Distribution of a Persistent Sodium Current Across the Ventricular Wall in Guinea Pigs

Bernhard F.A.S. Sakmann, Anthony J. Spindler, Simon M. Bryant, Klaus W. Linz, Denis Noble

Abstract—A tetrodotoxin-sensitive persistent sodium current, \( I_{\text{pNa}} \), was found in guinea pig ventricular myocytes by whole-cell patch clamping. This current was characterized in cells derived from the basal left ventricular subendocardium, midmyocardium, and subepicardium. Midmyocardial cells show a statistically significant (\( P<0.05 \)) smaller \( I_{\text{pNa}} \) than subendocardial and subepicardial myocytes. There was no significant difference in \( I_{\text{pNa}} \) current density between subepicardial and subendocardial cells. Computer modeling studies support a role of this current in the dispersion of action potential duration across the ventricular wall. (Circ Res. 2000;87:910-914.)

Key Words: persistent sodium current ■ guinea pig ventricle ■ regional differences

First indications for a slowly inactivating, persistent sodium current in cardiac cells came from studies on Purkinje fibers of dogs and rabbits.\(^1\),\(^2\) This was followed by the discovery of slowly inactivating sodium channels in ventricular myocytes of rats.\(^3\)

Subsequently, a small late sodium current was described in guinea pig ventricular myocytes using single-channel and whole-cell patch clamping.\(^4\) This study also suggested a significant effect of the late sodium current on action potential duration in ventricular cells: application of 60 \( \mu \)mol/L tetrodotoxin (TTX) reversibly shortened action potential duration at 95% repolarization (ADP\(_{95}\)) by about 10%. Kiyosue et al\(^5\) speculated that this was because of block of slowly inactivating sodium channels.

Additional studies investigated the changes in the I-V relation of a slowly inactivating, TTX-sensitive persistent sodium current \( I_{\text{pNa}} \) in rat ventricular myocytes in the absence and presence of hypoxia.\(^5\),\(^6\) This persistent sodium current increased during hypoxia, and, thus, it was suggested that it could be involved in the development of early after depolarizations (EADs) and arrhythmias during hypoxic states. Most recently, Maltsev et al\(^7\) showed that a persistent sodium current (which they called the late sodium current) is present in ventricular myocytes from human midmyocardium in normal donor hearts and heart failure patients. Interestingly, they found a similar (15% to 20%) reduction in action potential duration (APD) after application of 1.5 \( \mu \)mol/L TTX, as previously described\(^8\) in guinea pigs. They also showed that TTX abolished EADs in myocytes isolated from heart failure patients. Computer modeling studies fit these data surprisingly well. It has been demonstrated\(^9\) that EADs can be induced in a cell model by increasing \( I_{\text{pNa}} \); this mechanism also requires that the inactivation curve of the fast sodium current is shifted in the depolarizing direction, as has been observed in an SCN5A missense mutation.\(^9\) An increase in late sodium current is also present in some well-known genetic predispositions to arrhythmias, for example, one type of the long-QT syndrome.\(^10\)

\( I_{\text{pNa}} \) is distinct from the sodium background current \( I_{\text{Na}} \), which is TTX-insensitive.\(^11\) Furthermore, \( I_{\text{Na}} \) has a linear voltage dependence, whereas \( I_{\text{pNa}} \) has an I-V relation similar to that of the fast sodium current. \( I_{\text{pNa}} \) is also different from the sodium window current,\(^12\) which is attributed to the overlap of the activation and inactivation curves of the fast sodium current in this region. The window current is TTX-sensitive but present only at much more negative voltages.

Computer modeling studies suggest that the magnitude of \( I_{\text{pNa}} \) in guinea pig ventricular myocytes can have very large effects on the action potential plateau duration.\(^13\) Therefore, it is important to determine the exact magnitude of \( I_{\text{pNa}} \) in such cells.

It is well-known that APD varies in myocytes from across the ventricular wall. The slow component of the delayed rectifier current \( I_{\text{K}} \) is at least in part responsible for the differences in APD. However, another possible mechanism could be a differential distribution in the transmural levels of \( I_{\text{pNa}} \), because it significantly prolongs APD in computer models, a finding supported by the available experimental evidence described above.

The aim of this study was, firstly, to determine the size and current-voltage relation of the persistent sodium current in guinea pig ventricular myocytes and, secondly, to test the hypothesis that differences in transmural distribution of persistent sodium current may contribute to the variation in APD across the ventricular wall.
Materials and Methods

Myocyte Preparation

Myocytes from subendocardium, midmyocardium, and subepicardium were prepared as previously described.\(^4\)

Solutions

The following solutions were used for all experiments, unless stated otherwise.

External solution (mmol/L): NaCl 140, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 1.0, Na\(_2\)PO\(_4\) 0.33, glucose 5, and HEPES 5; added to the external solution.

The standard Tyrode solution contained (mmol/L): NaCl 140, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 1.0, Na\(_2\)PO\(_4\) 0.33, glucose 5, and HEPES 5; pH 7.4 with NaOH.

Internal solution (mmol/L): NaF 10, CsF 109, MgCl\(_2\) 1, BAPTA 10, TrisATP 5, TEA-Cl 20, TrisCrP 5, and HEPES 5; pH 7.4 with CsOH.

For the Li-replacement experiments, 130 mmol/L LiCl was used to replace 130 mmol/L NaCl in the external solution, and 10 mmol/L LiCl replaced the internal NaF.

For the cadmium (Cd)-block experiments, 100 \(\mu\)mol/L CdCl\(_2\) was added to the external solution.

The standard Tyrode solution contained (mmol/L): NaCl 140, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 1.0, Na\(_2\)PO\(_4\) 0.33, glucose 5, and HEPES 5; adjusted to pH 7.4 with NaOH; osmolarity: 310 mmol/L.

Electrophysiological Procedures

Myocytes were allowed to settle for \(\sim\)5 minutes onto the cover slip of a perfusion chamber situated on the stage of an inverted microscope (Diaphot, Nikon). Initially, the cells were superfused with standard Tyrode solution (flow rate \(\sim\)1 to 2 mL/min) at 36°C.

The cells were then voltage clamped in the whole-cell configuration using 1-mm-square borosilicate glass electrodes of 2 to 5 MΩ resistance filled with internal solution. Superfusion with the external solution specified above was started. The currents were recorded using Axon Instruments equipment (Axopatch 200B and Digidata 1200) and software (Clampex, sampling rate 5 kHz, signal Bessel filtered at 1 kHz).

The size of the transient sodium current was monitored every 10 seconds with a 100-ms clamp step from a holding potential of \(-100\) to \(0\) mV.

After \(\sim\)2 minutes, the voltage step-protocol shown in Figure 1 (inset) was applied. TTX 63 \(\mu\)mol/L was added to wash on for about 3 minutes, and the protocol was repeated.

For the calculation of membrane capacitance, a voltage ramp (from \(-40\) to \(-50\) mV, slope: 5.5 V/s) was applied, and the capacitance was calculated offline by dividing dQ/dt, which corresponds to the maximal current measured \((I_0)\) by dV/dt.

Data Analysis

Currents were leak subtracted offline using pClamp 6. The difference currents (control and TTX) were calculated. The size of the difference current was measured as the average between 300 and 350 ms after the onset of the depolarizing pulse. Data collected from the same region of the ventricle were averaged, and SEM was calculated. Student’s \(t\) test was performed (using Sigma Plot 5.0) on the averages of the 3 different regions. \(P<0.05\) was taken as statistically significant.

Results

Isolated guinea pig ventricular myocytes from subendocardium, midmyocardium, and subepicardium were voltage-clamped using the ruptured whole-cell patch-clamp technique described above.

Examples of original traces, recorded using the protocol described above, are shown under control conditions and after application of TTX (Figure 1).

Figure 2 shows the magnitudes of the difference currents (before and after application of 63 \(\mu\)mol/L TTX) determined as the average between 300 and 350 ms after the onset of depolarization. The size of the difference currents did not change significantly when measured as the average between 700 and 900 ms. However, the difference currents were larger when measured at 150 ms after the onset of depolarization (data not shown).

The \(I-V\) relations indicate that this late TTX-sensitive current \((I_{\text{pNa}})\) is about equally large in subepicardial \((n=20)\) and subendocardial \((n=18)\) cells and smaller in midmyocardial \((n=20)\) cells.

The differences are statistically significant for the midmyocardium and endocardium and midmyocardium and epicardium means between \(-50\) and 0 mV \((P<0.01\) for the region \(-50\) to \(-20\) mV and \(P<0.05\) for \(-10\) and 0 mV). Subepicardial \(I_{\text{pNa}}\) is indistinguishable from subendocardial \(I_{\text{pNa}}\). The maximal current at membrane potentials of \(-30\) to \(-20\) mV is subendocardium \(-0.36\pm0.03\) pA/pF, midmyocardium \(-0.23\pm0.02\) pA/pF, and subepicardium \(-0.40\pm0.04\) pA/pF.

There was no significant difference between the membrane capacitances of subendocardial \((149\pm8\) pF, \(n=18)\), midmyoco

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Figure 1. Current recordings obtained during depolarizing voltage-clamp pulses from \(-130\) to \(-20\) mV (see inset). The traces show recordings during control conditions (control) and after application of 63 \(\mu\)mol/L TTX (+TTX) and the difference current (Diff).

Figure 2. Current-voltage relations of \(I_{\text{pNa}}\) in guinea pig ventricular myocytes from the subendocardium (○, \(n=18)\), midmyocardium (▌, \(n=20)\), and subepicardium (▼, \(n=13)\). Data points were measured as current density averages (±SEM) between 300 and 350 ms after the onset of depolarization.

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cardial (153±8 pF, n=20), and subepicardial (152±10 pF, n=13) cells.

**Control Experiments**

To exclude the influence of the Na⁺-Ca²⁺ exchanger on $I_{pNa}$ measurement in our conditions, NaCl was replaced by LiCl. Sodium channels are highly permeable to lithium, which, on the other hand, has been reported to block the Na⁺-Ca²⁺ exchanger.₁₅ Ventricular myocytes isolated from bulk ventricular tissue were tested after LiCl was used to replace NaCl in the external solution. The experiments described above were repeated, and the resultant I-V relation (Figure 3) was found to be very similar to the I-V relation for midmyocardial cells shown in Figure 2 (n=8).

To test the hypothesis that the TTX-sensitive current observed is attributable to influx through TTX-sensitive calcium channels that has recently been described,₁₆ 100 µmol/L Cd was added to the external solution. Cd at this concentration has been reported to produce a near-complete block of calcium currents.₁₇ However, the $I_{pNa}$ I-V relationship in the presence of 100 µmol/L Cd is not significantly altered (n=3; average current density at −20 mV with 100 µmol/L Cd: −0.18±0.02 pA/pF versus −0.23±0.02 pA/pF in midmyocardial cells under control conditions).

Additional evidence that $I_{pNa}$ is not carried by calcium ions was obtained under experimental conditions with reduced (0.2 mmol/L) calcium in the external solution. The I-V relation of $I_{pNa}$ obtained under these conditions is qualitatively similar to those obtained in 1.8 mmol/L calcium (n=4; average current density at −20 mV with 0.2 mmol/L calcium: −0.21±0.08 pA/pF versus −0.23±0.02 pA/pF in midmyocardial cells under control conditions).

Previous work⁵,⁷ suggested a block of the persistent sodium current by small (0.1 to 1.5 µmol/L) concentrations of TTX in rat and human myocytes. Thus, we determined the sensitivity of $I_{pNa}$ to block by TTX in guinea pig ventricular myocytes (Figure 4). TTX 1.5 µmol/L was sufficient to block $I_{pNa}$; however, application of 0.5 µmol/L TTX failed to produce a block.

**Computer Modeling Analysis**

The experimentally obtained I-V relations were fitted by the least-squares method to the general function: $I=G(V−E)/\left(1+\exp[V−V_i/k]\right)$, where $I$ is the current, $V$ is the voltage, $G$ is the maximum $I_{pNa}$ conductance, $E$ is the sodium equilibrium potential, $V_i$ is the voltage at half-maximal activation, and $k$ is the slope factor. For the purposes of the calculation, a reversal potential of +50 mV was assumed to avoid outward sodium current at potentials positive to the experimentally observed reversal potentials (which are around +33 mV). Incorporation of these I-V relationships into the standard guinea pig ventricular cell model in OXSOFT Heart 4.8 produced an increase in APD (Figure 5). The extent of the increase is related to the amplitude of the persistent sodium current. Note that the different $I_{pNa}$ formulations were applied to the same basic cell model, ie, there was no difference in the models used for the 3 regions, except for $I_{pNa}$. Obviously, this is an oversimplification to illustrate specifically the particular contribution of $I_{pNa}$ to APD differences in subendocardium, midmyocardium, and subepicardium.

The computed change in APD is most likely an underestimation, because in the computation it was assumed that $I_{pNa}$ has no time dependence and was taken as the experimentally observed average current between 300 and 350 ms after depolarization. $I_{pNa}$ is larger if defined as the TTX-sensitive current present earlier on during depolarization. Nevertheless, even relatively small amounts of $I_{pNa}$ would significantly prolong APD (Figure 5). Differences in $I_{pNa}$ across the ventricle could thus contribute to APD variations.

**Discussion**

The results presented here establish the presence and relative magnitude of $I_{pNa}$ in guinea pig myocytes from the basal free
left ventricle. There is a clear difference in the size of $I_{pNa}$ transmurally. Perhaps surprisingly, $I_{pNa}$ is smallest in the midmyocardium and of about equal size in the subepicardial and subendocardium.

It is interesting to compare our results with those on rat myocytes (B.N. Honen and D.A. Saint, unpublished data, May 2000). There is agreement in both species on the experimental side in showing that there are no large gradients of persistent sodium current between the epicardial and endocardial surfaces. However, when computations are done on the very different action potential shapes in the 2 species, the results are quite different. As shown here in the guinea pig (and presumably also in humans and in other species in which the action potential plateau is high and long lasting), the contribution of $I_{pNa}$ is significantly large. In the rat, however, with a much faster early repolarization phase and a low plateau, computations suggest virtually no contribution to determining action potential duration (P. Noble, unpublished data, May 2000).

$I_{pNa}$ is smallest in midmyocardial tissue, where it has been assumed to be largest, particularly in M cells. This discrepancy can be explained in several ways. $I_{pNa}$ could be smallest in M cells despite their long APD, assuming that differences in other currents, such as $I_k$, are responsible for their long APD. Alternatively, M cells could be a small subset of all midmyocardial cells and, therefore, only occasionally observed in midmyocardial single-cell preparations. In agreement with this, several studies failed to show significant evidence of M cells in guinea pig, rat, and pig hearts.

$I_{pNa}$ is more TTX-sensitive than $I_{Na}$ but has similarly shaped I-V relation. It is identified as a current carried by sodium ions. This is on the basis of the following evidence: $I_{pNa}$ is reversibly blocked by TTX and is still present when sodium is replaced by lithium, a blocker of the Na$^+$-Ca$^{2+}$ exchanger; it is still present when 100 $\mu$mol/L Cd is added, a blocker of Ca$^{2+}$ channels; and lowering the external calcium concent-

$\textbf{Figure 5. OXSOFT HEART 4.8 output.}$ Membrane voltage is shown in the top traces, and the total sodium current ($I_{Na}$ and $I_{pNa}$) is shown in the bottom traces. APD varies depending on the size of $I_{pNa}$: subepicardium (*), subendocardium (●), midmyocardium (○), and no persistent sodium current included in the calculation (□). The same basic cell model was used for all 3 regions, the only difference being the size of $I_{pNa}$.
and background were identified in guinea pig ventricular myocytes and rabbit Purkinje and ventricular myocytes. These discrete channel openings could represent different modes of function of the same Na\(^+\) channel. The burst sodium channel activity decreased with time, and inactivation was complete; however, this was not the case with background channel activity. Inactivation was slow with time, and inactivation was complete; however, this was not the case with background channels, because these only partly inactivated. Thus, it is possible that fast sodium channels in the background mode are the source of \(I_{\text{pNa}}\). Alternatively, it has been proposed that a naturally occurring isof orm of the fast sodium current could account for the persistent sodium current. The techniques used in the present paper, which enabled \(I_{\text{pNa}}\) to be recorded in close to normal physiological conditions, do not allow accurate recording of the fast sodium current, so these questions must be left to future studies.

In conclusion, this study establishes the presence of \(I_{\text{pNa}}\) in guinea pig ventricular myocytes and demonstrates that midmyocar dial myocytes have a lower density of \(I_{\text{pNa}}\) than subendocardial and subepicardial cells. These differences could be important in contributing to the variation in APD across the ventricle.

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References

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