Effect of simulated $I_{to}$ on guinea pig and canine ventricular action potential morphology

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Dong, Min, Xiaoyin Sun, Astrid A. Prinz, and Hong-Sheng Wang. Effect of simulated $I_{to}$ on guinea pig and canine ventricular action potential morphology. Am J Physiol Heart Circ Physiol 291: H631–H637, 2006. First published March 24, 2006; doi:10.1152/ajpheart.00084.2006.—The transient outward current ($I_{to}$) is a major repolarizing current in the heart. The influence of $I_{to}$ density on action potential duration (APD) prolongation. To understand the species-dependent role of $I_{to}$ in regulating the ventricular action potential morphology and duration, we simulated $I_{to}$ conductance in guinea pig and canine endocardial ventricular myocytes using the dynamic clamp technique and perforated patch-clamp recordings. The effects of simulated $I_{to}$, in both types of cells were complex and biphasic, separated by a clear density threshold of $\sim 40$ pA/Pf. Below this threshold, simulated $I_{to}$ resulted in a distinct phase 1 notch and had little effect on or moderately prolonged the APD. $I_{to}$ above the threshold resulted in all-or-none repolarization and precipitously reduced the APD. Qualitatively, these results agreed with our previous studies in canine ventricular cells using whole cell recordings. We conclude that 1) contrary to previous gene transfer studies involving the Kv4.3 current, the response of guinea pig ventricular myocytes to a fully inactivating $I_{to}$ is similar to that of canine ventricular cells and 2) in animals such as dogs that have a broad cardiac action potential, $I_{to}$ does not play a major role in setting the APD.

The transient outward potassium current ($I_{to}$) is a key repolarizing current in the heart. The influence of $I_{to}$ on cardiac action potential waveform and duration is remarkably dependent on species. In smaller animals such as mouse and rat, $I_{to}$ is the dominant repolarizing current, and the high levels of $I_{to}$ in these animals are mostly responsible for the short duration and triangular shape of the action potential (2, 23, 27). In large animals, including humans, it is well known that $I_{to}$ is responsible for phase 1 repolarization of the action potential. However, the influence of $I_{to}$ on action potential duration (APD) is less clear. The use of a pharmacological approach in studying the problem is hampered by the lack of specific $I_{to}$ blockers. An alternative method, mathematical simulation, has produced somewhat inconsistent results, predicting that physiological levels of $I_{to}$ do not affect (5, 34) or significantly prolong (11) the APD.

In an earlier study, we used the dynamic clamp technique to study the role of $I_{to}$ in shaping the action potential in canine left ventricle (27). The dynamic clamp combines computer simulation with experimental electrophysiology and allows the introduction of programmable artificial conductances in living cells. We have shown that $I_{to}$, while being a key regulator of phase 1 repolarization, does not play a major role in regulating the APD over a wide current density range that encompasses the $I_{to}$ densities found in canine ventricular cells. At densities above a threshold (a value that is much higher than the physiological $I_{to}$ levels in canine ventricle), $I_{to}$ precipitously shortens the APD and results in all-or-none repolarization.

Our study also suggests that the difference in the effect of $I_{to}$ on action potential morphology in small and large animals is due, at least partially, to the dramatic difference in $I_{to}$ density in these species (27). Interestingly, our results in canine ventricular cells contrast with previous studies (9, 10) in guinea pig ventricular myocytes. Guinea pigs are unusual in their lack of native $I_{to}$ in the heart (4, 36), and in the studies by Hoppe et al., exogenous Kv4.3 current was introduced into guinea pig ventricular myocytes by either gene transfer or cell fusion techniques. Introduction of such an $I_{to}$-type current did not generate a phase 1 notch but progressively suppressed the action potential plateau and shortened the APD over the same $I_{to}$ density range as what we have tested in canine ventricular cells. The strikingly different observations in canine and guinea pig ventricular cells raise the possibility that guinea pig cells are unique not only in their total lack of native $I_{to}$ but also in their electrophysiological response to an exogenous $I_{to}$. It is plausible that due to the peculiar electrophysiological profile of the guinea pig cells, the threshold for all-or-none repolarization in these cells is much lower. As a result, the presence of even low to moderate densities of $I_{to}$ suppresses the development of the action potential plateau and shortens the APD. To test this possibility and to further understand the species-dependent role of $I_{to}$ in shaping the action potential, we performed in this study dynamic clamp simulations of $I_{to}$ in guinea pig ventricular cells.

A notable limitation of our earlier study (27) is the use of the whole cell patch clamp and intracellular Ca$^{2+}$ buffer, which can alter Ca$^{2+}$ intracellular handling and affect the action potential properties. In the present study, to preserve physiological intracellular Ca$^{2+}$ handling, we used the perforated patch clamp for all action potential recordings. We carried out simulation studies of $I_{to}$ in canine endocardial myocytes using the perforated patch clamp to verify our earlier results and to allow a side-by-side comparison of canine and guinea pig ventricular cells.

MATERIALS AND METHODS

Preparation of isolated guinea pig and canine ventricular myocytes. Handling and usage of animals were in accordance with protocols approved by the University of Cincinnati Institutional Animal Care and Use Committee. Adult guinea pigs, weighing 200–250
mg, of either sex were anesthetized by intraperitoneal injection of pentobarbital sodium (150 mg/kg body wt). Hearts were then quickly excised and mounted on a Langendorff perfusion apparatus and perfused with oxygenated Ca2+-free and then Ca2+-containing (1.5 mM) solution for 5 min each. The perfusion solution contained (in mM) 112 NaCl, 5.4 KCl, 1.7 NaH2PO4, 1.63 MgCl2, 4.2 NaHCO3, 20 HEPES, 5.4 glucose, and 10 taurine (pH = 7.6). This was followed by perfusion with the same solution containing zero Ca2+ and 85 U/ml collagenase (type II, Worthington) at 37°C until the hearts became flaccid. The ventricles were removed, minced, and pipette-triturated in collagenase (type II, Worthington) at 37°C until the hearts became flaccid. The ventricles were removed, minced, and pipette-triturated in oxygenated Kraft-Bru¨he (KB) solution containing (in mM) 83 KCl, 30 K2HPO4, 5 MgSO4, 5 Na-pyruvate, 5 CdCl2 (0.2 mM) was added to the external solution to block the Ca2+ current recordings, whole cell patch-clamp recordings were used. Pipette solution contained (in mM) 110 K-aspartate, 20 KCl, 85 NaCl, 10 HEPES, 0.1 Na-GTP (pH adjusted to 7.2 with KOH). Both perforated and whole cell patch-clamp recordings were performed with an Axo-patch-1B amplifier. Data were collected using pCLAMP® software through an Axon Digidata 1322A data acquisition system. All experiments were performed at 34°C with the exception of delayed rectifier K+ current (I_K) recordings, which were carried out at room temperature (24°C). All chemical and drugs were from Sigma unless otherwise stated.

Implementation of the dynamic clamp. Dynamic clamp experiments were performed as previously described (27). A modified version of the Windows-based DynClamp software was used in the dynamic clamp studies (21). Voltage sampling of the dynamic clamp software and output of the current injection command were through an Axon Digidata 2100 board.

I_{\text{in}} was defined as a rapidly and fully inactivating outward current and was formulated based on our previous canine epicardial I_{\text{Na}} model (27). Modifications were made to account for shifts of the voltage-dependence of I_{\text{Na}} gating properties by external Cd2+. The I_{\text{in}} conductance was given by the following:

$$I_{\text{in}} = g_{\text{Na}} m h R(V_m - E_{\text{K}})$$

(1)

$$R = \exp(V_m/300)$$

(2)

$$\alpha_m = 4 \times \frac{1}{1 + \exp(V_m - 25)/-20}$$

(3)

$$\beta_m = 3.5 \times \frac{1}{1 + \exp(V_m + 110)/29.5}$$

(4)

$$m = \frac{1}{1 + \exp(V_m + 13.9)/-8}$$

(5)

$$\alpha_h = 0.016 \times \frac{1}{1 + \exp(V_m + 59)/5.5}$$

(6)

$$\beta_h = 0.11 \times \frac{1}{1 + \exp(V_m + 27)/-6.5}$$

(7)

$$h = \frac{1}{1 + \exp(V_m + 50.3)/3.9}$$

(8)

where m is the activation variable, h is the inactivation variable, V_m is the membrane potential, and R is the rectification factor. Figure 1 shows the waveform, true peak conductance-voltage relationship, and voltage-dependencies of steady-state and time constants of the m and h gates of the model I_{\text{Na}}. E_{\text{K}}, the reversal potential for K+, was set to −85 mV. The electrode junction potential was 12 mV and was corrected online in the computation. Action potentials were triggered with just-threshold 2-ms current steps and recorded at steady state. Action potentials were recontrolled after each simulation. Because g_{\text{Na}} in Eq. 1 does not equal the true simulated I_{\text{Na}} conductance, the true peak current density in response to a depolarizing step from −80 to +40 mV is given to indicate the amplitude of the simulated I_{\text{Na}} unless otherwise stated.

Data analysis. Group data are presented as means ± SE. Statistical tests of the effects of the dynamic clamp simulation were performed.

![Fig. 1. Mathematical modeling of canine epicardial transient outward current (I_{\text{Na}}). A: waveform of the model I_{\text{Na}} in response to voltage steps ranging from −60 to 50 mV from a holding voltage of −80 mV. B: peak conductance and time constant of activation (m) gate of the model I_{\text{Na}} at various voltages (left) and steady-state and time constant of inactivation (h) gate at various voltages (right). g_{\text{Na}} peak conductance of simulated I_{\text{Na}}; V_m membrane potential, au, arbitrary units.](image-url)
using paired, two-tail Student’s t-tests. Other statistical tests used unpaired Student’s t-tests. A t value giving $P < 0.01$ was considered as significant.

**RESULTS**

**Electrophysiological properties of guinea pig ventricular myocytes.** We first examined the action potentials of guinea pig ventricular cells using the perforated current clamp. Figure 2A shows representative action potentials triggered at 1 and 3 Hz. Notably, the action potentials lacked phase 1 repolarization and had a waveform similar to that in canine endocardial ventricular cells (27). The average APD$_{90}$ at 1 Hz was 196.4 ± 12.1 ms ($n = 18$ cells) and was abbreviated to 137.4 ± 9.9 ms at 3 Hz ($n = 6$ cells). Consistent with their lack of phase 1 notch, whole cell voltage clamp recordings showed no detectable $I_{to}$ in guinea pig ventricular cells (Fig. 2B). By comparison, in response to the same voltage clamp protocol and under the same recording conditions, a robust $I_{to}$ was evident in canine epicardial cells (Fig. 2B, inset). Repolarizing currents in guinea pig ventricular cells were dominated by a large $I_K$ (Fig. 2C).

**Dynamic clamp simulation of $I_{to}$ in guinea pig ventricular cells.** To understand the role of $I_{to}$ in shaping the action potential, we introduced a simulated canine ventricular $I_{to}$ conductance in guinea pig ventricular cells using the dynamic clamp. Action potentials were triggered at 1 Hz and recorded at steady state. Simulation of a typical canine epicardial-sized $I_{to}$ in guinea pig ventricular cells produced a distinct phase 1 notch and a “spike-and-dome” action potential configuration but did not significantly affect the APD (Fig. 3A). The use of the perforated patch clamp presented a challenge for the implementation of the dynamic clamp. We only studied cells with stable series resistance of <7 MΩ, which was carefully monitored and fully compensated. The dynamic clamp output (Fig. 3A, inset) and the voltage traces were without oscillation or distortion.

We examined the density-dependent effect of simulated $I_{to}$ on the action potential. Over a wide density range, simulated $I_{to}$ produced a distinct phase 1 notch and a spike-and-dome action potential waveform (Fig. 3B). Low densities of $I_{to}$ did not significantly affect the APD (Fig. 3B, left); as the $I_{to}$ density...
was further increased, the APD was moderately prolonged (Fig. 3B, left, bold line) before reaching a threshold at which point the action potential alternated between one with a deep notch and a markedly prolonged APD and all-or-none repolarization (Fig. 3B, right, bold lines). Any simulated \( I_{\alpha} \) larger than the threshold produced a brief, spike-like action potential. To allow comparison between guinea pig and canine myocytes.

Guinea pigs have a heart rate >200 beats/min. At a higher and more physiological rate, cumulative activation of \( I_K \) would favor repolarization of the action potential. We therefore performed simulation studies of \( I_{\alpha} \) at pacing rates of 1 and 3 Hz. Simulation of \( I_{\alpha} \) at 3 Hz had generally similar effects on guinea pig action potential as observed at 1 Hz (Fig. 5E), although a higher density of \( I_{\alpha} \) was needed at 3 Hz to achieve all-or-none repolarization (Fig. 5F). This shift of the \( I_{\alpha} \) density-APD90 curve at 3 Hz toward a higher \( I_{\alpha} \) density probably reflects a higher degree of cumulative \( I_{\alpha} \) inactivation at a higher pacing frequency.

Effect of sustained outward current on APD. Our dynamic clamp simulation results in guinea pig ventricular cells differ markedly from those using the gene transfer or cell fusion approaches to introduce Kv4.3 currents in the cells (9, 10). In these earlier studies, a sustained outward current was generated in the ventricular cells along with the \( I_{\alpha} \)-like current. A previous computer modeling study suggested that the introduction of such a noninactivating current contributed to the shortening of the APD by the exogenous Kv4.3 current (6). To test this prediction in real guinea pig ventricular cells, we performed simulations of a modified canine epicardial \( I_{\alpha} \) model that had a noninactivating component. Again, simulation of a fully inactivating \( I_{\alpha} \) had the typical biphasic effect on action potential waveform and duration in guinea pig cells (Fig. 6A).
In the same cell, simulation of an \( I_{\text{to}} \) with a 10 to 16% sustained component (a fraction similar to those in the studies of Hoppe et al.) progressively and significantly suppressed the plateau and shortened the APD over the entire density range we tested (Fig. 6B). With the presence of the sustained current, \( I_{\text{to}} \) density had a monotonic inverse relationship with APD90, instead of the biphasic relationship when the simulated current fully inactivated (Fig. 6C). Similar results were obtained in a total of four guinea pig cells. Results shown in Fig. 6, B and C, closely reproduced the observations of Hoppe et al., suggesting that the presence of a sustained outward current accounted for the monotonic shortening of APD by \( I_{\text{to}} \) in these earlier studies.

DISCUSSION

Although the action potential waveform in guinea pig ventricular cells shares similarities with those of endocardial ventricular myocytes in large animals, such as dog, the under-
lying ionic current distribution profile in guinea pig and large animals is significantly different. $I_K$ is more prominent in guinea pig ventricular cells than in canine ventricle (15, 17) and is balanced by a significantly larger L-type Ca$^{2+}$ current ($I_{Ca,L}$) (8, 30). Indeed, it was shown that the effect of exogenous Kv4.3 current on action potential in guinea pig ventricular cells (9, 10) is distinct to that of $I_{to}$ in canine ventricular cells (27). In the present study, we used the dynamic clamp technique to simulate $I_{to}$ conductances in guinea pig and canine ventricular myocytes and have shown that, despite the significant differences in cellular electrophysiological background of the two myocytes, the effects of a fully inactivating $I_{to}$ on the action potential morphology and duration of these two types of myocytes were remarkably similar. Importantly, a distinctive threshold (~40 pA/pF) existed that separated the effect of $I_{to}$ into two phases. Below this threshold, increasing amounts of simulated $I_{to}$ generated a progressively increasing delay in the onset of phase 1 notch (1, 2) and did not significantly affect the APD at low densities and moderately prolonged the APD at higher densities. $I_{to}$ above the density threshold led to all-or-none repolarization, resulting in spike-like action potentials with dramatically reduced APD.

These effects of $I_{to}$ are not linked to the particular mathematical formulations of the $I_{to}$ model or a specific stimulation rate. Our experimental results are in agreement with the predictions of a modeling study of canine and guinea pig ventricular cells (6) and probably reflect a general role of the $I_{to}$ conductance in regulating the morphology of broad, plateau-possessing cardiac action potentials.

The underlying mechanism of the biphasic APD-$I_{to}$ relationship probably lies in the interplay between $I_{to}$ and $I_{Ca,L}$, as suggested by the modeling study (6). It is clear from our simulation studies that the voltage trajectory at the end of phase 1 repolarization depends on the potential of the notch. $I_{to}$, via its regulation of phase 1 notch potential, either allows reactivation of $I_{Ca,L}$ at the end of phase 1 and the development of the plateau (or the “dome”) or turns off $I_{Ca,L}$ by moving the notch potential below the activation voltage range of $I_{Ca,L}$ and completely suppresses the plateau. Prolongation of the APD occurs when $I_{to}$ causes a delay in the reactivation of $I_{Ca,L}$ and a shift in $I_{Ca,L}$ time course. In our experiments, the average notch potential for such transition between reactivation and deactivation of $I_{Ca,L}$ was ~20 mV for guinea pig ventricular cells and ~24 mV for canine endocardial myocytes.

In our present study, we used perforated patch-clamp recording to preserve physiological intracellular Ca$^{2+}$ handling and reexamined the effect of $I_{to}$ in canine endocardial cells. Under these conditions, simulation of $I_{to}$ in canine endocardial cells generated action potential waveforms that were remarkably similar to those recorded from canine epicardial tissue or myocyte using microelectrode recordings (13, 14, 16, 18). The APD-$I_{to}$ density relationship is qualitatively similar to our previous findings using whole cell recordings (Fig. 4 of Ref. 27) but with a few noticeable differences. The small depression of APD when $I_{to}$ was ~30 pA/pF was not found in the current study and was probably an artifact associated with intracellular Ca$^{2+}$ buffering. Instead, progressive APD prolongation was observed over the entire $I_{to}$ density range from ~28 pA/pF to the threshold for all-or-none repolarization. Importantly, in our present study, we found that simulation of canine epicardial level of $I_{to}$ (~20 pA/pF) did not significantly affect the endocardial APD, supporting our previous conclusion that physiological levels of $I_{to}$ do not play a significant role in regulating the APD in canine left ventricle. The same, however, cannot be said about $I_{to}$ in canine right ventricular cells. $I_{to}$ density is significantly higher in canine right epicardium than in the left (3, 25). With the assumption that the APD-$I_{to}$ density relationship shown in Fig. 4 is shared in right ventricular cells, $I_{to}$ probably moderately prolongs the APD in the right ventricular cells.

$I_{to}$ is conventionally defined in modeling studies as the fully and rapidly inactivating component of the total outward current. Such definition is supported by experimental evidence. It is shown that in mouse ventricular cells, $I_{to}$ inactivates fully and is separate from the noninactivating outward currents (35). In human atrial and mouse ventricular cells, antisense suppression or functional knockout of the Kv4-generated $I_{to}$ leaves the noninactivating current intact (1, 7, 31), arguing that in these native systems, $I_{to}$ and the noninactivating component are carried by channels with different molecular identities. For these reasons, we defined and modeled $I_{to}$ as a fully inactivating conductance in our simulation studies. With the simulation of even a small sustained conductance, the biphasic APD-$I_{to}$ relationship in guinea pig cells was dramatically changed to a monotonic inverse relationship, strikingly similar to those reported earlier (9, 10). Simulation of a sustained conductance also eliminated the notch and suppressed the plateau, closely reproducing the results of Hoppe et al. These results point to the importance of the ultrarapid $I_K$-type sustained current in regulating the action potential morphology. The marked effects of the sustained current are not surprising considering the small net current during the plateau phase. In canine ventricular cells, $I_{to}$ inactivation is rapid and near complete (Fig. 2B). We argued in an earlier study that the large $I_{to}$ size in smaller animals such as mouse is responsible for their brief action potentials (27). In addition to a large $I_{to}$, prominent slowly inactivating and noninactivating currents are present in mouse ventricle (35). These currents, when combined, can make up over half of the total outward current amplitude (35). It is likely that the presence of such slow and sustained currents also contributes importantly to the brief, spike-like action potential in mouse ventricle. In this case, species difference in cardiac action potential morphology is achieved by changes in both channel expression level and function.

Sudden cardiac death, mostly caused by ventricular arrhythmias, is responsible for about half of the mortalities in heart failure patients (20). One of the most characteristic electrophysiological changes in heart failure is the prolongation of the APD, which is believed to predispose the heart to afterdepolarization and reentrant arrhythmias (29). Accompanying the prolongation of APD, downregulation of $I_{to}$ is consistently observed in heart failure patients as well as animal models (20, 28, 32). Based on their close correlation, it was proposed that $I_{to}$ downregulation is an important contributor to APD prolongation in failure (12, 32). This is likely to be true in smaller animals, where $I_{to}$ is much beyond the density threshold for all-or-none repolarization (27) and is the major determinant of APD. The same conclusion may not apply to large animals, such as humans (22), because the role of $I_{to}$ in regulating action potential morphology differs significantly between small and large animals. $I_{to}$ in canine (27) and human (19) left ventricle is well below the density threshold for all-or-none repolarization, and our APD-$I_{to}$ density curves show that within this...
density range, reduction of $I_{to}$ regardless of the magnitude, will not prolong the APD under physiological conditions. In heart failure, a concerted change in ion channel expression occurs (33), and the APD-$I_{to}$ density relationship reported in our study may be altered as a result of the remodelings of the cellular electrical background. Also, it is possible that down-regulation of the Kv4 channel causes a concomitant decrease in the sustained component of the outward current, thereby resulting in APD changes. It would be important to expand our simulation studies to hypertrophy and failure settings.

REFERENCES


