Late Na Channels in Cardiac Cells: The Physiological Role of Background Na Channels

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ABSTRACT Two types of the late Na channels, burst and background, were studied in Purkinje and ventricular cells. In the whole-cell configuration, steady-state Na currents were recorded at potentials (−70 to −80 mV) close to the normal cell resting potential. The question of the contribution of late Na channels to this background Na conductance was investigated. During depolarization, burst Na channels were active for periods (up to ~5 s), which exceeded the action potential duration. However, they eventually closed without reopening, indicating the presence of slow and complete inactivation. When, at the moment of burst channel opening, the potential was switched to −80 mV, the channel closed quickly without reopening. We conclude that the burst Na channels cannot contribute significantly to the background Na conductance. Background Na channels undergo incomplete inactivation. After a step depolarization, their activity decreased in time, approaching a steady-state level. Background Na channel openings could be recorded at constant potentials in the range from −120 to 0 mV. After step depolarizations to potentials near −70 mV and more negative, a significant fraction of Na current was carried by the background Na channels. Analysis of the background channel behavior revealed that their gating properties are qualitatively different from those of the early Na channels. We suggest that background Na channels represent a special type of Na channel that can play an important role in the initiation of cardiac action potential and in the TTX-sensitive background Na conductance.

INTRODUCTION

In addition to generation of the action potential upstroke, the Na conductance in cardiac cells is involved in the regulation of the action potential duration and the resting membrane potential. The best support for these roles has been obtained using the specific Na channel blocker tetrodotoxin (TTX). TTX decreased the action potential duration (Dudel et al., 1967; Attwell et al., 1979; Coraboeuf et al., 1979) and induced a hyperpolarization of the resting membrane potential (Coraboeuf et al., 1979; Deitmer and Ellis, 1980; January and Fozzard, 1984; Imanishi et al., 1987). With step depolarizations, Na currents were detected at times that exceeded the duration of typical action potentials in ventricular (Patlak and Ortiz, 1985; Kunze et al., 1985; Grant and Starmer, 1987; Kiyosue and Arita, 1989; Lee, 1992; Saint et al., 1992) and Purkinje (Gintant et al., 1984; Carmeliet, 1987) cells. During action potentials recorded from spontaneously active cardiac cells, late opening Na channels were identified (Liu et al., 1992). At the single-channel level, two patterns of late Na current were observed: isolated brief openings (background currents) and repetitive openings (burst currents) lasting throughout most of the depolarization. Although both patterns of channel activity might contribute to the plateau duration, the role of either channel type in the membrane conductance in the range of the resting potential is unknown. The prior studies examined only potentials positive to threshold.

The studies of Patlak and Ortiz (1985) and Grant and Starmer (1987) showed that during depolarization trials with burst of openings occurred in clusters. This suggests that the burst state is long-lived. In these earlier studies, the burst lasted throughout the depolarizing pulses. If the burst channels do not undergo deactivation on repolarization, they could contribute to resting Na conductance. It is not known whether bursting channels undergo inactivation if depolarization is maintained.

Kiyosue and Arita (1989) reported that isolated brief openings (mean open time 0.2 ms) persist during maintained depolarization at −50 mV. Their rare occurrence and brief duration suggested that they played a minor role in the control of action potential duration. The behavior in the range of the normal resting potentials has not been described.

Using single sodium channel recordings in Purkinje and ventricular cells, we determined the occurrence of deactivation and inactivation in burst Na channels. The single channel nature of the Na conductance in the region of the resting membrane potential was also analyzed. The channels observed in the region of normal resting potential appear to be kinetically distinct from those responsible for the early transient current. In whole-cell voltage clamp experiments in rat ventricular myocytes, Saint et al. (1992) observed a component of sodium current activated at hyperpolarized potentials and having increased TTX sensitivity. The results are consistent with prior studies of Gilly and Armstrong (1984) describing "threshold" Na channels in the region of the threshold potential.

MATERIALS AND METHODS

Experiments were performed on enzyme-dissociated rabbit Purkinje and ventricular myocytes. The procedure of cell isolation has been described elsewhere (Grant and Starmer, 1987).

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For whole-cell recording, the micropipettes were filled with the following solution (in mM): CsF 120, $MgCl_2$ 0.1, K_2ATP 5, EGTA 5, KH_2PO_4 1, HEPES 5, and Glucose 5. The pH was adjusted to 7.3 with CsOH. The external solution was composed of (in mM): NaCl 130, KCl 5, $CaCl_2$ 0.2, $MgCl_2$ 1, Glucose 5, and HEPES 10. The pH was adjusted to 7.4 with NaOH.

For cell-attached recordings, the myocytes were superfused with a high potassium solution of the following composition (in mM): KCl 70, K-aspartate 80, NaCl 5, $MgCl_2$1, EGTA 0.05, HEPES 5, NaH_2PO_4 12, and glucose 10. The pH was adjusted to 7.4 with KOH. The micropipettes were filled with solution of the following composition (in mM): NaCl 180, CsCl 1, $MgCl_2$ 1, $BaCl_2$ 0.1, and HEPES 5. The pH was adjusted to 7.4 with NaOH. All experiments were performed at room temperature (22–23°C).

Single-channel currents were obtained with a patch clamp amplifier (EPC-7, List Medical System Corp., Darmstadt, Germany), filtered at 2.1 KHz (8 pole Bessel, Model 902 LPF, Frequency Devices, Haverhill, MA), and recorded on tape (Racal Instruments, 4DS, Vienna, VA). They were subsequently digitized at 20 KHz (TLI interface, Axon Instruments, Burlingame, CA) and stored on fixed drive of a computer (SPARC, Sun Microsystems, Inc.) for further analysis.

Because the total duration of bursts was not known a priori, the duration of test pulses could not be preselected. Depolarizing pulses were initiated manually and continued until there was closure of any opening channels.

Analysis of the background channel openings was performed from data acquired after a step depolarization. Each trial (a step depolarization from a holding potential) was analyzed by dividing it into two segments: an early segment associated with "normal" fast (early) Na channels, and a late segment, thought to be associated with late channels. Definitions of early and late currents have been suggested previously by Patlak and Ortiz (1985). The value of the transition point (separating hypothesized early events from late events) was evaluated from the inactivation time of fast sodium currents reported elsewhere (Gilliam et al., 1989; Brown et al., 1981; Zilberter and Timin, 1982). It was dependent on the membrane potential ranging from 30 ms at −30 mV to 150 ms at −60 to −80 mV. Additional analyses were based on channel activity during constant depolarization. Here we set the membrane holding potential to a certain value (e.g., −80 mV), waited at least 10 s, and then started analysis of events after this time. The duration of the constant potential epochs ranged from 2 to 10 min.

An automatic detection scheme with the threshold set at 0.5 times the single-channel amplitude was used to identify channel openings. Single-channel current amplitude was determined as a mean of clearly resolved events. The performance of algorithm was always checked by comparing raw current traces with the idealized records.

Histograms of channel open time distributions were fitted to single exponentials using a least-squares procedure. Validity of fitting of exponentials to such fits has been provided by theoretical analysis and simulation done in our laboratory (Hurwitz et al., 1991). The mean channel open time measured in each experiment is quoted as mean ± SE. Data collected from different experiments are equated as mean ± SD. Statistical significance of differences was determined using paired sign test.

RESULTS

The existence of a steady-state Na conductance in the range of the normal resting potential was examined by application of TTX in the whole-cell voltage clamp configuration. Fig. 1 shows holding currents recorded from a myocyte before and during TTX application at constant depolarization of −70 and −80 mV. These potentials were negative to that required for activation of a transient Na current. At −80 mV, application of TTX resulted in a reduction of inward current by 25 pA at −80 mV and by 30 pA at −70 mV. In five cells, the average current change was 22.3 ± 12.4 and 15.3 ± 9.1 pA at −70 and −80 mV, respectively. These experiments confirm the presence of a steady-state sodium conductance in the range of the resting potential. The remainder of the experiments in this study were designed to examine the nature of this conductance using single-channel recordings in the cell-attached configuration.

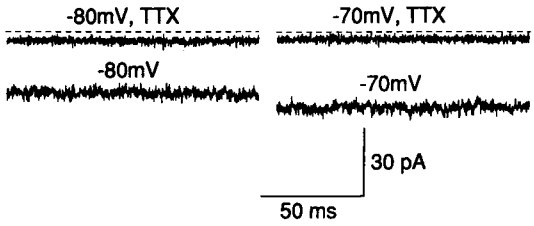

FIGURE 1 Whole-cell currents at constant potentials recorded before and after application of 200 μM TTX. The holding potential was fixed at −80 or −70 mV. TTX decreased the inward currents by 25 and 30 pA at −80 and −70 mV, respectively.

Recordings with at least 100 trials at one potential were obtained in 17 Purkinje and 33 ventricular cells. With the low resistance microelectrodes employed in these studies, the initial ensemble averaged peak current was 30–500 pA at −40 mV depolarization. The late openings of Na channels were qualitatively similar in both cell types. Bursting and background channels were TTX-sensitive; with micropipette solution containing 50 μM TTX, both early and late channel activity were abolished.

Response of bursting Na channels to maintained depolarization and repolarization

The response of burst Na channel to prolonged step depolarization was determined at −40 mV. Each depolarizing trial was terminated only after it was evident that the channel had closed for several seconds. Examples of bursting selected in different Purkinje and ventricular cells are illustrated in Fig. 2.

Burst channel activity often continued for more than 3 s. In some cases, Na channels demonstrated different gating modes during prolonged depolarization (see traces 1, 6, and 8). For example, in the trace 1 there are four long openings in the time interval marked by arrows. Their averaged open time is 368 ms. The time distribution of other openings in this burst (551 events) is well approximated by one exponent (τ_o = 5.4 ms) giving estimated probability density

$$f(t) = 1/\tau_o \cdot \exp(-t/\tau_o) = 0.18 \cdot \exp(-0.18 \cdot t);$$

The probability of obtaining an opening with the duration of $\tau_1 > 368$ ms, therefore, is

$$P(\tau > \tau_1) = \int_{\tau_1}^{\infty} f(t) \cdot dt = 4.1 \cdot 10^{-30}$$

This result suggests that the prolonged openings arise from the switching of channel gating between two modes rather than a part of a single distribution of open times. Qualitatively similar results have been obtained for long channel openings in traces 6 and 8.

Because of the heterogeneity of open and closed times within burst, we do not present data of their averaged kinetics. In many cases, however, the difference in channel

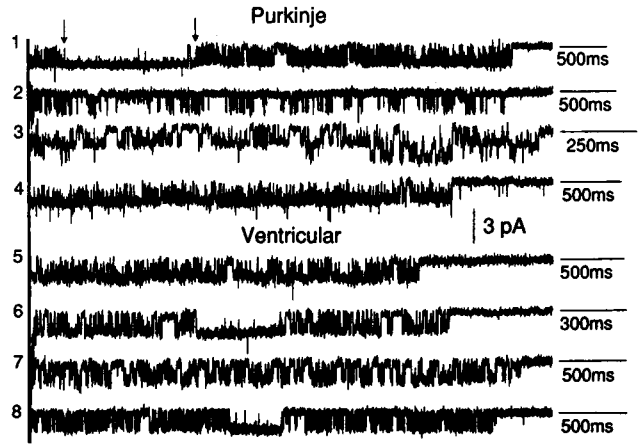

FIGURE 2 Selected examples of bursts of Na channel activity recorded from Purkinje and ventricular cells. Currents were elicited by depolarizations from a holding potential of −120 mV to a test potential of −40 mV. Each trial was recorded from separate myocytes, and the pulses were terminated only after it was evident that the channel had closed for several seconds. In each case, the channel closed finally without reopening, demonstrating the existence of slow and complete inactivation. The apparent mode of gating changed during trials 1, 6, and 8 (amplified in text). For each trial, the time calibration is shown on the far right; it was adjusted according to the total duration of the burst.

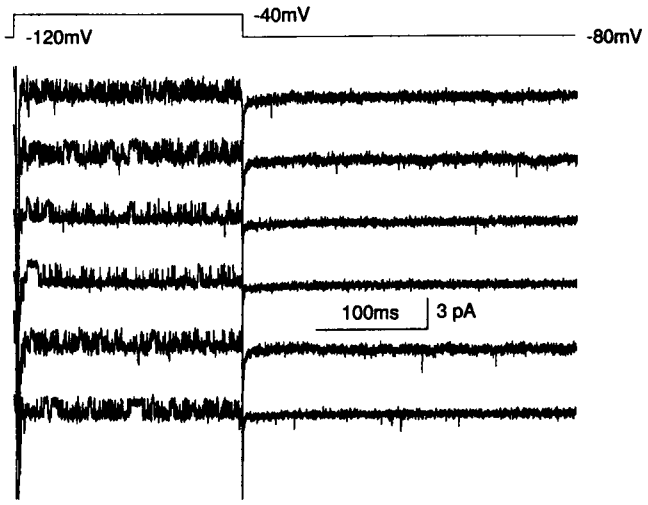

FIGURE 3 Burst Na channels close quickly after repolarization. Selected trials of membrane currents during bursts recorded from ventricular myocytes. Trials in which the channel was open at the end of the voltage pulse (depolarization from a holding potential of −120 mV to a test potential of −40 mV) were selected for illustration. In each trial, the bursts of openings ended promptly on repolarization to −80 mV. This potential is close to the resting value in those cells. Note the occurrence of brief background openings on repolarization to −80 mV.

modes was prominent. In all cases, channels finally closed and did not reopen for many seconds during maintained depolarization. Average duration of 73 bursts of at least 40-ms duration was 720 ± 1204 ms. Thus, we conclude that the burst channels undergo some kind of slow and complete inactivation.

The prolonged duration of the bursts suggests that they could contribute to the resting Na conductance in the interval between action potentials. Such a role would be dependent on the incomplete or delayed channel deactivation on repolarization. We examined the response of bursting channels to repolarization in four cells. In 910 depolarizing epochs, 22 channel bursts with duration exceeded 200 ms were recorded. In each case, the burst terminated promptly on repolarization to −80 mV that is close to the normal resting potential (Fig. 3). We conclude that the bursting mode of channel gating does not contribute to resting Na conductance because of rapid deactivation on repolarization.

Background Na channels

Unlike the burst Na channel openings, background Na channel openings were observed as unclustered, brief single openings. For many depolarizing epochs, these openings were seen more frequently than burst activity. For example, in five experiments the average rate of appearance of burst and background channel openings at −40 mV, 400-ms depolarizations were $(8.58 \pm 4.08)\cdot 10^{-4}$ and $(2.13 \pm 0.73)\cdot 10^{-2}$ openings per pulse per pA of peak early current (ensemble averaged), respectively.

We determined the occurrence of background Na channel openings during sustained depolarization without preceding hyperpolarization to remove inactivation. At potentials ranging from −120 to 0 mV, channel openings were detected. Fig. 4 represents current traces at −40 and −70 mV. Sections marked by arrows are shown with a larger time scale. Note that many channel openings could not be resolved completely at 2.1 KHz recordings because of their short open time. Amplitude of ensemble averaged early peak Na current was 500 pA in this experiment and τ_o was 0.34 ms for −40 mV (818 events) and 0.42 ms for −70 mV (570 events).

Channel activity during constant depolarization might mean that background Na channels lack inactivation. To test this, we first compared the average number of late channel

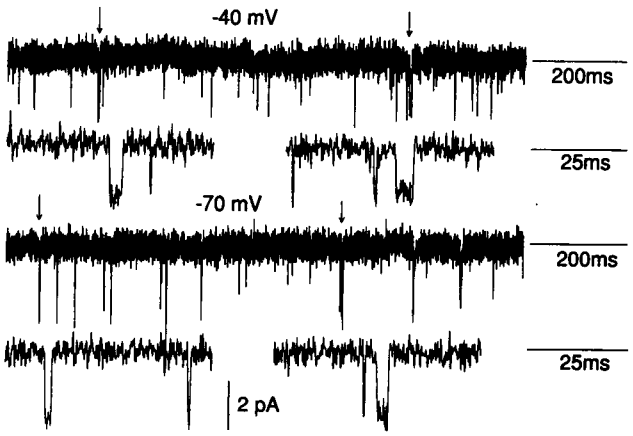

FIGURE 4 Background openings of single Na channels during maintained depolarization at −40 and −70 mV. The current at the instant of each arrow in traces 1 and 3 is shown on an expended time scale in traces 2 and 4.

openings (N_o) during 400-ms depolarizations to −40 mV with or without preceding hyperpolarization to holding potential of −120 mV. In five experiments, N_o was 2.02 ± 1.9 after the conditioning hyperpolarization and 1.07 ± 1.13 without preceding hyperpolarization ($p < 0.03$). Thus, the initial hyperpolarization markedly increased the probability that the late Na channels would open in response to depolarization. This suggests that a fraction of channels recovered from inactivation during hyperpolarization.

The fact that, on one hand, N_o was dependent on preliminary hyperpolarization and, on the other hand, that channels could be active at constant potentials for long periods of time led us to hypothesize that late Na channels undergo incomplete inactivation. In this case a macroscopic late Na current in response to depolarization should decay and approach a steady-state level. To explore this hypothesis for an ensemble of channels, we first idealized current traces (to avoid the background noise) and then summed an equal number of traces at each potential in six cells. Fig. 5 represents the results of this procedure in a typical experiment. One hundred 400-ms current epochs were idealized and summed for each potential. The blank region at the beginning of each epoch includes the initial capacitative and transient currents excluded from the summary data. Late channel activity decreases in time at each potential, i.e., channels undergo inactivation. However, the opening probability was not zero even after a 400 ms.

As indicated above, it is important to distinguish background channel openings from openings related to the initial transient current occurring with long latency. We employed a number of approaches to distinguish these types of openings. As an initial approach, we measured τ_o over a range of test potentials. At −70 and −80 mV background channels were arbitrarily defined as those openings occurring between 150 and 400 (or 500) ms after the onset of depolarization. Fig. 6 illustrates the typical dependencies of τ_o on membrane potential. At most potentials, the early channel mean open time exceeded that for background channels. Both curves were bell-shaped with maxima separated by 20–30 mV. At potentials more positive than −70 mV, we conclude that these two voltage dependencies represent Na channels exhibiting different gating properties. However, open times were similar at −70 to −80 mV, suggesting that background channel openings and early channel openings occurring with long latency cannot be distinguished solely on the basis of open time. In five experiments, τ_o for early Na channels was 0.89 ± 0.15 ms at −40 mV and 0.66 ± 0.12 ms at −70 mV ($p < 0.03$); for background Na channels τ_o was 0.34 ± 0.061 ms at −40 mV and 0.49 ± 0.082 ms at −70 mV ($p < 0.03$).

To obtain more detailed information on channel heterogeneity at −70 and −80 mV, in six experiments we compared τ_o at three different intervals after the onset of depolarization: 20–50, 50–150, and 150–400 (or 500) ms. In Fig. 7, current traces at −70 and −80 mV are shown. Each trace was obtained by summing 100 idealized epochs generated from a holding potential of −120 mV. All epochs that contained more than two overlapping channel openings during the interval analyzed were discarded from the calculation of τ_o. At −70 mV, τ_o was 0.69 ± 0.015 ms (1717 analyzed events) in the range of 20–50 ms, 0.60 ± 0.015 ms (1805 analyzed events) in the range of 50–150 ms, and 0.61 ± 0.019 ms (244 analyzed events) in the range of 150–400 ms. At −80 mV, τ_o was 0.43 ± 0.015 ms (511 analyzed events) in the range of 20–50 ms, 0.41 ± 0.02 ms (555 analyzed events) in the range of 50–150 ms, and 0.39 ± 0.018 ms (343

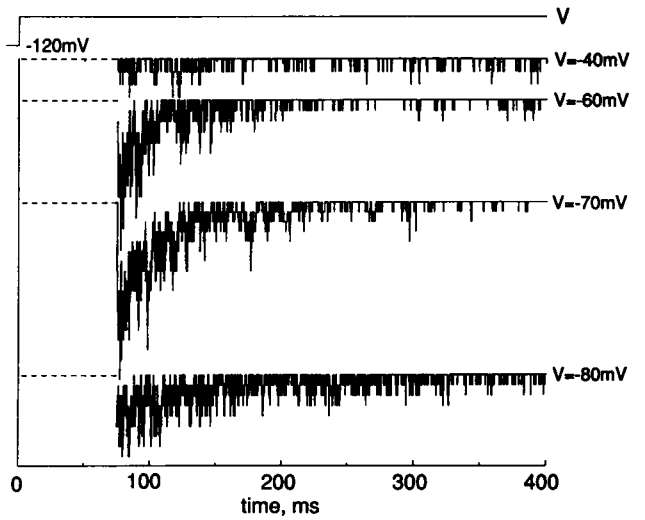

FIGURE 5 Background Na channels undergo inactivation. Each trace represents the sum of 100 idealized currents in response to 400-ms depolarizations from a holding potential of −120 to −80, −70, −60, and −40 mV. At each test potential, the single-channel current amplitude has been normalized. The blank region at the beginning of each trial includes the capacitative and initial transient current that is excluded from the summary data. Note the time-dependent decrease in late channel activity at each potential.

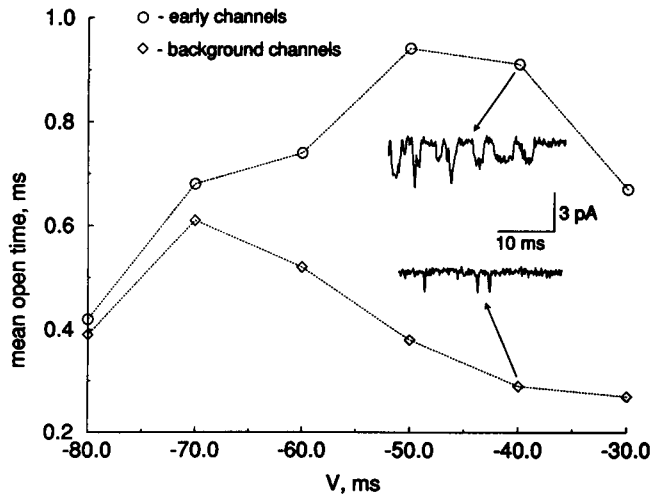

FIGURE 6 Dependence of the mean open time of early (○) and background (◇) openings on membrane potential. The mean open times were estimated from openings in 100 or more trials recorded from ventricular myocytes at each of the test potentials shown. The openings at −40 mV are shown in the insets and are representative of early and background channels.

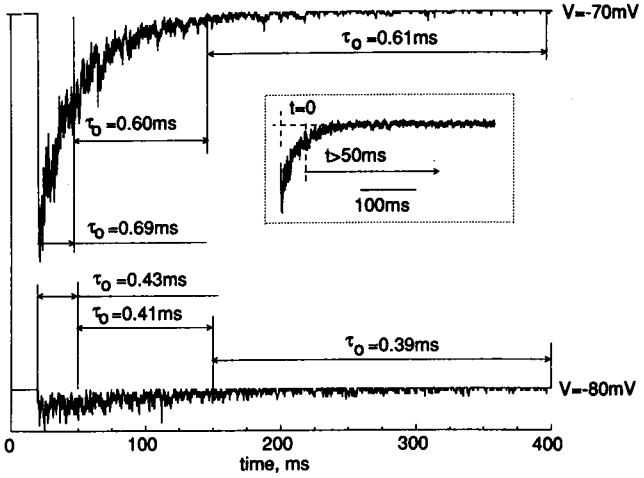

FIGURE 7 The mean open time of Na channels in different time ranges of step depolarization to −70 or −80 mV. Each trace reproduces a sum of 100 idealized currents in response to depolarization from −120 mV holding potential. All epochs that contained more than two overlapping channel openings during the interval analyzed were discarded from the calculation of τ_o. At both potentials, τ_o at the beginning of depolarization differs significantly from that at the end of depolarization (see text). Ensemble averaged record of 100 original current epochs at −70 mV is shown in the inset. After 50 ms of depolarization (where τ_o becomes homogeneous), current amplitude is about 20% of peak current amplitude.

analyzed events) in the range of 150–400 ms. From the data obtained at −70 mV, we conclude that 50 ms after the onset of depolarization, τ_o became stationary, suggesting a single gating mechanism at these times. During the first 50 ms of depolarization, we hypothesize that there is a mixture of early and background Na channel activity contributing to the longer τ_o. Ensemble averaged record of 100 original current epochs is shown in the inset. After 50 ms of depolarization, current amplitude is about 20% of peak current amplitude. At −80 mV, there is also significant difference between τ_o in the beginning and in the end of depolarization. Qualitatively similar results were obtained in other experiments (see Table 1).

Other arguments in favor of prominent channel heterogeneity at potentials close to the fast channel activation threshold were obtained in three experiments where we analyzed τ_o after a step depolarization to −70 mV (from −120 mV holding potential) with or without a 10-ms prepulse to 0 mV. The presence of the prepulse caused inactivation of most of the early Na channels and, presumably, also a large fraction of the background Na channels. We predicted that the mean open time of late openings at −70 mV would be the same as that occurring throughout the test pulse to −70 mV, which was preceded by the 10-ms prepulse. In Fig. 8, the sum of 140 idealized epochs obtained either without (*top*) or with (*bottom*) the prepulse are shown. In the first case, τ_o is 0.47 ± 0.019 ms in the range of 15–50 ms after the beginning of depolarization (432 analyzed events) and 0.40 ± 0.028 ms in the range of 150–500 ms (220 analyzed events). After the prepulse, fewer channel openings (106 events in comparison to the 220 events measured from 150–500 ms after depolarization) were detected in the 25–500 ms time range (lower current trace). However, τ_o for these openings was 0.40 ± 0.040 ms, which is equal to the τ_o in the tail of the upper trace.

As a last test to confirm that background channels provide currents in the inactivating region of macroscopic currents induced by step depolarizations in the vicinity of the fast Na current activation threshold and the cell resting potential, we compared τ_o at step depolarizations with τ_o measured after several seconds (>10) of maintenance of a constant potential ($n = 5$). Examples of open time distribution during different time ranges of depolarization and at constant potentials are shown in Fig. 9. As is seen from these results and from data in Table 1, at both −70 and −80 mV potentials τ_o measured 150 ms after the onset of depolarization differ significantly from τ_o measured at the beginning of depolarization and is similar to the τ_o at constant depolarization. These data also suggest similarity of the background channels detected either in response to step depolarization or after long duration of depolarization at a constant potential.

TABLE 1 τ_o in different time ranges of depolarization

Cell	V (mV)	τ_o (ms) in time range (ms) of step depolarization			τ_o (ms) at constant V
		20–50	50–150	150–500	
01.29.93	−70	0.69 ± 0.015	0.60 ± 0.015	0.61 ± 0.019	
		n = 1717	n = 1605	n = 244	
	−80	0.43 ± 0.015	0.41 ± 0.02	0.39 ± 0.018	
		n = 511	n = 555	n = 343	
02.15.93	−70	0.33 ± 0.018	0.28 ± 0.01	0.27 ± 0.01	0.30 ± 0.041
		n = 362	n = 403	n = 258	n = 71
	−80	0.29 ± 0.031	0.25 ± 0.014	0.22 ± 0.014	
		n = 90	n = 85	n = 131	
03.15.93	−70	0.47 ± 0.019	0.42 ± 0.016	0.40 ± 0.28	
		n = 432	n = 475	n = 220	
04.01.93	−70	0.86 ± 0.023	0.53 ± 0.019	0.42 ± 0.021	0.42 ± 0.019
		n = 1228	n = 864	n = 557	n = 570
10.20.93.1	−70	0.50 ± 0.029	0.43 ± 0.045	0.32 ± 0.041	0.29 ± 0.025
		n = 268	n = 100	n = 51	n = 138
	−80	0.48 ± 0.01	0.38 ± 0.008	0.30 ± 0.015	0.28 ± 0.012
		n = 1672	n = 1645	n = 366	n = 417
10.20.93.2	−80	0.26 ± 0.012	0.26 ± 0.009	0.23 ± 0.010	0.21 ± 0.011
		n = 304	n = 498	n = 412	n = 332

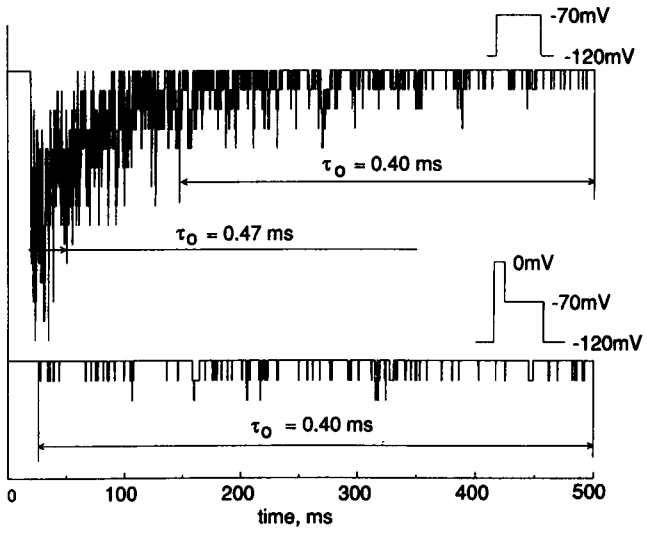

FIGURE 8 The mean open time of Na channels in control (*top*) and after the 10-ms prepulse to 0 mV (*bottom*). Each trace reproduces the sum of 140 idealized currents. After inactivation of early Na channels by the prepulse (*bottom*), τ_o of the remaining channels is similar to that of channels in the 150–500 ms range of the upper trace.

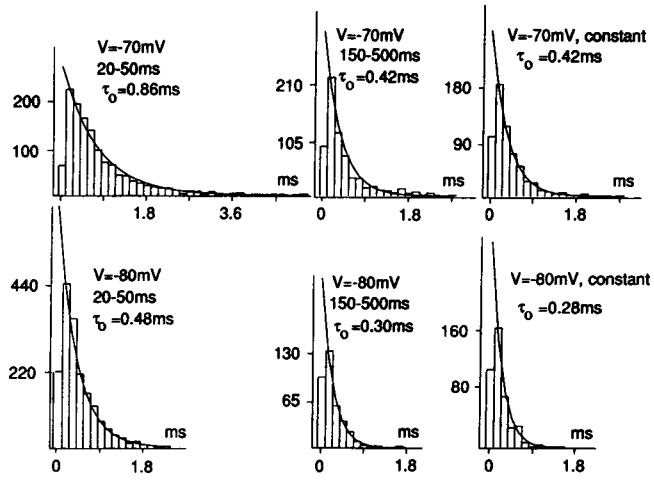

FIGURE 9 Distributions of the background Na channel mean open time in different time ranges of step depolarization and at constant potential. At each histogram, the time range is noted with respect to the depolarization onset. At −70 and −80 mV step depolarization, τ_o at the beginning of depolarization is significantly larger than at the end of depolarization and is similar to τ_o at constant potential.

To estimate the average number of background channels active in the range of cell resting potential, we measured N_o at a −80 mV constant potential in three patches with a similar transient Na current amplitude in response to −40 mV step depolarization (215 ± 8 pA). N_o was 3.2 ± 0.6.

DISCUSSION

In the present paper, we demonstrate the existence of steady-state Na currents in the range of normal cardiac cell resting potential. From two types of late Na channel activity, burst and background, burst channel openings unlikely contribute to the background Na conductance. Bursts terminate on repolarization to the resting potential level. Background channels, on the contrary, sustain activity at this potential. Moreover, they underlie the significant fraction of Na current in the range of activation threshold.

We observed background Na channel activity much more frequently than activity of burst Na channels. Similar observations have been reported by Patlak and Ortiz (1986) and Kiyosue and Arita (1989). However, in these studies the open probability of background Na channels was much less than that for the early Na channel. It was suggested that background channels represent an additional "mode" of the early Na channel gating in which they can rarely reopen from the inactivation state (Patlak and Ortiz, 1986). One of the main arguments in favor of this hypothesis was based on the existence of a correlation between the amplitude of early Na current and the background channel activity (Patlak and Ortiz, 1986). However, one can suggest an alternative explanation of this correlation based on variations in the patch size that exist within the pipette: if we assume different channel populations (fast and background), then large patches will reveal both large early and large background currents, whereas small patches will reveal less current from both classes.

The fact that early and background channels have similar conductance (Patlak and Ortiz, 1986; Kiyosue and Arita, 1989) and are both TTX-sensitive (our results; see also Kiyosue and Arita, 1989) complicates investigation of a relationship between them. However, our results show that kinetic properties of these two potential classes of Na channel activity are very different. We used the mean open time, τ_o, as the main criterion to distinguish between the early and the background channel openings. For most potentials tested (−80 to −20 mV), τ_o for background Na channels appeared to be much less than for early Na channels (see Fig. 8). Moreover, the voltage dependence of τ_o was different for the two channel types. Another property of the background Na channels that distinguishes them from early ones is their ability to open at a constant membrane potential. Background channel openings could be recorded for many minutes at steady-state potentials in the range from −120 to 0 mV. Finally, the background channels underlay a significant fraction of total Na current at −70 and −80 mV, suggesting a little correlation between activity of early and background channels. This fact and the results discussed above lead us to suggest that background and early Na channels are distinct types and are independent of each other.

Channel openings at constant potentials might indicate a lack of an inactivation mechanism or incomplete inactivation. Our data suggest that the background Na channels undergo incomplete inactivation. Partial inactivation was clearly revealed by analysis of ensemble channel behavior (see Fig. 5). At each potential, the background channel activity decreased with time, approaching a steady-state level. In previous studies (Kiyosue and Arita, 1989; Patlak and

Ortiz, 1986), the background channel activity was investigated for a relatively positive potential range (−50 to −20 mV). These authors estimated the number of the background channel openings without consideration of channel inactivation. The true number of the background channels could have been significantly underestimated.

At potentials in the range of fast Na current activation threshold, the background channels contributed significantly to total Na channel activity. We applied three different approaches to distinguish between channel types at −70 mV potential. First, we established that after step depolarization, τ_o measured within the initial 50 ms was significantly larger than that in the range of 150–400 (500) ms. We interpret this result in the following way: mixed channel activity predominates at the beginning of depolarization, whereas after 150 ms background channels dominate. We can state also that background channels dominate in the mixed activity in the range of 50–150 ms. This is evident in the experiment in Fig. 9 (*top*), where τ_o is similar for 50–150 and 150–400 ms time ranges. Let us consider results of another experiment (cell 04.01.93 in Table 1) in which τ_o in the time range 50–150 ms is intermediate between those in 20–50 and 150–500 ms. We suggest for simplicity that channel population in the range of 20–50 ms consists of early Na channels (that leads to underestimation of the real number of background channels) and in the range of 150–500 ms of background channels only. Then for number of early channels, n_e, and background channels, n_b, in the range of 50–150 ms we have

$$\tau_o = \frac{0.86 \cdot n_e + 0.42 \cdot n_b}{864} = 0.53$$

$$n_e + n_b = 864$$

From here, $n_e = 216$ and $n_b = 648$. Again, in this experiment mixed channel activity in the time range of 50–150 ms consists of at least 75% background channel openings. It is important to note that at such a negative potential the current amplitude after 50 ms of depolarization is significant compared with the total peak current amplitude (see Fig. 9, *inset*).

Second, we inactivated most of the early channels by applying a prepulse to 0 mV. The τ_o of the remaining active channels (background channels) was similar to the τ_o measured in the range 150–500 ms in the absence of the prepulse. The background channel activity was also strongly depressed by this 10-ms prepulse, suggesting that during the prepulse the majority of the active background Na channels coexists with the active early Na channels and inactivates during the prepulse time.

Third, we found that τ_o in the range 150–500 ms after the onset of depolarization was equal to that obtained after prolonged (more than 10 s) depolarization. This fact suggests that the same type of channels underlay the background openings measured during constant and step depolarization. Qualitatively similar results were obtained at −80 mV.

It was shown that under normal conditions the resting membrane potential in cardiac tissue is several millivolts more positive than the potassium equilibrium potential (Baumgartenet al., 1984; Shen et al., 1980). In the present study, we recorded steady-state Na currents at −80 mV under voltage-clamp conditions. Could the background Na channels contribute significantly to this depolarizing background conductance? To evaluate this possibility, consider the following parameters measured at potentials near the rest potential: $N_o = 3.2$ (average data at −80 mV constant potential and sample time, $T = 500$ ms), $\tau_o = 0.3$ ms, current through a single channel, $i = 2$ pA, cell membrane surface, $S = 14{\cdot}103$ μm^2 (Shattock and Matsuura, 1992), and that in the pipette tip a membrane forms a hemisphere with the diameter $d = 2$ μm. In this case, the whole-cell current through background Na channels can be evaluated as

$$I = \frac{2 \cdot \tau_o \cdot S \cdot i \cdot N_o}{\pi \cdot d^2 \cdot T} = 8.6 \text{ pA}$$

This estimation is in a good agreement with steady-state Na currents measured in whole-cell configuration at −80 mV (15.3 ± 9.1 pA).

From these studies, we suggest that the background channel represents a special type of Na channel. At positive potentials (>−40 mV), the short channel openings detected after inactivation of early Na channels represent only a small fraction (free from inactivation) of the total background Na channel population. At potentials in the vicinity of activation threshold and the cell resting potential (−70 to −80 mV), background channels underlie the significant fraction of total Na current. They can play an important role in the initiation of the cardiac action potential. They can also significantly contribute to the background Na conductance and resting potential maintenance.

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