Pacemaker Current Exists in Ventricular Myocytes

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I\textsubscript{f}, the “cardiac pacemaker current,” is a nonselective cation channel activated on hyperpolarization in primary and secondary pacemaker regions of the mammalian heart. The cardiac pacing rate can be modulated by shifting the activation of I\textsubscript{f} to more positive (faster pacing) or more negative (slower pacing) voltages. We report for the first time the presence of this pacemaker current in ventricular myocytes. The potential importance of this observation to the mechanism of differentiation of nonpacing regions in the heart is discussed. (Circulation Research 1993;72:232–236)

**Key Words** • ventricular myocyte • pacemaker current • pacing current

The rhythm of the mammalian heart is myogenic in origin.\textsuperscript{1} One current that contributes to pacing in both the primary (SA node) and secondary pacemakers (Purkinje fibers) is a nonselective cation current activated on hyperpolarization and called I\textsubscript{f}.\textsuperscript{2-4} I\textsubscript{f} provides a time-dependent inward current that drives the membrane toward threshold. Although I\textsubscript{f} is present in both primary and secondary pacemaking cells, it is activated at more positive voltages in the primary pacemaker,\textsuperscript{5,6} and positive shifts of its voltage range of activation can dramatically speed auxiliary pacemaking in Purkinje fibers.\textsuperscript{7} In the present study we report for the first time the presence of this pacemaker current in ventricular myocytes. It is activated at potentials more negative than the normal physiological voltage range. The voltage gradient of I\textsubscript{f} activation from sinus node through Purkinje fibers to working ventricle suggests the hypothesis that a negative shift of I\textsubscript{f} activation may initiate the loss of pacemaker activity in auxiliary pacemaker or nonpacing cardiac tissue.

**Materials and Methods**

**Cell Preparation**

Guinea pig ventricular myocytes were prepared as described by Gao et al\textsuperscript{8} and Isenberg and Klockner.\textsuperscript{9} During this procedure the heart was placed in a calcium-free Tyrode’s solution containing (mM) NaCl 137.7, NaOH 2.3, MgCl\textsubscript{2} 1, glucose 10, HEPES 5, and KCl 5.4 (pH 7.4). Canine Purkinje and ventricular myocytes were also used. The Purkinje myocytes were dissociated as previously described.\textsuperscript{10} Small chunks of ventricle were dissected in most cases from the epicardium where Purkinje contamination should be least. The ventricular myocytes were dissociated in the same manner and in the same solutions as the Purkinje myocytes with one exception: The Ca\textsuperscript{2+} in the Tyrode’s solution was 2 mM instead of 4 mM.

**Electrophysiologic Studies**

The isolated cells were placed in a Lucite bath in which the temperature was maintained at 36±0.5°C by a temperature controller.\textsuperscript{11} An Axopatch 1B amplifier and the whole-cell patch clamp technique were used to observe I\textsubscript{f} currents. The pipettes had a resistance of 2–4 MΩ when filled with solution containing (mM) NaCl 6, K-aspartate 130, MgCl\textsubscript{2} 2, CaCl\textsubscript{2} 5, EGTA 11, Na\textsubscript{2}-ATP 2, Na-GTP 0.1, Na-cAMP 0.2, and HEPES 10 (pH adjusted to 7.2 by KOH). The external solution was similar to calcium-free Tyrode’s solution except it contained in addition (mM) KCl 19.6, BaCl\textsubscript{2} 8, MnCl\textsubscript{2} 2, CaCl\textsubscript{2} 1.8, CdCl\textsubscript{2} 0.2, isoproterenol 0.008 (absent in Figure 3A) (pH 7.4). We used the divalent Ba\textsuperscript{2+}, Mn\textsuperscript{2+}, and Cd\textsuperscript{2+} to block overlapping currents. The background K\textsuperscript{+} current activates and inactivates in the same voltage range as I\textsubscript{f}. Ba\textsuperscript{2+} blocks this current.\textsuperscript{4} The Mn\textsuperscript{2+} and Cd\textsuperscript{2+} are used to reduce Ca\textsuperscript{2+} currents, which can overlap with and obscure I\textsubscript{f} tail currents. Ba\textsuperscript{2+} and Mn\textsuperscript{2+} were included in previous studies of I\textsubscript{f} in Purkinje fibers.\textsuperscript{5} I\textsubscript{f} activates at extremely negative potentials in ventricle. We used isoproterenol outside and cAMP inside to shift I\textsubscript{f} more positive on the voltage axis\textsuperscript{12,13} and make the study more experimentally accessible. In the absence of these additives the I\textsubscript{f} activation threshold was −100 mV (n=6) in canine Purkinje myocytes and −137 mV (n=5) in canine ventricular myocytes; in their presence it was −89 mV (n=10) in the canine Purkinje myocytes and −129 mV (n=18) in the canine ventricular myocytes. The data were recorded on an FM tape recorder (Hewlett-Packard Co., Palo Alto, Calif.; 3964a, 600-Hz bandwidth) and simultaneously acquired by CLAMPEX (pCLAMP, version 5.5, Axon Instrument, Inc.) for later analysis by CLAMPFIT (pCLAMP, Axon). Data was low-pass filtered with a cutoff of 10 or 15 Hz. Each pulse was applied twice and averaged to increase the signal-to-noise ratio. Values are presented as mean±SD.

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Results

The studies presented here were performed on guinea pig ventricular myocytes, as well as canine ventricular and Purkinje myocytes with the whole-cell patch clamp technique. We used isoproterenol (8 μM) and an elevated [K]o (25 mM) and added 0.2 mM cAMP to the pipette solution to maximize our chances to observe I_v. (In later experiments we removed isoproterenol, the pipette cAMP, and the elevated [K]o, and I_v was still observed [see “Materials and Methods” and Figure 4].) Figure 1 illustrates our basic finding, first obtained in guinea pig ventricular myocytes. On hyperpolarization to potentials more negative than −130 mV a slow, time-dependent increase in inward current was observed. The magnitude of I_v increased for hyperpolarizations up to −175 mV (Figure 1B). This time-dependent current was Ba^2+ insensitive (since the Tyrode’s solution contained 8 mM BaCl_2) but was completely eliminated by 4 mM CsCl (Figure 1A, inset). I_v is a Cs^+ sensitive and largely Ba^2+-insensitive current. Therefore, we concluded that the ventricular current might be I_v (in Figure 3 we show that the reversal potential of this current demonstrated the expected selectivity of I_v). We observed this slow, time-dependent current in 12 of the 16 guinea pig ventricular myocytes that we could hyperpolarize to at least −140 mV. In 10 of these 12 cells Cs^+ blocked the time-dependent current; the remaining two died before Cs^+ application. The average voltage threshold of I_v activation was −148±13 mV (n=12).

Although I_v was activated at extremely negative potentials in guinea pig ventricular myocytes, this activation range could be an artifact of the cell isolation procedure. To confirm the relatively negative position of I_v we required a species in which ventricular myocytes could be compared with a natural pacemaking region in which I_v is known to activate at more positive potentials. We chose the canine because of the ability to isolate both ventricular and Purkinje myocytes. A typical set of results from both canine ventricular and Purkinje myocytes are provided in Figure 2. In both preparations a Ba^2+ insensitive, Cs^+ sensitive current exists (Figures 2Ai and 2Bi). However, the voltage range of activation for the I_v current in the two preparations differs markedly. In the Purkinje myocyte of Figure 2A, the I_v current begins activating between −70 and −80 mV, whereas in the ventricular myocyte activation occurs only negative to −110 mV. I_v was observed in all 10 Purkinje myocytes; the average voltage at which I_v began to activate was −89±16 mV. I_v was present in 18 of 21 canine ventricular myocytes that withstood hyperpolarization to at least −140 mV. In the canine ventricular myocytes the average threshold activation voltage was −129±7 mV (n=18). The normalized current–voltage relations for I_v in the canine ventricular and Purkinje myocytes of Figures 2A and 2B are plotted in Figure 2C. The canine as well as the guinea pig ventricles contains an I_v-like current, but it activates at potentials nearly 40 mV more negative than a similar current in canine Purkinje myocytes.

To further confirm the identity of the ventricular current as I_v, we obtained the reversal potential of this current in canine ventricular myocytes. A typical set of experimental results is illustrated in Figure 3. In these experiments our Tyrode’s solution contained either 5.4 mM [K]o (Figure 3A) or 25 mM [K]o (Figure 3B), and 140 mM [Na]o. The reversal potential was −47 mV in Figure 3A and −20 mV in Figure 3B. The average reversal potential was −41±6 mV in three experiments in 5.4 mM [K]o, and −13±5 mV in four experiments in 25 mM [K]o. This is consistent with the known selectivity of I_v to Na^+ and K^+.6

Finally, we confirmed that the I_v current in canine ventricular myocytes can be observed in the absence of cAMP added to the pipette solution and in the absence of isoproterenol added to the perfusate. One sample result from a total of five cells is provided in Figure 4. The I_v threshold was 8 mV more negative under these conditions (see “Materials and Methods” for details).
Discussion

We have demonstrated that an I_f-like current exists in ventricular myocytes from both the guinea pig and canine heart. This current typically activates at potentials more negative than -120 mV, is Cs⁺ sensitive and Ba⁺ insensitive, and has a reversal potential consistent with selectivity to Na⁺ and K⁺. We compared the canine ventricular myocytes to canine Purkinje myocytes studied with the same solution. I_f activated at more negative potentials (roughly 40 mV) and appeared to activate more slowly (although due to the difficulty of pulsing to extremely negative potentials, steady state comparisons were not possible). Also, like the Purkinje I_f, the ventricular I_f showed some evidence of a delay in activation (e.g., see Figure 3B). Clearly, at these extremely negative potentials, this current serves no physiological role. Why is it there? Evidence now suggests that I_f plays a major role in diastolic depolarization in Purkinje fibers¹,⁶,¹⁵ and the sinus node.⁵,⁶,¹⁵ I_f is also present in pacing regions of atrium¹⁶,¹⁷ and the atroventricular node.¹⁸ Increases in pacemaker rate are accomplished by catecholamines in at least part by positive shifts of I_f along the voltage axis,¹² while the negative chronotropic action of acetylcholine is medi-
Figure 3. Reversal potential of \( I_f \) in canine ventricular cells measured at 5.4 mM (panel A) and 25 mM (panel B) external potassium concentrations. Four (panel A) and six (panel B) superimposed current records of \( I_f \) are shown. The cell was hyperpolarized to \(-125\) mV (panel A) and \(-145\) mV (panel B) from a holding potential of \(-50\) mV and clamped back to the test pulses shown in the insets. The reversal potential of \( I_f \) was determined by linear interpolation to zero current of the tail current amplitude on either side of the reversal. The value in 5.4 mM \( K^+ \) was \(-47\) mV and that in 25 mM \( K^+ \) was \(-20\) mV. The holding current at \(-50\) mV was \(-144\) pA in panel A and \(-183\) pA in panel B. The insets show the time-dependent tail currents expanded (with DC levels moved closer together to allow for expansion).

Figure 4. \( I_f \) recorded in a canine ventricular myocyte in the absence of isoproterenol in the Tyrode's solution and in the absence of cAMP in the pipette solution. The \([K]_o\) was 5.4 mM, and the holding potential was \(-55\) mV. Whole-cell currents in response to a 2-second hyperpolarization to \(-155\) mV to activate \( I_f \) followed by a 200-msec depolarization to rapidly deactivate \( I_f \). \( I_f \) was reversibly blocked by 4 mM Cs\(^+\). The holding current at \(-55\) mV was \(-140\) pA. The Tyrode's solution contained 8 mM Ba\(^{2+}\) to block background \( K^+ \) current and 2 mM Mn\(^{2+}\) and 0.2 mM Cd\(^{2+}\) to block Ca\(^{2+}\) current.

ated in part by voltage shifts of \( I_f \) activation in the negative direction.\(^9\) Thus, a major mechanism for controlling heart rate has been modulating the voltage dependence of \( I_f \).

On the basis of our study, one possibility is that pacemaking can be terminated by shifting \( I_f \) to a level so negative that it can play no physiological role. This approach is wasteful, because the alternative of turning off the \( I_f \) gene and not synthesizing the \( I_f \) channel protein would be just as effective. It is possible that under certain pathological circumstances the Purkinje pacemaker system may fail; then a ventricular \( I_f \) can be used as a backup, but only if it can be shifted back to more physiological potentials.

We know little about how the voltage dependence of \( I_f \) is controlled. Increases in cAMP levels shift \( I_f \) in the positive direction.\(^1\) In Purkinje fibers this is thought to involve kinase A and phosphorylation,\(^2\) whereas in the sinus node direct cAMP-dependent gating has been proposed.\(^3\) We also know that in sinus node myocytes, rundown of the current can occur.\(^4\) This involves a continuous negative shift of the voltage of \( I_f \) activation and so may provide another means of studying the control of \( I_f \) voltage dependence. Whatever the final answer, our results provide evidence for a pacemaker current in ventricle and the interesting observation that this current can occur 50–100 mV more negative than it does in the primary pacemaker, the sinus node.

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