayed outward rectification with reversal potentials similar to the calculated Nernst potential for Cl\textsuperscript{−}. The reversal potentials tracked the \(E_{\text{Cl}}\) closely when the Cl\textsuperscript{−}/C\textsubscript{0} gradient was changed, suggesting that Cl\textsuperscript{−}/C\textsubscript{0} was the major charged carrier.

Conclusions: We have shown for the first time that \(I_{\text{Cl,swell}}\) activates in guinea pig heart in endotoxic shock. The change in this membrane current, together with the activation of ATP-sensitive K\textsuperscript{+} current, contributes to the electrophysiological derangement in endotoxic shock.

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1. Introduction

The hemodynamic changes in endotoxic shock were characterized by decreased vascular tone and depressed myocardial function [1]. Myocardial dysfunction was mainly caused by increased nitric oxide production and related to shortened action potential duration (APD) [2,3]. The ionic mechanisms responsible for the shortened APD were not fully understood. Decreased L-type Ca\textsuperscript{2+} channel currents was one of the proposed mechanisms [4]. We recently observed in guinea pig model that after 6-h endotoxic shock, the shortened APD recorded from papillary muscle could persist for more than 1 h in vitro, and glibenclamide could hasten the process of the recovery of APD to the control level, suggesting a role of ATP-sensitive K\textsuperscript{+} channel in the shortening of APD during endotoxic shock [3]. However, it took about 1 h to achieve the restoration of APD by glibenclamide. It is possible that other ionic mechanisms are involved.

Activation of swelling-activated Cl\textsuperscript{−} current (\(I_{\text{Cl,swell}}\)) contributes to the shortening of APD during cell swelling in guinea pig ventricular myocytes [5], and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) partially antagonized the APD-shortening effect of cell swelling [5]. We
hypothesized that $I_{\text{Cl,swell}}$ activates during endotoxic shock and contributes to APD shortening.

In the present study, we used 9-anthracene carboxylic acid (9-AC) and DIDS to explore the role of $I_{\text{Cl,swell}}$ in endotoxic shock. We found that during endotoxic shock, $I_{\text{Cl,swell}}$ was activated in ventricular myocytes from guinea pig and might play a role in the APD-shortening and myocardial dysfunction.

2. Methods

This study conforms to NIH Guidelines for the Care and Use of Laboratory Animals. Throughout the studies, all efforts were taken to minimize animal pain and suffering. Adult Hartley guinea pigs (300–450 g) of either sex were used in this study.

2.1. Lipopolysaccharide-induced endotoxic shock

We used the lipopolysaccharide (LPS)-induced endotoxic shock model in guinea pigs, which has been described previously [3]. In brief, guinea pigs were anesthetized with urethane (1.2 g/kg, intraperitoneal injection). Escherichia coli LPS (serotype 0127:B8) (10 mg/kg) was injected via right femoral vein [3,6]. Sham experiments were performed in the same way as the LPS-treated ones except that LPS was not given.

2.2. Recording of cardiac action potentials

Guinea pigs were sacrificed by cervical dislocation at the fifth hour after injection of LPS. A strand of free-running papillary muscle from left ventricle was dissected, mounted into a 2-ml perfusion chamber, and perfused with Tyrode solution oxygenated with a gas mixture of 3% CO$_2$ and 97% O$_2$ at 37 °C. The composition of normal Tyrode solution was (in mM) NaCl, 137, KCl, 4, CaCl$_2$, 1.8, MgCl$_2$, 0.5, NaH$_2$PO$_4$, 1, NaHCO$_3$, 12, and glucose, 5, with pH titrated to 7.4. Conventional microelectrode technique applied to multicellular preparation from guinea pig papillary muscles was performed [3,7,8]. Borosilicate glass micropipettes were made by a pipette puller (David Kopf, Model 720, Tujunga, CA, USA). The tip resistance of pipette was around 20–40 MΩ when filled with 3 M KCl pipette solution. The tissue preparation is electrically compensated by 60–90%. Square-wave electrical stimulations with pulse duration of 2 ms and a frequency of 1 Hz were delivered from the stimulator (model S88, Grass, W. Warwick, USA). The resting membrane potential (RMP), $V_{\text{max}}$ of phase 0 upstroke of action potential (obtained by a differentiator), and action potential duration at 90% repolarization (APD90) were recorded. Data were also recorded by a digital data recorder (model VR-10B, Instrutech, Great Neck, NY, USA) for off-line analysis.

Action potentials were continuously monitored for 60 min without any pharmacological intervention (control) or under perfusion of different concentrations of $I_{\text{Cl,swell}}$ blockers: 9-AC [9] and DIDS [10], to examine their effects on the action potentials. The role of inducible nitric oxide synthase (iNOS) in the shortening of the APD was also investigated by using specific iNOS inhibitors: L-N$^\omega$-(1-iminoethyl)lysine (L-NIL) [11] and N-[3-(aminomethyl)phenyl]methyl]-ethanimidamide (1400 W). We also tested the role played by protein kinase C (PKC) activation in the APD shortening in the endotoxic shock by using 4β-phorbol 12-myristate 13-acetate (PMA) and phorbol 12,13-didecanoate (PDD) [10,12].

2.3. Voltage-clamp experiments

Enzymatic digestion method was used to isolate single ventricular myocytes from the papillary muscles of guinea pig left ventricle after 5-h endotoxic shock [13,14]. Guinea pigs were killed by cervical dislocation. The heart was immediately excised. After the aorta was cannulated, the heart was mounted onto a Langendorff apparatus. All solutions used for the cell isolation procedure were oxygenated and maintained at 37 °C. The hearts were perfused retrogradely with normal Tyrode solution with the following composition (in mM): NaCl, 137, KCl, 4, CaCl$_2$, 1.8, MgCl$_2$, 0.5, HEPES, 10, and glucose, 5.5 (pH=7.4, titrated with NaOH). Immediately after the blood was washed out, the perfusate was switched to the nominally calcium-free Tyrode solution for 5 min. Subsequently, nominally calcium-free Tyrode solution containing 1 mg/ml collagenase (Class 2, Worthington, NJ, USA) and 0.04 mg/ml protease (type XIV, Sigma, St. Louis, MO, USA) were perfused for 2–10 min. The perfusion solution was then shifted to low-calcium (0.18 mM) enzyme-free Tyrode solution for 5 min. The papillary muscle was dissected into small pieces and gently agitated. The dispersed cells were stored in normal Tyrode solution, and only the quiescent ones with clear striation were used. We performed single-pipette, whole-cell, voltage-clamp technique with a patch clamp amplifier (Axopatch 1-D, Axon, Foster City, USA) to record membrane currents [15,16]. All experiments were performed at room temperature ($=22$ °C) [9]. Cell capacitance was measured by integrating the capacitive transient evoked by applying a 5-mV hyperpolarizing step (from −40 to −45 mV) [12,16]. The cell capacitance and series resistance were electrically compensated by 60–90%.

The $I_{\text{Cl,swell}}$ was recorded by stepped voltage-clamp pulses (200 ms) between 80 and −80 mV in 20-mV steps from a holding potential of −80 mV. The effects of different $I_{\text{Cl,swell}}$ blockers were examined. The modified Tyrode solution for perfusion contains (in mM) NaCl, 130, CsCl, 5.4, MgCl$_2$, 0.5, CdCl$_2$, 1, HEPES, 10, glucose, 5, ouabain, 20 μM, and verapamil, 0.02, with a pH of 7.4 (titrated with CsOH). The pipette solution contains (in mM) CsCl, 110, TEA-Cl, 20, MgATP, 5, EGTA, 20, creatine, 5, and HEPES.
10, with a pH of 7.2 (titrated with CsOH). To test if Cl⁻ is the charged carrier of the current, we replaced part of the CsCl in the pipette solution with Cs-aspartate to measure the reversal potential and to calculate the Nernst potential for Cl⁻ ($E_{Cl} = RT/F \ln[C_i]/[C_o]$) in some experiments [17]. The bath was grounded via a 3 M KCl agar bridge. The final osmolarity of both solutions was between 292 and 296 mOsm/l.

2.4. Drugs and data analyses

E. coli LPS (serotype 0127:B8), 9-AC, DIDS, PMA, PDD, 4α-PDD, and glibenclamide were purchased from Sigma. L-NIL and 1400 W were purchased from Tocris (Northpoint, Avonmouth, UK). Lipo polysaccharide, DIDS, L-NIL, and 1400 W were dissolved in normal saline, while other agents were dissolved in DMSO. The final concentration of DMSO in the perfusate was <0.1%.

All values are expressed as mean±S.E.M. Two-way ANOVA was used to analyze the experimental data, with one way being between groups and the other way being within group. If significance was found, a post hoc analysis (Newman–Keul’s) was used. The difference was considered statistically significant when P value was less than 0.05.

3. Results

3.1. Effects of 9-AC on the action potentials in endotoxic shock

After 5-h endotoxic shock, the APD₉₀ of LPS-treated guinea pigs was significantly shortened compared to those receiving sham experiments (146.2±2.3 vs. 195.1±1.9 ms, P<0.001). In the following 60-min perfusion of various drugs or normal Tyrode solution (defined as control), the APD₉₀ was continuously monitored for a total of 60 min. Fig. 1A shows the original action potential tracings from guinea pig papillary muscles. The APD₉₀ was progressively prolonged by 9-AC, and the effect was concentration dependent. The summary of the changes by 9-AC was shown in Fig. 1B. The APD₉₀ also prolonged in the control group, but to a much lesser extent than that by 9-AC. The RMP did not significantly change in the control (0 min=86.4±1.2 mV vs. 60 min=85.7±1.5 mV; P>0.05), nor by 9-AC (for 300 μM, 0 min=86.2±1.3 mV vs. 60 min=86.5±1.4 mV; for 1 mM, 0 min=86.1±1.1 mV vs. 60 min=86.8±1.3 mV; for 3 mM, 0 min=86.0±1.3 mV vs. 60 min=87.2±1.5 mV; all P>0.05). The $V_{\text{max}}$ was not significantly changed in the control (0 min=208.1±6.8 V/s vs. 60 min=204.2±6.4 V/s; P>0.05), nor in those receiving 9-AC (for 300 μM, 0 min=204.5±5.2 V/s vs. 60 min=209.2±7.6 V/s; for 1 mM, 0 min=202.5±4.8 V/s vs. 60 min=213.2±8.9 V/s; for 3 mM, 0 min=208.2±7.2 V/s vs. 60 min=216.6±9.6 V/s; all P>0.05). There was a trend for the RMP to become more negative, and the $V_{\text{max}}$ became larger with higher doses of 9-AC.

3.2. Effects of DIDS on the action potentials in endotoxic shock

DIDS had similar effects as 9-AC. Fig. 2A shows the action potential tracings from guinea pig papillary muscle. DIDS dose-dependently prolonged APD₉₀. Fig. 2B was the summary of the effects by DIDS. The APD₉₀ also pro-
longed in the control group, but to a much lesser extent than that by DIDS. The RMP did not change significantly in the control group (0 min=−85.6±2.4 mV vs. 60 min=−86.1±2.1 mV; P>0.05), nor by DIDS (for 100 µM, 0 min=−85.8±1.6 mV vs. 60 min=−86.1±2.4 mV; for 300 µM, 0 min=−86.2±1.6 mV vs. 60 min=−86.8±1.5 mV; for 1 mM, 0 min=−86.1±1.7 mV vs. 60 min=−87.7±2.5 mV; all P>0.05). The V_max was not significantly changed in the control (0 min=201.9±3.8 V/s vs. 60 min=203.1±4.9 V/s; P>0.05), nor in those receiving DIDS (for 100 µM, 0 min=205.8±5.2 V/s vs. 60 min=210.3±6.6 V/s; for 300 µM, 0 min=208.7±5.9 V/s vs. 60 min=217.8±9.4 V/s; for 1 mM, 0 min=207.9±7.3 V/s vs. 60 min=221.7±11.7 V/s; all P>0.05). Similarly, there was a trend for the RMP to become more negative, and the V_max became larger with higher doses of DIDS.

3.3. The effects of $I_{Cl,swell}$ blockers on basal APD90

Fig. 3 shows the effects of 9-AC and DIDS on basal APD90. The baseline value (196.7±1.5 ms) is that before the sham operation. There was no significant change after the sham operation (197.7±2.1 ms). In the following 60-min perfusion with normal Tyrode solution containing $I_{Cl,swell}$ blockers: 9-AC and DIDS, the APD90 prolonged to 223.3±4.6 ms (9-AC, 3 mM) and to 224.8±4.6 ms (DIDS, 1 mM), suggesting a basal activation of $I_{Cl,swell}$ in guinea pig ventricular myocytes.

3.4. Effects of iNOS inhibitors on the action potentials in endotoxic shock

In order to define the involvement of iNOS in the action potential shortening in endotoxic shock, we used specific iNOS inhibitors, L-NIL and 1400 W, in this study. L-NIL (30 µM) and 1400 W (10 µM) had no effect on the APD90 in the sham group (Fig. 4), suggesting that there is no basal iNOS activity, or that basal iNOS activity did not play a role in the APD modulation. In the papillary muscle from guinea pig after 5-h endotoxic shock, 60-min perfusion of 30 µM L-NIL significantly prolonged the APD90 to a similar level as that in
the sham group (Fig. 4). Lower concentration of L-NIL (3 μM) was less effective. Similarly, 1400 W, another specific iNOS inhibitor, concentration-dependently prolonged APD90 in papillary muscles from endotoxic shock guinea pigs. The number of experiment was shown in the parentheses. *P<0.05 vs. LPS-treated group; †P<0.01 vs. LPS-treated group.

3.5. The role of PKC in the APD shortening in endotoxic shock

To assess the role of PKC activation in the APD shortening in endotoxic shock, phorbol esters, including PMA and PDD, were used in the present study. As shown in Fig. 5, 1 μM PMA significantly prolonged the APD90. PDD (1 μM) had similar effect. 4α-PDD, which has similar chemical structure as PDD but is ineffective in activating PKC, could not prolong APD90. These findings suggest that PKC activators are capable of prolonging APD, possibly through their inhibitory effect on I_{Cl,swell}.

3.6. Additive effects of I_{Cl,swell} blockers and glibenclamide on the action potentials in endotoxic shock

We previously reported that ATP-sensitive K+ channel current activated during endotoxic shock and contributed partly to the APD-shortening phenomenon [3]. We therefore tested if there is additive effect of blockers of I_{Cl,swell} and ATP-sensitive K+ current in reversing the shortening of APD. Fig. 6 shows that the time needed for the APD90 to

![Fig. 4. Effects of iNOS inhibitors on the APD90 in endotoxic shock. Specific iNOS inhibitors, L-NIL and 1400 W, had no effect on the APD90 in the sham group. In contrast, L-NIL and 1400 W dose-dependently prolonged APD90 in papillary muscles from endotoxic shock guinea pigs. The number of experiment was shown in the parentheses. *P<0.05 vs. LPS-treated group; †P<0.01 vs. LPS-treated group.

Fig. 5. The role of PKC in APD shortening in endotoxic shock. The number of experiment was shown in the parentheses. +P<0.01 vs. LPS-treated group.

Fig. 6. Additive effects of I_{Cl,swell} blockers and glibenclamide on the action potentials in endotoxic shock. Glibenclamide hastened the recovery of APD90, and the time needed for the APD90 to reach 190 ms is significantly shorter than I_{Cl,swell} blockers alone. n=16 and 10 for 3 mM 9-AC and 3 mM 9-AC+100 μM glibenclamide, respectively; n=15 and 11 for 1 mM DIDS and 1 mM DIDS+100 μM glibenclamide, respectively. *P<0.05.
recover to 190 ms was abbreviated further by the addition of glibenclamide to $I_{\text{Cl,swell}}$ blockers, suggesting that both $I_{\text{Cl,swell}}$ and ATP-sensitive K$^+$ channel current contribute to the APD shortening in endotoxic shock.

3.7. $I_{\text{Cl,swell}}$ activated in ventricular myocytes in endotoxic shock

The cell capacitance of the isolated ventricular myocytes from guinea pigs undergoing endotoxic shock was larger than those from the sham operation ($268.3\pm10.3$ vs. $224.1\pm8.1$ pF, $P<0.01$; $n=26$ and 30, respectively). Fig. 7 shows that time-independent currents were recorded in myocytes from endotoxic shock (panel (a)). These currents were sensitive to 1 mM DIDS (panel (b)), and the DIDS-sensitive currents (panel (c)) showed outward rectification (panel (d)). DIDS was more effective in inhibiting the outward current than the inward current. The reversal potential of the DIDS-sensitive currents was $-2.2$ mV, very close to the calculated $E_{\text{Cl}}$ ($-1.4$ mV). When we changed the chloride concentration of the pipette solution, the reversal potentials tracked the $E_{\text{Cl}}$, indicating Cl$^-$ is the major charged carrier (Fig. 8). Fig. 9 shows that these time-independent currents (panel (a)) induced in myocytes from endotoxic shock were also sensitive to 3 mM 9-AC (panel (b)). The 9-AC-sensitive currents (panel (c)) also demonstrated outward rectification characteristic (panel (d)).

Fig. 7. Original current tracings recorded in ventricular myocytes from endotoxic shock guinea pigs ($n=26$). (a) Currents were recorded by stepped voltage clamp pulses (200 ms) between 80 and $-80$ mV in 20-mV steps from a holding potential of $-40$ mV; (b) residual currents after 1 mM DIDS; (c) DIDS-sensitive currents (a–b) showing a time-independent characteristic; (d) Current–voltage ($I$–$V$) curve. The DIDS-sensitive current showed outward rectification. The reversal potential ($-2.2$ mV) was very close to the calculated $E_{\text{Cl}}$ ($-1.4$ mV), suggesting that this DIDS-sensitive current, which was activated in endotoxic shock, was an $I_{\text{Cl,swell}}$. ■: currents from panel (a); ●: currents from panel (b); ○: currents from panel (c).

Fig. 8. The correlation of the reversal potentials with the $E_{\text{Cl}}$. When the Cl$^-$ concentrations in the pipette solution were changed, the reversal potentials tracked the $E_{\text{Cl}}$ closely. The number in the parentheses is the cell number.

Fig. 9. Original current tracings recorded in ventricular myocytes from endotoxic shock guinea pigs ($n=16$). (a) Currents were recorded by stepped voltage clamp pulses (200 ms) between 80 and $-80$ mV in 20-mV steps from a holding potential of $-40$ mV; (b) residual currents after 3 mM 9-AC; (c) 9-AC-sensitive currents (a–b) showing a time-independent characteristic; (d) Current–voltage ($I$–$V$) curve. The 9-AC-sensitive current showed outward rectification. ■: currents from panel (a); ●: currents from panel (b); ○: currents from panel (c).
4. Discussion

We have shown for the first time that cardiac $I_{Cl,swell}$ activates during endotoxic shock. The change in this membrane current may contribute to the electrophysiological and contractile derangement in endotoxic shock.

Histomorphometrical analysis in canine endotoxic shock revealed myocardial cell swelling and capillary compression [18]. In a porcine model of septic shock, created by intermitted application of E. coli LPS, the earliest lesions were ischemic alterations of the muscle fibers with myocardial swelling [19]. The breakdown of high-energy phosphates and macromolecules increases the osmolarity of the extracellular space and causes cellular swelling [19]. Similar findings occur in myocardial ischemia and reperfusion [21,22]. In the present study, the cell capacitance of myocytes from endotoxic shock was about 20% larger than that from sham operation, suggesting an increase in cell volume.

$I_{Cl,swell}$ is stimulated by an increase in cell volume. Cardiac $I_{Cl,swell}$ was initially identified by Tseng [23] and is expressed throughout the heart in many species. The current is time independent over the physiologic voltage range [12,24] and outwardly rectifying with either a physiologic [25] or a symmetric [17] $Cl^-$ gradient. $I_{Cl,swell}$ reverses near $E_{Cl}$ and tracks the $Cl^-$ gradient almost ideally. The current, which was activated by endotoxic shock in this study, has several characteristics. They were (1) dependent on $Cl^-$ gradient with the reversal potential near $E_{Cl}$, (2) exhibited outward rectification, and (3) were sensitive to $I_{Cl,swell}$ blockers. These features match those of $I_{Cl,swell}$ described by others [10,23].

At positive membrane potentials, $I_{Cl,swell}$ is outwardly rectifying and contributes to enhancement of the plateau phase (phase 2) and the rapid repolarization phase (phase 3) of the action potential and will thus shorten the action potential [5,9]. The shortening of the action potential could be attenuated by $I_{Cl,swell}$ blocker, such as DIDS [5]. In single cardiomyocytes isolated from the LPS-treated rabbits, $I_{Cl,swell}$ was persistently activated in ventricular myocytes isolated from a rabbit aortic regurgitation model [9,27]. The current is also fully turned on in ventricular myocytes isolated from a rabbit aortic regurgitation model of dilated cardiomyopathy [10] or from a rat aortic constriction model of ventricular hypertrophy [28]. In LPS-treated rats, $Cl^-$ conductance increased in hepatocytes, and the voltage dependence and inhibitor specificity of this conductance were similar to that of a swelling-activated $Cl^-$ conductance [29].

Previously, we have shown that in guinea pigs undergoing endotoxic shock, the increase in plasma nitrate and tissue cGMP content correlated with the decrement of the APD in papillary muscles [3]. In the present study, we further demonstrated that specific iNOS inhibitors, L-NIL and 1400 W, prolonged the APD. Swelling-activated $Cl^-$ current can be modulated by NO and NO donors in rabbit portal vein myocytes through cGMP-dependent and cGMP-independent mechanisms [30]. The detail-controlling mechanisms in guinea pig ventricular myocytes remain to be determined.

It is proposed that $I_{Cl,swell}$ is regulated by the balance between PKC-mediated phosphorylation of the channel and its dephosphorylation by serine/threonine protein phosphatases [31]. Inhibition of $I_{Cl,swell}$ by PKC activation was observed in guinea pig atrial and ventricular myocytes [32]. In rabbit atrial myocytes, PDD, but not 4α-PDD, produced strong inhibition of $I_{Cl,swell}$ [12]. Furthermore, we found that PKC activators, PMA and PDD, but not 4α-PDD, could reverse the APD shortening in endotoxic shock, in line with the hypothesis that PKC-mediated phosphorylation can inhibit $I_{Cl,swell}$.

In the present study, we measured the APD90 from papillary muscles by conventional microelectrode technique instead of from single ventricular myocytes by current clamp technique. This is based on the following reasons. First, for a given osmotic perturbation, isolated myocytes swell to a greater extent than do myocytes in intact tissue [20], i.e., 50–60% of predicted for isolated myocytes [33,34] compared to 25–30% for cells in situ [35]. This is because of the space constraints imposed by the relatively inelastic extracellular matrix [35,36] and pericardium. Since the extent of activation of $I_{Cl,swell}$ correlates with the extent of cellular swelling [9,20], it is likely that the actual magnitude of $I_{Cl,swell}$ activation in intact tissue is less than that from isolated cells. It seems appropriate to measure the APD90 from multicellular preparation such as papillary muscles. Second, the model-simulated action potential changes responding to stretch correlate better with action potential changes in guinea pig papillary muscle than with those from isolated myocytes [37]. For example, the resting APD90 for guinea pig ventricle calculated from modeling is 193 ms [37], very close to what we recorded from papillary muscle. On the other hand, the APD90 recorded by current clamp technique in isolated guinea pig ventricular myocytes ranged from 154 to 748 ms [5,38–40].

In the present study, we have demonstrated that $I_{Cl,swell}$ blockers prolonged APD90 in sham group, suggesting a basal activation of $I_{Cl,swell}$. This might explain why the...
final APD90 after 60-min perfusion of $I_{Cl,swell}$ blockers were longer than the averaged value from an intact guinea pig ventricle (about 190 ms when stimulated with 1 Hz) [16]. Indeed, basal activation of $I_{Cl,swell}$ has been reported in both atrial and ventricular myocytes [12,23,25]. In addition, $I_{Cl,swell}$ is the primary basal Cl$^-$ conductance [10].

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References


