Identification of Gating Modes in Single Native Na\textsuperscript{+} Channels From Human Atrium and Ventricle

Thomas Böhle, Mathias C. Brandt, Michael Lindner, Dirk J. Beuckelmann

Abstract—The aim of the present study was to investigate the single-channel properties of different gating modes in the native human cardiac Na\textsuperscript{+} channel. Patch-clamp experiments were performed at low noise using ultrathick-walled pipettes. In 17 cell-attached patches containing only one channel, fast back and forth switching between five different Na\textsuperscript{+}-channel gating modes (F-mode, M1-mode, M2-mode, S-mode, and P-mode) was identified, but no difference in the gating properties was found between normal and diseased cardiomyocytes from atrium or ventricle, respectively. Hodgkin-Huxley fits to the ensemble-averaged currents yielded the activation-time (\(\tau_a\)) and inactivation-time (\(\tau_i\)) constants. \(\tau_a\) was comparably fast in the F-mode, M1-mode, M2-mode, and S-mode (0.15 ms) and slow in the P-mode (0.3 ms). \(\tau_i\) ranged from 0.35 ms (F-mode) to 4.5 ms (S-mode and P-mode). The mean open-channel lifetime (\(\tau_o\)) was shortest in the F-mode and P-mode (0.15 ms) and longest in the S-mode (1.25 ms). The time before which half of the first channel openings occurred (\(t_{0.5}\)) was comparably short in the F-mode, M1-mode, M2-mode, and S-mode (0.3 ms) and long in the P-mode (0.9 ms). It is concluded that (1) a single native human cardiac Na\textsuperscript{+} channel can be recorded at low noise, (2) this channel can change its gating properties at a time scale of milliseconds, (3) lifetimes of the observed gating modes are short ranging from milliseconds to seconds only, and (4) the gating modes are characterized by specific activation and inactivation kinetics and differ at least in their mean open time and first latency. (Circ Res. 2002;91:421-426.)

Key Words: patch clamp ★ human myocardial sodium channels ★ modal gating ★ channel activation ★ channel inactivation

Although Na\textsuperscript{+} channels have been subject to intense electrophysiological investigation in various tissues,\textsuperscript{1} little is known about the native human cardiac Na\textsuperscript{+} channel. Previously published studies have been conducted in heterologous expression systems, where channel proteins are removed from their physiological environment.\textsuperscript{2–4} In these experiments, the Na\textsuperscript{+} channel \(\alpha\)-subunit (hH1) was expressed alone, or coexpression with the \(\beta\)-subunit was performed. Other methods used the reconstituted Na\textsuperscript{+}-channel protein fused into planar lipid bilayers.\textsuperscript{5} A major disadvantage of these methods is the artificial environment, in which electrophysiological experiments on the channel were performed. In the present study, results on single native human cardiac Na\textsuperscript{+} channels are presented. Our approach may lead to important insights into the physiological function of this channel protein.

Using this approach, arrhythmias may be understood in more detail. It has been shown that the LQT3 syndrome and the Brugada syndrome are based on different mutations in the gene (SCN5A, 3p21) encoding for hH1.\textsuperscript{5–8} In heterologous expression systems, a distinct mutation (\(\Delta K P Q\)) underlying the LQT3 syndrome is associated with late Na\textsuperscript{+} currents.\textsuperscript{9} From fits of the results of these experiments to different kinetic models, it was postulated that modal gating of the channel protein may be the reason for the late currents.\textsuperscript{9,10} Hence, genetic defects may underlie different rates of switching between gating modes. Therefore, the analysis of modal gating in native human cardiac Na\textsuperscript{+} channels may also help to better understand pathophysiological processes.

For the characterization of different gating modes, it is necessary to record at low noise from a patch, which contains no more than one channel. By using thick-walled patch pipettes\textsuperscript{11} with ultraclean tips,\textsuperscript{12} five distinct gating modes of Na\textsuperscript{+}-channel action (F-mode, M1-mode, M2-mode, S-mode, and P-mode) have been described in ventricular cells from adult white mice.\textsuperscript{13–15} They differ at least in activation and/or inactivation kinetics, open-channel lifetime, and first latency. In the present report, we identified these gating modes in atrial and ventricular cells of normal and diseased human myocardium. Durations of these modes were observed to be short.

Materials and Methods

Cell Isolation
Right atrial appendage specimens were obtained from patients suffering from coronary heart disease or aortic-valve disease under-
going heart surgery. The procedure for isolation of cardiomyocytes from human atrial appendages has been described in detail by Brandt et al.\textsuperscript{16} Single cells of nonfailing (not suitable for transplantation) and terminally failing (ischemic/dilated cardiomyopathy) ventricles were isolated according to the procedure described by Beuckelmann et al.\textsuperscript{17} The experimental protocol was designed to conform with the recommendations from the Declaration of Helsinki and approved by the ethical committee of the University of Cologne.

Solutions and Temperature
The cardioplegic solution used for transport of the tissue was composed of (in mmol/L) NaCl 15, KCl 9, MgCl\textsubscript{2} 4, histidine hydrochloride 18, histidine 180, tryptophan 2, mannitol 30, CaCl\textsubscript{2} 0.015, glutaric acid 1, and KOH 2. The modified Tyrode’s solution for cell isolation and storage contained (in mmol/L) NaCl 135, KCl 4, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 0.1, NaH\textsubscript{2}PO\textsubscript{4} 0.33, HEPES 10, and glucose 10 (pH 7.3). The experiments were performed at 23±1°C in a bath solution that contained (in mmol/L) KCl 230, CsCl 20, MgCl\textsubscript{2} 1, EGTA 10, and HEPES 5 (pH 7.3) and a pipette solution that contained (in mmol/L) NaCl 255, BaCl\textsubscript{2} 2.5, KCl 4, and HEPES 5 (pH 7.3). The elevated Na\textsuperscript{+} concentration was used to enhance unitary current amplitudes.\textsuperscript{18}

Patch Pipettes
For low-noise recordings,\textsuperscript{11} patch pipettes were prepared from thick-walled borosilicate glass tubing (Hilgenberg) with an external diameter of 2.0 mm and an internal diameter of 0.25 mm. The patch pipettes were inserted into a pipette holder of small dimension made completely of silver.\textsuperscript{11} Pipette tips were prepared only seconds before start of recording by breaking off the final tip region at the glass bottom of the bath chamber.\textsuperscript{12} Gigaohm seals were formed after touching the cell membrane by application of slight suction.

Data Acquisition and Analysis
Unitary Na\textsuperscript{+}-channel currents were recorded at a sampling rate of 100 kHz in the cell-attached patch configuration with an Axopatch 200B amplifier (integrating head stage, intrinsic noise 0.045 pA rms at 5 kHz, Axon Instruments, Inc). Analog filtering was performed with an intrinsic filter at a bandwidth of 10 kHz. When data needed further filtering for the analysis, an offline gaussian filter algorithm was used. The holding potential was −120 mV, duration of prepulses was 20 ms, and 4-ms pulses were applied at a rate of 10 Hz. Capacitive transients were compensated for carefully via compensation circuits containing two exponentials. Leak and residual capacitive currents were removed by subtracting averaged blank traces, which were formed exclusively from traces in the neighborhood of the time of the actual sweep. None of the patches in the present study showed any sign of containing more than one active Na\textsuperscript{+} channel. This was verified by inspecting several thousands of consecutive traces at pulses to −40 mV or more positive potentials to exclude the existence of any superimposition of opening events. Histograms of the open-channel lifetime were constructed by using the baseline method.\textsuperscript{11} Herein single-channel open times were calculated from the distribution of the opening-induced gaps in the middle of the baseline noise. In contrast to usual open-time histograms, the distribution of dwell times measured at the baseline contains both channel events (t\textscript{c}) and noise events (t\textscript{n}). For separating the t\textscript{c} from the t\textscript{n} distribution, an exponential can be fitted to the t\textscript{c} distribution, and the corresponding t\textscript{n} bins can be clipped for fitting the t\textscript{n} distribution. To improve resolution, amplitude histograms were formed by eliminating the transition points with the variance-mean technique.\textsuperscript{19} For curve-fitting, a derivative-free Levenberg-Marquardt routine\textsuperscript{20} was used. Recording was performed on a PC-80486 with P-clamp software (Axon Instruments, Inc). Analysis was performed on a Pentium personal computer with ISO2 software (MFk Computer) after converting the data with PCV software (MFk Computer).

Gating modes were selected by reidentifying blocks of traces from the average-of-interval plots. In these diagrams, the time course of an experiment is illustrated by plotting the averaged current of each individual sweep as a dot. The method of separating different gating modes is illustrated in Figure 1.

Figure 1A depicts a narrow part of an average-of-interval plot, in which switching between two different gating modes is detected. In Figure 1B, 16 consecutive sweeps in the F-mode (left) and 16 sweeps after switching to the S-mode (right) are illustrated. Both before and after the switch, empty sweeps, ie, traces without openings were excluded. Next (Figure 1C), ensemble-averaged currents of each suspected gating mode were formed from the traces in panel B.

Different blocks of traces in each suspected gating mode were combined to increase the number of openings and to yield smooth ensemble-averaged currents. This is demonstrated later in Results (see Figure 3). The mode-specific kinetics of the currents were then fitted (see also Figure 3) with a Hodgkin-Huxley model of the type \textit{Am}h.\textsuperscript{21} In this model, \textit{A} represents a scaling factor, and \textit{m} and \textit{h} denote parameters describing activation and inactivation, respectively, of the Na\textsuperscript{+} channel. \textit{m} is raised to the third power as activation is assumed to appear in several steps, ie, several conformational changes of the Na\textsuperscript{+}-channel protein are necessary for activation. This fitting procedure yields the activation-time constant (t\textscript{a}) and the inactivation-time constant (t\textscript{i}). We have chosen only one inactivation gate (\textit{h}) rather than two (\textit{h}, j), as often used in the more common Amh scheme, because we believe that \textit{h} and \textit{j} may represent two different gating modes. In contrast, fitting one gate to our ensemble-averaged current represents fitting of a single gating mode. The gating modes were further differentiated by the mean open-time

\begin{figure}[h]
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\includegraphics[width=0.5\textwidth]{figure1.jpg}
\caption{Method for the separation of different gating modes (ventricular cell from a nonfailing human heart, prepulse potential −120 mV, pulses to −40 mV; empty sweeps were excluded). A, Narrow part of the average-of-interval plot, in which switching from one gating mode to another was identified (each square dot represents the averaged current of an individual sweep of 4-ms duration; the narrow line indicates zero current, filter 10 kHz). B, Sixteen consecutive sweeps before and after switching from the F-mode to the S-mode (the arrow indicates beginning of the test pulse; the amplitude of short openings may be reduced by the filter of 5 kHz). C, Ensemble-averaged currents formed from the respective traces in panel B (the arrow indicates beginning of the test pulse, filter 5 kHz).}
\end{figure}
In both patches, long and short openings were observed. The amplitude of short openings may be reduced due to filtering.

Ensemble-averaged currents (Figure 2B; illustrated for the F-mode; left, atrial cell of a patient with coronary heart disease; right, ventricular cell from a nonfailing human heart), which have been constructed according to the method illustrated in Figure 1, reveal no obvious differences. Subsequently, the currents were fitted with a Hodgkin-Huxley model of the type Am³h (see Materials and Methods for details). The activation-time constant (τₐ) and the inactivation-time constant (τᵢ) are indicated in both fits.

To define the mean single-channel current amplitude at −40 mV, amplitude histograms were built. Figure 2C illustrates graphics from recordings of single Na⁺-channel currents from an atrial cell of a patient with coronary heart disease (left) and from a ventricular cell from a nonfailing human heart (right). Each distribution could be fitted with the sum of two gaussian curves, and the peak of the baseline noise is truncated. In both cell types, the mean amplitude of the open-channel current is essentially the same, −2.368 pA in the atrial cell and −2.309 pA in the ventricular cell. Furthermore, the single-channel conductance was not altered by mode switches (not illustrated).

Figure 3 shows ensemble-averaged currents of single Na⁺-channel openings at test pulses to −40 mV in the F-mode, M₁-mode, M₂-mode, S-mode, and P-mode, respectively. Gating modes were reidentified according to the method illustrated in Figure 1. Macroscopic kinetics of the Na⁺ current are characteristic in each mode. The kinetics of activation in the F-mode, M₁-mode, M₂-mode, and S-mode are not fully resolved, but the data show that in the P-mode τᵢ is slower by a factor of about two. Comparison of the first latency yields a respective factor of about three (see Figure 5). More accurate comparison is possible for τᵢ. Compared with the F-mode, the inactivation-time constant is nearly 2 times slower in the M₁-mode, more than 4 times slower in the M₂-mode, and S-mode are not fully resolved, but the data show that in the P-mode τᵢ is slower by a factor of about two. Comparison of the first latency yields a respective factor of about three (see Figure 5). More accurate comparison is possible for τᵢ. Compared with the F-mode, the inactivation-time constant is nearly 2 times slower in the M₁-mode, more than 4 times slower in the M₂-mode, and approximately 13 times slower in the S-mode, and P-mode, respectively.

The mode-specific kinetics of the currents were fitted with a Hodgkin-Huxley model of the type Am³h (see Materials and Methods for details). The magnitude of the parameters, activation-time constant (τₐ) and inactivation-time constant (τᵢ), is indicated for each mode. The kinetics of activation in the F-mode, M₁-mode, M₂-mode, and S-mode are not fully resolved, but the data show that in the P-mode τᵢ is slower by a factor of about two. Comparison of the first latency yields a respective factor of about three (see Figure 5). More accurate comparison is possible for τᵢ. Compared with the F-mode, the inactivation-time constant is nearly 2 times slower in the M₁-mode, more than 4 times slower in the M₂-mode, and approximately 13 times slower in the S-mode, and P-mode, respectively.

Figure 4 shows histograms of the open-channel lifetime in single-channel patches at test pulses to −40 mV in the F-mode, M₁-mode, M₂-mode, S-mode, and P-mode, respectively. Gating modes were reidentified according to the method illustrated in Figure 1. Macroscopic kinetics of the Na⁺ current are characteristic in each mode. The kinetics of activation in the F-mode, M₁-mode, M₂-mode, and S-mode are not fully resolved, but the data show that in the P-mode τᵢ is slower by a factor of about two. Comparison of the first latency yields a respective factor of about three (see Figure 5). More accurate comparison is possible for τᵢ. Compared with the F-mode, the inactivation-time constant is nearly 2 times slower in the M₁-mode, more than 4 times slower in the M₂-mode, and approximately 13 times slower in the S-mode, and P-mode, respectively.

Results

Figure 2 compares single Na⁺-channel currents of cells isolated from different regions of human hearts, which have been either healthy or in different disease states (prepulse potential −120 mV, pulses to −40 mV). A, Selected single Na⁺-channel currents of an atrial cell from a patient with aortic valve incompetence (left) and a ventricular cell from a nonfailing human heart (right). In both patches, long and short openings were observed (the arrows indicate beginning of the test pulses; the amplitude of short openings may be reduced by the filter of 5 kHz). B, Ensemble-averaged currents (shown here for the F-mode) of an atrial cell from a patient with coronary heart disease (left, 253 sweeps, filter 10 kHz) and a ventricular cell from a nonfailing human heart (right, 71 sweeps, filter 5 kHz), which have been constructed according to the method illustrated in Figure 1. The currents were fitted with a Hodgkin-Huxley model of the type Am³h (see Materials and Methods), yielding the activation-time (τₐ) and inactivation-time (τᵢ) constants (the arrows indicate beginning of the test pulses; empty sweeps were excluded). C, Amplitude histograms from single Na⁺-channel current recordings of an atrial cell from a patient with coronary heart disease (left, 752 sweeps) and of a ventricular cell from a nonfailing human heart (right, 893 sweeps). Each distribution was fitted with the sum of two gaussian curves, and the peak of the baseline noise is truncated, and the mean amplitude of the open-channel current is indicated (filter 10 kHz).

constant (τᵢ) and the time before which half of the first channel openings occurred (tₒ₁₃), as is also illustrated in Results (see Figures 4 and 5).
ms) are dominated by a short mean open time. The M1-mode ($\tau_o = 0.28$ ms) and the M2-mode ($\tau_o = 0.40$ ms) are dominated by intermediate mean open times. In the S-mode, two mean open times were found. One was long ($\tau_{o1} = 1.24$ ms) and the other was short ($\tau_{o2} = 0.12$ ms). These are addressed later (see Discussion).

Figure 5 shows histograms of the cumulative first latency in one-channel patches at test pulses to $-40$ mV in the F-mode, M1-mode, M2-mode, S-mode, and P-mode, respectively. Gating modes represent the same traces as those in Figures 3 and 4.

Cumulative first-latency distributions for the F-mode, M1-mode, M2-mode, and S-mode differ only slightly. The time before which half of the first channel openings had occurred ($t_{0.5}$) in each of these modes is $\approx 0.3$ ms. The time course of the cumulative first latency in the P-mode was much slower, yielding $t_{o5}$ of $\approx 0.9$ ms. In this mode, $t_{o5}$ may be even larger, because late first channel openings may have been cut off by the pulse duration of only 4 ms.

Because only traces with openings were reidentified from the average-of-interval plots, the probability of nonempty traces is zero in all plots.

Discussion

To our knowledge, this is the first description of the gating characteristics of single native human cardiac Na⁺ channels in atrium and ventricle. We looked for distinct Na⁺-channel gating modes (F-mode, M1-mode, M2-mode, S-mode, and P-mode), which have previously been characterized in myocardial cells from adult white mice. For this purpose, patches containing only a single Na⁺ channel were investigated. In previous studies, gating modes were identified from the long time course of the averaged current per trace (average-of-interval plots). There it was found that modal gating may happen in at least two different ways: (1) The lifetime of a specific mode may be rather long, ie, about some seconds to several minutes or (2) the lifetime of a specific mode may be rather short, ie, less than about a few seconds. The second possibility was derived from the fact that in some experiments, from only one large block of traces of the average-of-interval plots, two different mean open-time constants were found. We suggested that fast back and forth switching between two different gating modes was present in these recordings. Both in (1) and (2), the time course of switching itself was not resolved. We observed that it appeared from one pulse to the next, which means that at a pulse rate of 10 Hz, switching may have happened faster than 100 ms. Sometimes switching even appeared within one trace, indicating a time course of less than a few milliseconds. The gating modes in the present report were also identified from the average-of-interval plots. Because only fast back and forth switching between gating modes according to type 2 was observed, a new method had to be found to resolve different gating modes. The results indicate that identification of relatively small blocks of sweeps inherent in individual gating modes and their subsequent combination facilitates the analysis of characteristics of human Na⁺-channel gating modes.

We found that the mean open time was short and nearly identical in the F-mode ($\tau_o = 0.15$ ms) and in the P-mode ($\tau_o = 0.14$ ms). It was prolonged in the M1-mode ($\tau_o = 0.28$ ms) and in the M2-mode ($\tau_o = 0.40$ ms). In the S-mode, two different mean open-time constants were found. The first was
long ($\tau_n=1.25$ ms) and the second was short ($\tau_n=0.12$ ms). Because we were not able to separate these two time constants by selecting different traces, we conclude that only in this case switching between two different gating modes happened within the same trace. We assume that the long open time represents the S-mode, and the short open time represents the P-mode.

Cumulative first-latency distributions of different gating modes allowed determination of the time before which half of the first channel openings occurred ($t_{0.5}$). This was fast and nearly identical in the F-mode ($t_{0.5}=0.26$ ms), the M1-mode ($t_{0.5}=0.31$ ms), the M2-mode ($t_{0.5}=0.36$ ms), and the S-mode ($t_{0.5}=0.30$ ms). In contrast, it was slow in the P-mode ($t_{0.5}=0.92$ ms).

From the Hodgkin-Huxley fits of the ensemble-averaged currents, activation and inactivation kinetics of the different gating modes were calculated. The activation-time constant ($\tau_n$) was fast and similar in the F-mode ($\tau_n=0.14$ ms), the M1-mode ($\tau_n=0.17$ ms), the M2-mode ($\tau_n=0.17$ ms), and the S-mode ($\tau_n=0.14$ ms), and it was slow in the P-mode ($\tau_n=0.29$ ms). The inactivation-time constant ($\tau_i$) was fast in the F-mode ($\tau_i=0.35$ ms) and successively slower in the M1-mode ($\tau_i=0.62$ ms), the M2-mode ($\tau_i=1.56$ ms), and the S-mode ($\tau_i=4.57$ ms). In the P-mode ($\tau_i=4.50$ ms), it was identical to the S-mode.

The mean single Na$^+$-channel current amplitude was identical in atrial and ventricular myocytes, in normal and diseased tissue, and in different gating modes, ie, it was not altered by mode switching.

When the five gating modes of native human cardiac Na$^+$ channels are compared with those of adult white mice (see Table 1 in Böhle and Benndorf13; see Figure 3 in Böhle et al15), only slight differences can be found. The mean open times ($\tau_o$), the activation-time constants ($\tau_n$), and the inactivation-time constants ($\tau_i$) are almost identical. Whereas in human cardiac Na$^+$ channels the time before which half of the first channel openings occurred ($t_{0.5}$) was slightly slower in the F-mode, M1-mode, M2-mode, and S-mode, it was slightly faster in the P-mode. In tissue from adult white mice, fast back and forth switching was observed only between the S-mode and the P-mode. In contrast in human tissue, we found fast back and forth switching between all five gating modes. Thus, the main difference in modal gating of myocardial cells from adult white mice compared with human tissue is the respective lifetime of the five gating modes. In human tissue, in all five modes it was found to be only short. In tissue from adult white mice, a long lifetime could be shown in the F-mode, M1-mode, M2-mode, and the P-mode, but in the S-mode it was found to be only short. In the P-mode, it could be short, too.

All gating modes were found in both normal and diseased human atrium and ventricle, but no regulation site is yet detected. Modal gating may be regulated by (1) phosphorylation through protein kinase C$^{22}$ (brain Na$^+$ channels),$^{23-24}$ (cardiac Na$^+$ channels), and/or by protein kinase A$^{24-26}$ (cardiac Na$^+$ channels),$^{27-30}$ (cardiac L-type Ca$^{2+}$ channels), and (2) mechanisms involving intracellular Ca$^{2+}$,$^{31-33}$ or (3) mechanical stress on the plasma membrane during contraction of the heart muscle cell. In this context, it is important that the contraction-relaxation cycle of single heart-muscle cells is altered in terminal heart failure. Compared with nonfailing cells, the intracellular Ca$^{2+}$ concentration during relaxation, ie, during the diastolic phase, is increased, and during contraction systole, it is depressed. Additionally, the diastolic decay of the intracellular Ca$^{2+}$ concentration is markedly slowed.$^{17}$ When regulation sites (2) or (3) are discussed, it might be of further importance that different types of Na$^+$ current, presumably reflecting different gating modes, have been identified in single beating cells of chick embryo heart and have been correlated to different phases of the action potential by direct measurement.$^{34-36}$ From these results, we suspect that synchronous appearance of gating modes may be triggered by contraction of the muscle cell.

Acknowledgments

This research was supported by grants from the Bundesministerium für Bildung, Forschung und Technologie (BMBF 01 KS 9502, ZMMK, Projekt 4), and the M. and W. Boll-Stiftung. We thank Nadine Henn and Iris Berg for their excellent technical assistance, Harald Metzner and Jürgen Staszewski (Center of Physiology and Pathophysiology, University of Cologne) and their colleagues for their continual technical advice and support, and Prof DeVivie (Department of Cardiac Surgery, University of Cologne) and his colleagues for providing the myocardial tissue.

References

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Circ Res. 2002;91:421-426; originally published online August 15, 2002; doi: 10.1161/01.RES.000003521.38733.EF

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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