Differences in Transient Outward Currents of Feline Endocardial and Epicardial Myocytes

Tetsushi Furukawa, Robert J. Myerburg, Nanako Furukawa, Arthur L. Bassett, and Shinichi Kimura

Whole-cell voltage-clamp experiments were performed on enzymatically dissociated single ventricular myocytes harvested from feline endocardial and epicardial surfaces. The studies were designed to test the hypothesis that the differences in the amplitude of transient outward current (\( I_{\text{to}} \)) contribute to the difference in action potential configuration between endocardial and epicardial myocytes. In the control state, action potentials recorded from epicardial cells demonstrated a prominent notch between phases 1 and 2, and membrane current recordings displayed a prominent \( I_{\text{to}} \) whereas in endocardial cells the notch in action potentials and \( I_{\text{to}} \) were small. External application of 4-aminopyridine (2 mM) reduced the amplitudes of notch and \( I_{\text{to}} \) in epicardial cells but not in endocardial cells. After application of 4-aminopyridine (2 mM) and caffeine (5 mM), the notch and \( I_{\text{to}} \) were abolished completely in both endocardial and epicardial cells. The first component of \( I_{\text{to}} \) (\( I_{\text{to1}} \)) was present in all epicardial cells studied \((n=20)\); it was absent in 12 of the 20 endocardial cells, and a small \( I_{\text{to}} \) was present in the remaining eight endocardial cells. The mean amplitude of \( I_{\text{to}} \) was significantly greater in epicardial than in endocardial cells. At a test voltage of +80 mV, the amplitude of \( I_{\text{to}} \) was 102.0±47.7 pA/pF in epicardial cells and 3.3±3.3 pA/pF in endocardial cells \((p<0.01)\). The second component of \( I_{\text{to}} \) (\( I_{\text{to2}} \)) was present in all endocardial \((n=30)\) and epicardial \((n=30)\) cells studied. The amplitude of \( I_{\text{to2}} \) was significantly greater in epicardial than in endocardial cells. At a test voltage of +60 mV, the amplitude of \( I_{\text{to2}} \) was 10.8±4.1 pA/pF in epicardial cells and 8.1±4.9 pA/pF in endocardial cells \((p<0.05)\). The differential distribution of \( I_{\text{to}} \) in endocardial and epicardial myocytes may relate to regional heterogeneity of electrical properties of the heart. (Circulation Research 1990;67:1287–1291)

Since transient outward current (\( I_{\text{to}} \)) was recognized in sheep Purkinje fibers under voltage-clamp conditions,\(^1\) its existence has been documented in several cardiac preparations, and its characteristics have been studied.\(^2\)\(^–\)\(^1\(^5\)\) There is general agreement that \( I_{\text{to}} \) consists of two components,\(^7\)\(^,\)\(^1\(^2\)\(^,\)\(^1\(^4\)\(^,\)\(^1\(^5\)\) although in some preparations only one or the other component is documented. The first component of \( I_{\text{to}} \) (\( I_{\text{to1}} \)) is sensitive to the K\(^+\) channel blocker 4-aminopyridine (4-AP),\(^3\) is considered to be responsible for the early phase of repolarization (phase 1), and is strongly rate dependent.\(^6\)\(^,\)\(^8\) The second component (\( I_{\text{to2}} \)) is dependent on Ca\(^{2+}\) release from the sarcoplasmic reticulum and is abolished by the agents that block Ca\(^{2+}\) release from the sarcoplasmic reticulum, such as ryanodine and caffeine.\(^4\)\(^5\)\(^,\)\(^9\)

It has been shown in multicellular ventricular muscle preparations\(^1\(^6\)\(^–\)\(^1\(^8\)\) and in isolated myocytes\(^1\(^9\)\) that epicardial action potentials have a prominent notch between phases 1 and 2, resulting in a “spike and dome” configuration. This feature is not present in endocardial cells. Using conventional microelectrode techniques, Litovsky and Antzelevitch\(^1\(^7\)\(^,\)\(^1\(^8\)\) concluded from the responses of the epicardial notch to pharmacological interventions that \( I_{\text{to}} \) was prominent in canine ventricular epicardium but not in endocardium. However, this regional heterogeneity has never been demonstrated in single isolated cells. It is not known if this regional heterogeneity represents inherent cellular electrophysiological properties, because multicellular ventricular muscle preparations have extracellular ionic and electrotropic interactions. The purpose of this study was to test the hypothesis that the epicardial notch reflects a differential distribution of \( I_{\text{to}} \) in feline endocardial and epicardial myocytes. Single-pipette, voltage-clamp techniques were used to carry out these studies on isolated single myocytes.

From the Departments of Medicine (Cardiology) and Pharmacology, University of Miami School of Medicine, Miami, Fla.

Supported by grants HL-21735 and HL-19044 from the National Heart, Lung, and Blood Institute, by grants-in-aid from the American Heart Association, Florida Affiliate (S.K.) and Palm Beach Chapter of Florida Affiliate (R.J.M.), and by a project grant from American Heart Association, Florida Affiliate (A.I.B.).

Address for correspondence: Shinichi Kimura, MD, Division of Cardiology (R-94), Department of Medicine, University of Miami, School of Medicine, Miami, FL 33101.

Received January 31, 1990; accepted July 2, 1990.
Materials and Methods

Single endocardial and epicardial myocytes from feline left ventricles were harvested by an enzymatic dissociation procedure similar to that described by Kimura et al. Briefly, domestic cats of either sex, weighing 2.5–3.5 kg, were anesthetized with pentobarbital sodium (30 mg/kg i.p.) and anticoagulated with heparin sodium (400 IU/kg i.v.). The heart was excised rapidly and mounted on a Langendorff perfusion apparatus (60-cm height). It was perfused retrogradely via the aorta for 10 minutes and equilibrated with 100% O₂ at 37°C at a rate of 10–15 ml/min, followed by perfusion for 5 minutes with nominally calcium-free Tyrode’s solution. The Tyrode’s solution contained (mM) NaCl 143.0, KCl 4.0, CaCl₂ 1.8, MgCl₂ 0.5, Na₂HPO₄ 0.33, glucose 5.5, and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) 5.5 (pH 7.4 with NaOH). The preparation was then perfused with 0.04% collagenase (Type 1, Sigma Chemical Co., St. Louis) dissolved in Tyrode’s solution containing 50 μM Ca²⁺. Exposure to the enzyme was continued until the solution flowed freely (15–20 minutes), after which the collagenase was washed out with 150 ml of a Kraftbrühe (KB) solution containing (mM) KCl 40.0, glutamic acid 50.0, taurine 20.0, KH₂PO₄ 10.0, MgCl₂ 0.5, glucose 11.0, ethylene glycol-bis(β-aminoethyl ether N,N,N’N’-tetraacetate acid (EGTA) 0.5 and HEPES 10.0 (pH 7.4 with KOH). Small pieces of the left ventricular free wall tissues were dissected from the endocardial and epicardial surfaces (to a depth not exceeding 20% of the thickness of the ventricular wall), using a fine scissors. After the tissues had been agitated gently in a beaker containing KB solution, single cells were separated from tissue pieces by passing the cells through 140-μm mesh nylon gauze. Isolated cells were stored in the KB solution at 4°C for a minimum of 60 minutes and were studied within 24 hours after isolation.

Whole-cell currents and membrane potentials were recorded using the method of Hamill et al. through a Dagan 8900 patch-clamp amplifier (Dagan Corp., Minneapolis). The recording chamber was superfused continuously with filtered Tyrode’s solution at a temperature of 37°C at a rate of 3 ml/min. The Ni²⁺ solution had 2.0 mM NiCl₂ in the Tyrode’s solution. Tetrodotoxin, 4-AP, and caffeine (all from Sigma) were added to the Tyrode’s solution to the final concentrations described in the text. The internal solution in the patch electrode contained (mM) KCl 130.0, ATP 5.0 (as dipotassium salt, Sigma), creatine phosphate 5.0, and HEPES-KOH buffer 5.0 (pH 7.3). For the EGTA-containing solution, 20.0 mM EGTA was added to the above internal solution. The amplitude of Iₜₒ was measured as the difference between the peak of Iₒ and the minimum current level during the depolarizing pulse after the peak. The amplitude of Iₒ was normalized to cell membrane capacitance. The value for the membrane capacitance was 148±29 pF for endocardial cells (n=58) and 140±44 pF for epicardial cells (n=57) (p=NS).

Equal numbers of experiments for endocardial and epicardial cells for each heart were done in each protocol so that variations from animal to animal would not affect comparisons between endocardial and epicardial cells. Data were presented by consecutive experiments. Membrane potentials were digitized on-line with an A/D convertor (model PCM-1, Medical Systems Corp., Greenvale, N.Y.) and stored on a videocassette recorder (model SL-HF900, Sony, Tokyo). Membrane currents were digitized on-line by a 12-bit resolution Labmaster A/D convertor (TecMar Scientific Solutions, Burlingame, Calif.) under the control of an IBM-AT computer and were stored on a hard disk. Data were analyzed using the software program pCLAMP (Axon Instruments, Inc., Burlingame, Calif.). All data are expressed as mean±SD. Statistical significance was evaluated by Student’s unpaired t test, where appropriate. Differences with p<0.05 were considered significant.

Results

Transmembrane action potentials recorded from single epicardial cells demonstrated a prominent notch between phases 1 and 2, producing a “spike and dome” configuration (Figure 1B, left panel), whereas those recorded from endocardial cells demonstrated only a small notch (Figure 1A, left panel). Current recordings by voltage-clamp techniques displayed prominent Iₒ in epicardial cells (Figure 1B, left panel), whereas Iₒ was much smaller in endocardial cells (Figure 1A, left panel). External application of 4-AP (2 mM) substantially reduced the notch and Iₒ in epicardial cells (Figure 1B, middle panel), whereas it had only a minimal effect on the notch and Iₒ in endocardial cells (Figure 1A, middle panel). As a result, after treatment with 4-AP (2 mM), action potential configuration and Iₒ amplitude became similar in endocardial and epicardial cells (Figures 1A and 1B, middle panels). Application of 4-AP (2 mM) and caffeine (5 mM) completely eliminated the notch and Iₒ in both endocardial and epicardial cells (Figures 1A and 1B, right panels). These data were obtained from eight endocardial cells and seven epicardial cells and indicated that the feline epicardial cells had a greater Iₒ than did endocardial cells.

To compare the amplitude of Iₒ, we clamped the membrane voltage for 300 msec to a test voltage (Vₜ) between −30 and +80 mV in 10-mV steps from a holding voltage (V₀) of −60 mV at an interval of 5 seconds (Figure 2A). In all experiments, the external solution contained tetrodotoxin (15.7 μM) and NiCl₂ (2 mM). In all epicardial cells (n=20), a prominent Iₒ was recorded, whereas a small Iₒ was recorded in eight of the 20 endocardial cells, and no Iₒ was recorded in the remaining 12 endocardial cells. Averaged amplitude of Iₒ was significantly greater in epicardial cells than in endocardial cells at each Vₜ (Figure 2B). At a Vₜ of +80 mV, the amplitude of Iₒ
was 3.3±3.3 pA/pF in endocardial cells and 102.0±47.7 pA/pF in epicardial cells (p<0.01).

To compare the amplitudes of I_{to2}, we applied depolarizing pulses for 300 msec to \( V_t \) ranging from -30 to +80 mV in 10-mV steps from a \( V_h \) of -80 mV at intervals of 5 seconds. In all experiments, external solution contained tetrodotoxin (15.7 \( \mu \)M) and 4-AP (2 mM). I_{to2} was recorded in all endocardial (\( n=30 \)) and epicardial (\( n=30 \)) cells. The amplitude of I_{to2} was slightly but significantly greater in epicardial cells than in endocardial cells (Figure 3B). At a \( V_t \) of +60 mV (the \( V_t \) that elicited maximum I_{to2} in both endocardial and epicardial cells), I_{to2} amplitude was 8.1±4.9 pA/pF in endocardial cells and 10.8±4.1 pA/pF in epicardial cells (p<0.05).

**Discussion**

Using conventional microelectrode techniques, Litovsky and Antzelevitch\(^{17,18} \) previously suggested that I_{to} is prominent in canine left ventricular epicardium but not in endocardium. Their conclusions were based on the differences in action potential configuration and their responses to heart rate and various pharmacological interventions, but no direct evidence for differential density of I_{to} in endocardial and epicardial cells was demonstrated. In the present study, using single-pipette, voltage-clamp techniques, we have demonstrated directly the differential distribution of I_{to1} and I_{to2} in single myocytes isolated from endocardial and epicardial surfaces of the cat left ventricle, as well as their contribution to the differences in action potential configurations between two sites. I_{to1} was much more prominent in epicardial than in endocardial cells. I_{to1} was present in all epicardial cells studied, whereas it was absent in more than half of the endocardial cells; the remaining endocardial cells demonstrated a very small I_{to1}. In contrast, I_{to2} having similar amplitudes was present in all endocardial and epicardial cells studied. The amplitude of I_{to2} was only slightly greater in epicardial than in endocardial cells. The difference in action potential configuration between endocardial and epicardial cells appeared to be due to the difference in amplitude of I_{to1} between two cell types. When I_{to1} was blocked by external application of 4-AP, the notch between phases 1 and 2 in epicardial action potentials became small, and the configuration

---

**Figure 1.** Correlation between notch in action potential and transient outward current (I_{to}). Current traces were recorded during 300-msec depolarizing pulses from a holding potential of -80 mV to a test voltage of +30 mV. Internal solution contained no EGTA. Panel A: Data from an endocardial cell. Panel B: Data from an epicardial cell. To correlate notch in action potential with I_{to} action potentials and current tracings were obtained from the same preparation and were elicited at an identical pacing rate (0.5 Hz). Left panels show action potential and current tracings in the control state. Normal Tyrode's solution without 4-aminoypyridine (4-AP) or caffeine was used as external solution. A prominent notch in action potential and a prominent I_{to} in the current tracing were present in an epicardial cell (panel B), whereas a small notch and small I_{to} were present in an endocardial cell (panel A). In middle panels, application of 2 mM 4-AP substantially decreased the amplitudes of the notch in the action potential and I_{to} in an epicardial cell (panel B), but not in an endocardial cell (panel A). In right panels, after application of 2 mM 4-AP and 5 mM caffeine, the notches in the action potentials and I_{to} were completely abolished in both an endocardial and epicardial cell.
of endocardial and epicardial action potentials became similar.

Using voltage-clamp techniques, researchers have reported on the existence of $I_{\text{o}}$ in ventricular myocytes in rat,\textsuperscript{10} canine,\textsuperscript{13,14} and rabbit hearts.\textsuperscript{15} Even though it may be difficult to compare these data because of differences in experimental method, there appears to be a spectrum of differences in the relative amplitudes of components of $I_{\text{o}}$ among the species. Most remarkable is $I_{\text{o}}$ in rat myocytes reported by Josephson et al.\textsuperscript{10} Using voltage-clamp by conventional microelectrode impalement, they reported that the amplitude of $I_{\text{o,1}}$ was about 10-fold greater than other species and was responsible for a fast phase of repolarization in action potentials of that tissue.\textsuperscript{10} The patch-clamp technique was used to study $I_{\text{o}}$ in canine myocytes by Tseng and Hoffman,\textsuperscript{14} rabbit myocytes by Hiraoka and Kawano,\textsuperscript{15} and feline myocytes in this study. In canine myocytes, the $I_{\text{o,2}}$ amplitude is very small compared with the $I_{\text{o,1}}$ amplitude.\textsuperscript{15} In contrast, in rabbit myocardium, $I_{\text{o,2}}$ was relatively large, ranging 25–70% of the amplitude of $I_{\text{o,1}}$.\textsuperscript{15} In cat, the $I_{\text{o,2}}$ amplitude was approximately 20% of the $I_{\text{o,1}}$ amplitude in epicardial cells, and it was even greater than the $I_{\text{o,1}}$ amplitude in endocardial cells. However, comparison among these three studies requires some caution, because the amplitude of $I_{\text{o,2}}$ may vary according to differing amounts of Ca$^{2+}$ influx and/or Ca$^{2+}$ release from the intracellular stores, as well as differing heart rate. These experiments were done under differing heart rates: 0.143 Hz by Tseng and Hoffman,\textsuperscript{14} 0.1–0.0167 Hz by Hiraoka and Kawano,\textsuperscript{15} and 0.2 Hz by us. In addition, Tseng and Hoffman\textsuperscript{14} used an internal pipette solution containing 1 mM EGTA. The existence of EGTA in the internal pipette solution may suppress Ca$^{2+}$ transients, and thus $I_{\text{o,2}}$ might be underestimated in their study.

Several recent studies have suggested that electrophysiological heterogeneity between endocardium and epicardium may be an important factor for development of ischemic arrhythmias. $I_{\text{o,1}}$ is known to be strongly rate dependent; it is markedly reduced at high rates.\textsuperscript{4,8,10} Thus, presence of a strong $I_{\text{o,1}}$ in epicardial but not in endocardial myocytes may provide a heterogeneity of rate dependence of action...
potential durations and refractory period. Suggested is to be linked to excitation–contraction coupling, because it is strongly dependent on calcium release from the sarcoplasmic reticulum. The difference in $I_{o2}$ amplitude between endocardial and epicardial myocytes also may suggest the existence of a regional heterogeneity in excitation–contraction coupling. Two hypotheses to explain the underlying basis for the differential amplitude of $I_{o2}$ between endocardial and epicardial myocytes may be considered: 1) the density of channel protein is different, and 2) although channel density is not different, channel activity in endocardial cells is reduced either by a decrease in unitary conductance or by a decrease in open states of the channels. Experiments on single-channel properties in endocardial and epicardial myocytes are under way to test the latter hypothesis.

References

18. Litovsky SH, Antzelevitch C: Rate dependence of action potential duration and refractoriness in canine ventricular endocardium differs from that of epicardium: Role of the transient outward current. J Am Coll Cardiol 1989;14:1053–1066

KEY WORDS • transient outward current • left ventricle • endocardium • epicardium
Differences in transient outward currents of feline endocardial and epicardial myocytes.
T Furukawa, R J Myerburg, N Furukawa, A L Bassett and S Kimura

doi: 10.1161/01.RES.67.5.1287

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/67/5/1287

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org//subscriptions/