Na channels that remain open throughout the cardiac action potential plateau

Yuan-mou Liu, Louis J. DeFelice and Michele Mazzanti
Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322

ABSTRACT In this paper we report the direct measurement of rare Na channel events that occur during the cardiac action potential, viz., channels that open at the upstroke and remain open throughout the plateau and early repolarization phase. The technique we use allows us to record channel activity and action potentials at the same time; thus, we are certain of when the Na channels open and when they finally close. The slow Na channels have the same voltage dependence, single-channel conductance, and TTX sensitivity as the fast Na channels, and they conduct Li. It therefore seems likely that the fast and the slow currents flow through the same channel. If this interpretation is correct, then the Na channel not only initiates the action potential but also helps to maintain its plateau. It is possible that the slow Na currents represent a separate collection of channels rather than a low-probability state of the fast Na channels. Regardless of which interpretation is correct, the present experiments allow us to assess the effect of the slow currents on action potential shape and on sustained Na entry.

INTRODUCTION

The voltage-dependent Na channel, sometimes referred to as the fast Na channel, activates and inactivates rapidly in response to step depolarizations. Its function is usually associated with a fast inward current that both initiates the action potential and promotes its propagation. In heart, the Na depolarization opens Ca channels and K channels, and these currents determine the duration of the action potential plateau. The fast Na channels remain open only tens of milliseconds during the initial phase of the action potential, and they are thought to play little role in maintaining the plateau. In most models of the Na current, the overlap of the activation and inactivation variables in most models of the fast Na current describe a steady-state component (Hodgkin and Huxley, 1952; McAllister et al., 1975; Beeler and Reuter, 1977; DiFrancesco and Nobel, 1985). This macroscopic current, sometimes called the window current (Attwell et al., 1979), has never been fully explained in terms of the underlying single-channel events (Vandenberg and Bezanilla, 1991*a, b*).

In our previous work on beating heart cells, we have shown that this classical view of Na channels has to be augmented. The peak of the cardiac action potential actually exceeds the dynamic Na reversal potential (Mazzanti and DeFelice, 1987; Wellis et al., 1990). Since Ca channels open at about the same time as the Na channels (Mazzanti and DeFelice, 1990), and since the Ca reversal potential is much greater than the Na reversal potential, an outward Na current occurs just after the inward Na current. Although this outward current is small in amplitude, it may last for 30 or 40 milliseconds and can account for as much as one-third of the total Na that moves across the membrane.

In the present paper we report a much rarer event that implies an even slower Na channel kinetics. In approximately one out of every 100 beats, a Na-conducting channel remains open throughout the entire length of the action potential plateau. These extremely long openings are TTX sensitive and have voltage dependence and single-channel conductance similar to the fast Na channel. Nearly identical long openings are observed when Li replaces Na. Furthermore, these rare events resist all of the usual Ca channel blockers at concentrations that are high enough to block the whole-cell Ca currents in these same cells.

Patlak and Ortiz (1985, 1986) have previously observed slowly inactivating Na single-channel currents in adult rat heart and in frog skeletal muscle. The slow Na channels they recorded during voltage-clamp experiments had the same conductance, reversal potential, and TTX sensitivity as fast Na channels in the same cells. The slow Na channels in rat heart remain open for up to 150 ms during voltage steps to −30 mV. Patlak and Ortiz argue that the fast and the slow currents result from a single class of Na channels with two or more kinetic modes. The long openings (or long bursts of openings) are very rare, occurring on the order of once per 10^3 voltage-clamp steps. They have been observed in a wide variety of cardiac tissue, including guinea-pig ventricle (Nilius, 1987; Nilius et al., 1989*a, b;* Kiyosue and Arita, 1989), rat ventricle (Kirsch and Brown, 1989), and dog Purkinje fibers (Fozzard et al., 1987), where the specific suggestion was made that, in addition to underlying the slowly decaying late Na current, the slow Na channels ought to contribute to the action potential plateau. Grant and Starmer (1987) arrived at a very similar conclusion in their analysis of single Na channel kinetics in

Dr. Liu's present address is Department of Physiology, 2nd Medical School, 200025 Shanghai, Peoples Republic of China; and Dr. Mazzanti's present address is Physiology and General Biochemistry, University of Milan, 20133 Milan, Italy.

0006-3495/92/09/654/09 $2.00

rabbit ventricular myocytes, and Carmeliet (1987) made essentially the same point for rabbit Purkinje fibers. Indeed, it has been known for some time that TTX has an effect on action potential shape (Coraboeuf et al., 1979), and that this effect was "attributable, at least in part, to the existence of a TTX-sensitive inward Na current lengthening the plateau of the Purkinje fiber action potential."

This paper substantiates the role of Na channels in the maintenance of the cardiac action potential plateau by direct measurement. In the discussion, we present a descriptive model of the three components of the Na current: the fast inward Na current, the early outward Na current, and the rare late openings, and we speculate on their relative roles in cardiac function.

MATERIALS AND METHODS

Tissue culture and solutions

Embryonic ventricle cells were prepared by enzymatic digestion of seven-day chick embryo hearts, following the procedure of DeHaan (1967) as recently revised by Fujii et al. (1988). After 12–24 h in tissue culture medium, we washed the isolated cells with the standard bath solution just before the experiments. All the experiments were done at room temperature. The composition of the bath solution (in mM) was: 130 Na, 1.3 K, 1.5 Ca, 0.5 Mg, 133 Cl, 0.5 SO_4, 1.3 PO_4, 5 dextrose, 10 HEPES, pH 7.35. In some experiments, 1 μM TTX was added to the bath. In the same preparation, Fuji et al. (1988) report a 50% reduction in the fast Na current at 1 nM TTX. Ten Eick et al. (1984) found, in 11-day chick ventricle cells, that 3 nM TTX blocked rapidly-opening channels more than delayed-opening channels; however, 300 nM TTX blocked all Na currents. The cell-attached patch pipette solution was similar to the bath solution. In some experiments, the pipette solution contained 65 Na/65 Cs instead of 130 Na, or 130 Li instead of 130 Na, and in other experiments it contained the normal both solution plus 10 μM TTX, 300 μM Cd, 50 μM nifedipine, 3 mM Co, or 100 μM Ni. Cl replaced SO_4 and PO_4 in all pipette solutions. When we used a whole-cell electrode to record action potentials, it contained an intracellular-like solution consisting of 120 K, 0.1 Ca, 2 Mg, 124.2 Cl, 1.1 EGTA, 10 HEPES, pH 7.35. At seven days, about half of the cells beat spontaneously in the standard bath solution. The rate is highly variable, and beating cells may stop or change their beat rate when these are patched. We selected cells that were beating in the range 0.8 to 1.2 times/s after the cell-attached patch or whole-cell electrodes were in place.

Electrodes and recording techniques

The patch electrodes were made from borosilicate glass (Corning 7052) using a programmable puller (Sachs-Flaming, PC-84, Sutter Instruments, Novato, CA). Before pulling, we keep the glass at 470°C for at least 24 h. This procedure cleans the surface of the glass and gives the electrodes more uniform shapes with the same pulling program. We coat the tips with Sylgard (Dow Corning) and store the electrodes in a dry vacuum oven at 100°C for an indefinite period. The tips were fire-polished to 1–2 μm outside diameter just before using them. The filled pipettes had resistances of 4–10 megohms when dipped into the bath solution. The estimated surface area of the patch formed with these electrodes is between 5 and 7 μm^2 by capacitive measurements (Mazzanti and DeFelice, 1987). The action potentials from whole-cell electrodes are virtually identical to those from the cell-attached electrodes taken immediately after breaking the patch. In the present experiments we have used the latter method exclusively, except for Fig. 6, where we used whole-cell electrodes filled with intracellular-like solutions to record trains of action potentials. After breaking the patch in the cell-attached mode, the electrode solution and the intracellular solution eventually mix, and the action potential deteriorates.

Before performing an experiment, we identified patches that contained many Na channels by applying a positive potential of 20 or 40 mV to the cell-attached electrode. This hyperpolarizing voltage gave an appreciable inward current (>5 pA) at the upstroke of the action potential only if the patch contained dozens of Na channels (Wellis et al., 1990). It was these patches that we used in the present study. We used List EPC5 and EPC7 amplifiers to measure the voltage and current and a Panasonic VCR to store the data. We analyzed the data on a Nicolet 4094 oscilloscope and an IBM-AT computer. For more details on the recording technique see Fischmeister et al. (1984), Mazzanti and DeFelice (1987, 1988, 1990) and Wellis et al. (1990).

Analysis

After screening the patches for the presence of Na channels (and the absence of delayed rectifier or inward rectifier K channels), we recorded up to 1,000 action currents, $i_{Na}(t)$, through the isolated patch. We then broke the patch and recorded the first few action potentials, $V(t)$. Normally the patch potential, V_p, is zero, but in some experiments we offset the action potential to $V(t) - V_p$ to measure the voltage dependence of the patch currents.

The relationship between the Na action current and the membrane voltage is:

$$V_{Na}(t) = -\frac{N_{Na}}{C}\int_0^t \langle i_{Na}(t)\rangle\, dt,$$

where $V_{Na}(t)$ is the contribution of Na to the total action potential, $V(t)$, N_{Na} is the Na channel density, C is the membrane capacitance per unit area, and $\langle i_{Na}(t)\rangle$ is the average Na action current through the patch. Under the integral sign, t is a dummy variable. This method allows one to calculate the contribution to the action potential of any particular ion current by a direct measurement of that current during the beat (DeFelice, 1989; DeFelice et al., 1990).

Here we are interested primarily in the effect of the slow Na current on cardiac excitability. Since the long-lasting openings occur so rarely, it is practically impossible to obtain an average. Instead, we have isolated the rare events and considered the integral of a typical event, $i_{Na}(t)$. Thus, we evaluate:

$$-\int_0^t i_{Na}(t)\, dt,$$

where $i_{Na}(t)$ refers to a selected trace. This integral, appropriately scaled, measures the contribution of the rare event to the action potential.

RESULTS

If the bath solution and the patch pipette contain the same physiological solution (130 mM Na), and if $V_p = 0$ (so that the patch experiences the normal cell action potential), cell-attached patch recordings on spontaneously beating cells have three patterns of inward current (Fig. 1, *left*). The majority of the traces show the fast inward Na current at the beginning of the action potential. However, two types of rare events also occur: brief openings observed as isolated events throughout the plateau, and long-lasting openings that are the same amplitude as the brief openings. The long-lasting openings are interrupted by brief closings, and may also be described as bursts of openings. These three situations are shown in Fig. 1 (*left*) from top to bottom. On the same

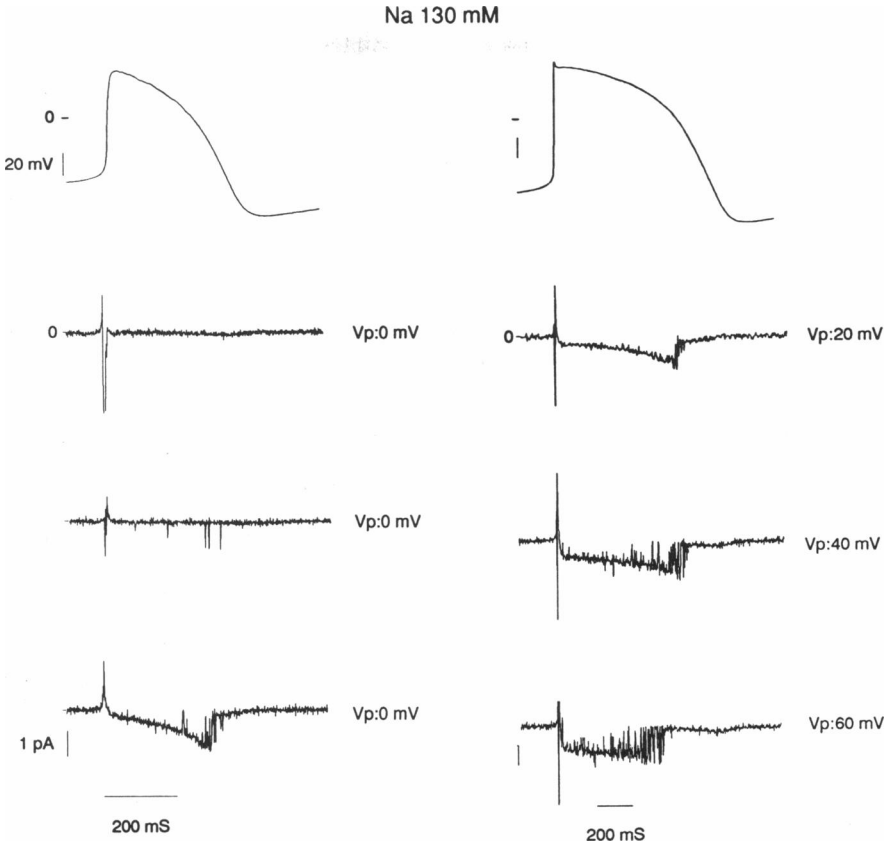

FIGURE 1 (*Left*) Selected inward patch currents from a cell-attached patch ($Vp = 0$) during spontaneous faring of seven-day chick ventricle. (*Top to bottom*) The action potential of the fast inward Na current; the late current showing brief, isolated events (*drizzle*); the long-lasting current that is open throughout the action potential plateau and repolarization phase. (*Right*) Voltage dependence of the current amplitude and the duration of the long opening. As the voltage in pipette (V_p) increases from 20 to 60 mV, single-channel current increases and the duration of long opening decreases (see Table 1).

time scale we see the cell action potential, which was measured just after the patch recordings through the same electrode (see Methods). The percent occurrence of the long openings, roughly defined by their appearance in the bottom trace of Fig. 1 is ~1% at $V_p = 0$ (Table 1). Notice that the amplitudes of the brief and the long openings increase as the action potential repolarizes. Since the rare events persist long after the fast, inward Na current, we call them late currents or slow currents. The principal focus of this paper is on the long-lasting openings seen at the bottom left of Fig. 1, what ions they carry and which channels they represent.

TABLE 1 The number of beats that occur before observing the first long opening (the major event lasting more than 200 ms)

V_p	Experiment 1	Experiment 2	Experiment 3
0	248	102	56
20	82	26	18
40	30	14	18
60	7		

The numbers are relative to the first action current (#1) after forming the seal. The pipette potential, V_p, is in mV.
For seven experiments, the average number of beats before the first long opening was 121 ± 68 at $V_p = 0$ mV, and 44 ± 28 at $V_p = 20$ mV.

The amplitude and frequency of occurrence of the long opening is voltage dependent. Applying positive voltages to the pipette solution, $V_p = 20$, 40, or 60 mV, hyperpolarizes the patch transmembrane potential during the action potential. Hyperpolarization increases the amplitude of the fast Na current (see Wellis et al., 1990 for a thorough analysis), and it increases the amplitude of the long opening (Fig. 1, *right*). Hyperpolarization also increases the frequency of the long openings (Table 1). In ten experiments, we observed a decrease in the duration of the long opening as V_p increased. Fig. 1 illustrates this effect, but we have not quantified it further.

When the pipette solution contains 10^{-5} M TTX, each category of current in Fig. 1 disappears: in 10 different cells, the first 1,000 beats (per cell) measured after forming the seal had zero long openings of the type depicted in Fig. 1 (0/10,000). Without TTX in pipette solution, we were always able to obtain the long-lasting openings if we waited long enough: in seven different cells, the first 2,541 beats (total) measured after forming the seal had a

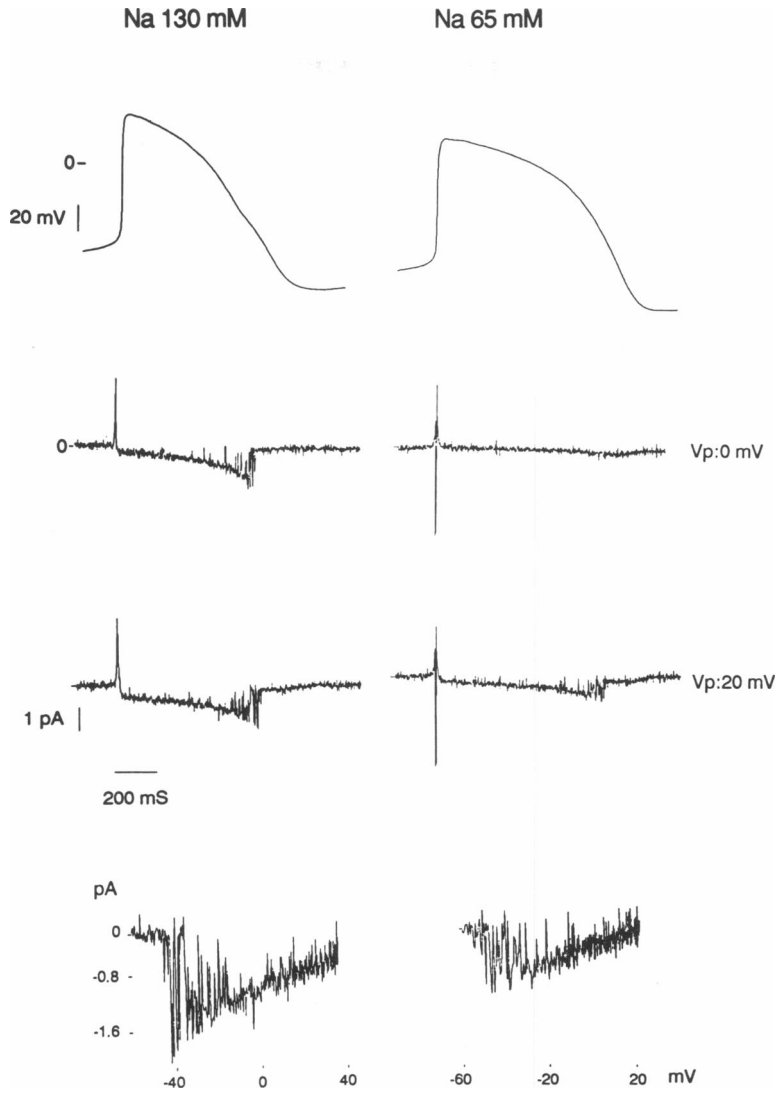

FIGURE 2 Change of single-channel current with the concentration of Na in pipette. The amplitude of long opening with 65 mM Na in pipette is smaller than that with 130 mM Na. It is difficult to observe long openings when V_p equals 0 mV in of 65 mM Na in the pipette. The bottom panel in each experiment shows the single-channel $i(V)$ curves of the long opening. With 130 mM Na, the conductance (taken as a straight line through the decaying ramp) is 16 pS and the extrapolated reverse potential is about 60 mV. With 65 mM Na in pipette, the conductance decreases to 11 pS, and the reverse potential to about 30 mV.

total of 21 long openings (21/2,541). These data are for 130 mM Na and V_p = 0 mV in the pipette. See Table 1 for additional data.

In five experiments we decreased the concentration of Na in the pipette solution from 130 mM to 65 mM. With lower Na in the patch, the amplitude of the long opening was smaller than with normal Na (Fig. 2). It was usually impossible to measure a long opening at V_p = 0, hence, in these experiments the patch was usually hyperpolarized by 20 mV or more. In three experiments in which the concentration of Na in pipette was elevated to 260 mM, the amplitude of the long opening nearly doubled when compared to a long opening in 130 mM Na (data not shown). Thus, the Na concentration in the pipette substantially influences the amplitude of the long-opening current. Since the action potential is different in different cells, we facilitated comparison from experiment to experiment by plotting $i_{Na}(t)$ versus $V(t)$. Thus, Fig. 2 also compares the open-channel $i(V)$ curves of the long opening with 130 mM Na or 65 mM Na in the patch pipette (*bottom*). In 130 mM Na, the single-channel conductance is 16 pS, and the reverse potential extrapolates to ~60 mV. In 65 mM Na, the single-channel conductance is 11 pS, and the reversal potential is about 30 mV. We made these estimates simply by drawing straight lines through the data between −20 and 20 mV. This change in reversal potential is more than expected by straight application of the Nernst equation. A factor of two in external Na concentration would imply a shift of ~16 mV. In five experiments, the estimated reversal potential for the long-lasting opening with 65 mM Na in the pipette ranged between 25 and 35 mV. In five compa-

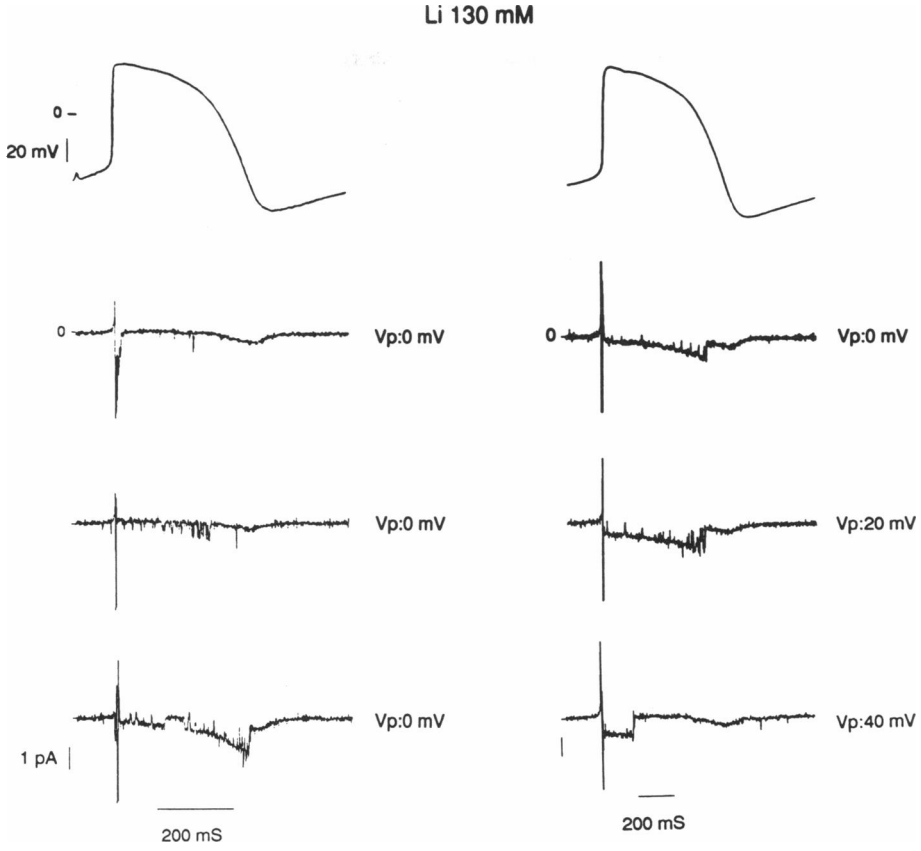

FIGURE 3 Selected inward patch currents with lithium in the patch. With 130 mM Li instead of 130 Na in pipette, the action current traces show fast inward current, isolated, brief openings, and the long opening. The current amplitude and the duration of the long opening also have the same voltage dependence. (Compare with Fig. 1.)

rable experiments with 130 mM Na, the range was 50 to 60 mV. For this comparison we selected cells with similar beat rates. The calculation of a 16-mV shift assumes the same internal concentration of Na for both external concentrations of Na. Wellis et al. (1990) have shown that $[Na]_{in}$ is not independent of external Na, especially in beating cells. Furthermore, the current through the long opening may itself influence the local Na concentration inside the cell. Therefore, although the shift in reversal potential is in the expected direction for a Na-selective channel, the absolute value of the shift is difficult to interpret (see Discussion).

With 130 mM Li replacing Na in the pipette solution, the cell-attached recordings show approximately the same current patterns. Fig. 5 shows the fast inward Li current, the rare brief openings, and the long opening. In four experiments qualitatively similar to the one in Fig. 3, we verified that Li may also carry the late currents. The amplitude and the frequency of occurrence of the Li long openings were also voltage dependent in the same sense as the Na long openings. For example, when V_p = 40 mV the amplitude increases and the duration of the long opening shortens compared to $V_p = 0$. Fig. 4 compares the single-channel conductance of Li to Na. The conductance in Li was 18 pS and the reversal potential was about 60 mV, nearly the same as in Na.

Different Ca channel antagonists were placed in the patch pipette solution to block Ca channels: 300 μM cadmium, 50 μM nifedipine, 3 mM cobalt, or 100 μM nickel. For each antagonist, we repeated the experiments illustrated in Fig. 5 three to six times. In spite of the presence of these Ca channel blockers, the cell-attached

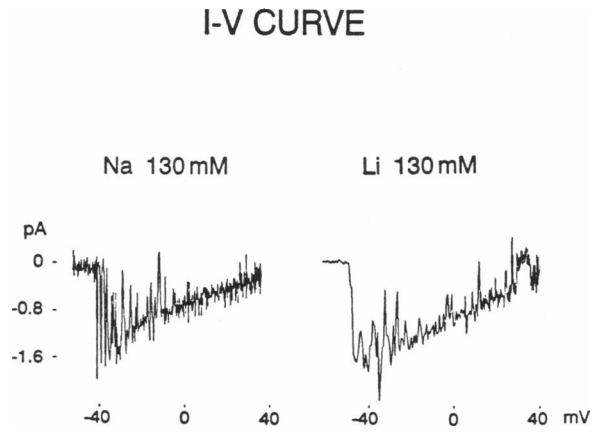

FIGURE 4 Comparison of single-channel $i(V)$ curves with 130 mM Na and 130 mM Li in the patch. The conductance of Na is 17 pS, and its reverse potential is ~50 mV. The conductance of Li is 18 pS, and its reverse potential is similar to that of Na.

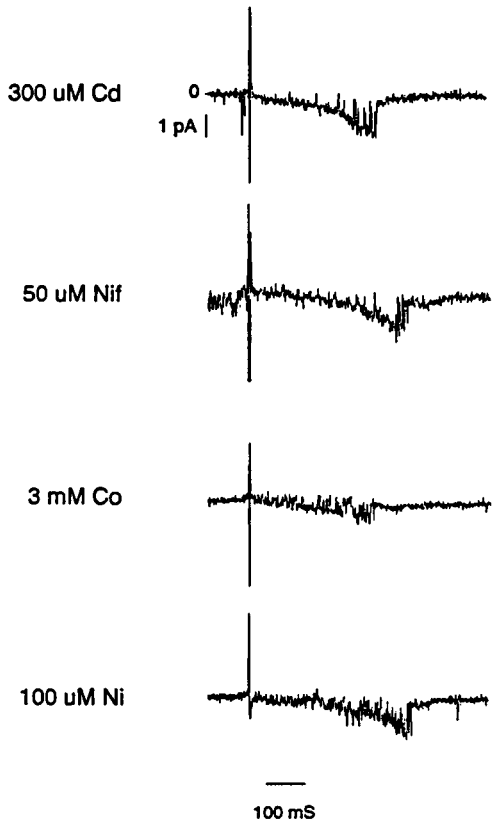

FIGURE 5 Ca channel blockers have little effect on the long openings. In the presence of cadmium, nifedipine, cobalt, and nickel, the action currents show similar long openings like those without blockers. Some traces, e.g., 300 μM Cd and 50 μM Nif, show what appear to be inward current channel activity before the beginning of the action potential. It is possible that these patches contain other types of channels in addition to Na channels. However, their amplitude and direction suggest that they are late dyastalic openings of the Na channel. The differences in shape are due to the differences in the action potentials from individual cells.

recordings show the characteristic long opening of the control experiment without Ca blockers (Fig. 1). The individual examples are different because the action potential is different in every cell. However, in each case, we observed that the amplitude and the frequency of the long openings are voltage dependent, just as they were in the control experiments. The concentrations of the Ca channel blockers Cd, Nif, and Co used in the patch were high enough to block completely the Ca current in whole-cell recordings on the same cells.

In a separate series of experiments, we measured the effect of TTX on the cell action potential using a whole-cell electrode configuration. After the spontaneous action potential became stable, we added 10^{-6} M TTX to bath solution. In the top panel of Fig. 6, the upper trace is the control action potential, and the lower trace is the action potential several seconds after adding TTX to the bath. The action potential duration decreases and the plateau potential is lower. The same data on a faster time scale show that the rapid upstroke of the action potential does not change initially. Several minutes after adding TTX, it too became slower and eventually the cell stopped beating. Since other cardiac currents are voltage dependent, it is not reasonable to conclude at this point that the observed change is brought about by blocking the long-lasting Na current. Therefore, it is of interest to calculate the effect on the action potential of only the long-lasting Na currents and compare this to the TTX experiments on whole cells.

To this end, Fig. 6 also shows a typical long-lasting opening observed under normal patch conditions (130 mM Na, $V_p = 0$). The integral of this slow current, $\int_0^t i_{Na}(t)\,dt$, is given on the same time scale. The vertical axis of the integral is in picocoulombs (pC). The bottom

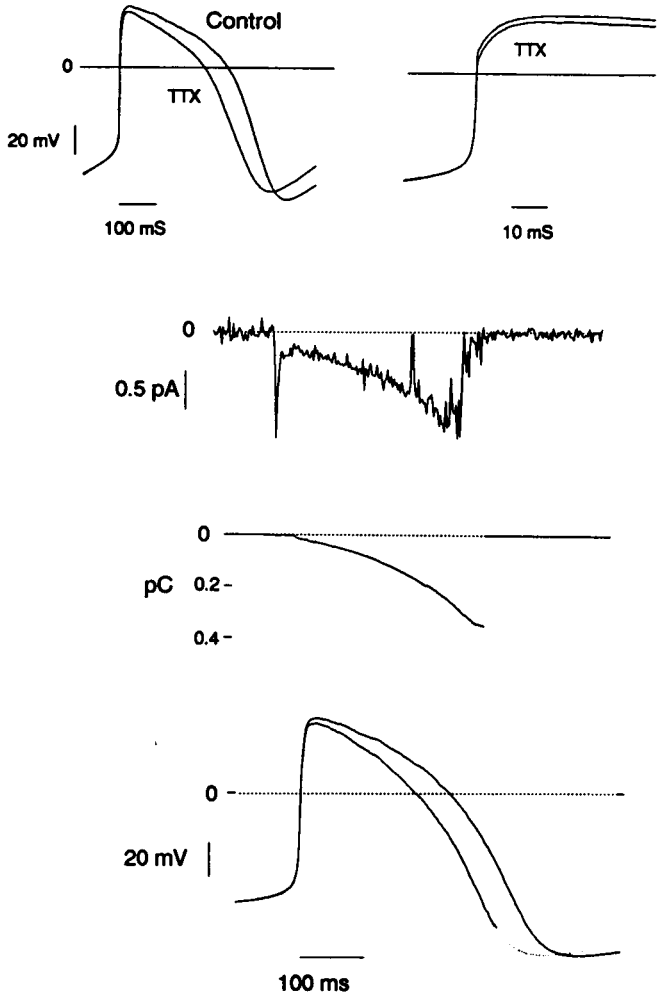

FIGURE 6 The effect of TTX on the action potential. (*Top left*) the upper trace is a control action potential. After adding 10^{-6} M TTX to bath solution, the action potential duration decreases and the plateau becomes lower. (*Top right*) the same trace on a faster time scale. The three panels in the bottom portion of the figure show how the long openings effect the shape of the action potential. The current trace is the total patch current after blank subtraction. Note the presence of the fast, inward current. The integral of this current is in units of picocoulombs (pC). The bottom panel shows the result of subtracting this integral, appropriately scaled, from the measured action potential. The scaling factor is 125 mV/pC.

panel shows the action potential that generated the long opening, and it shows the theoretical effect of blocking a certain fraction of these long-lasting, slow Na currents. The scaling factor used to reduce the action potential from its control level to the long-lasting, TTX-blocked level is 125 mV/pC. This number is approximate, but we may use it as a check on the fraction of channels displaying the long openings (see below).

DISCUSSION

Our experiments suggest that the long-lasting (slow) Na currents flow through the same channels that carry the fast Na current: (*a*) The slow current is inward and its amplitude increases as the action potential repolarizes, which is expected for a Na current. This same effect is seen if we hyperpolarize the patch by applying positive voltages to the patch pipette (Fig. 1). (*b*) The conductance of the long opening decreases, and its reversal potential shifts like a Na-conducting channel (Fig. 2). (*c*) Virtually all of the effects observed in Na are also observed in Li, a well-known permeant ion for the fast channel (Fig. 3). (*d*) In no case were we able to observe the long openings with 10^{-5} M TTX in the patch pipette.

We are confident of the TTX result for the following reason: we actually found the slow Na currents while looking for mode II Ca currents, not in high concentrations of Ba or Ca (Hess et al., 1984; Cavalie et al., 1986; Mazzanti and DeFelice, 1990), but in normal Ca concentrations. Our strategy was to add TTX to a physiological solution (1.5 mM Ca), apply this solution in a cell-attached patch, and wait for mode II Ca channel openings. We had already seen them in 10 mM Ca (Mazzanti and DeFelice, 1990) and were asking whether they existed in more normal conditions. We never observed any long openings under these physiological conditions. Hence, we obtained, somewhat inadvertently, quite a lot of negative data. As soon as we removed the TTX from the patch pipette, we began to see the late openings reported here.

This raises the question of whether the long-lasting, openings could be TTX-sensitive mode II-type Ca channel openings. As far-fetched as this seems, we tested the possibility by showing that none of the standard Ca channel blockers had any effect on the late openings (Fig. 5). In normal Ca concentrations, such as those used in this study, mode II Ca openings reported previously are apparently too small to observe. For an analysis of Ca antagonistic drugs on Na currents in young embryonic chick heart, see Kojima and Sperelakis (1983, 1985).

What is the relation of the slow Na current to the more usual Na current? The fast inward current, and the outward current, were analyzed in detail in previous papers (Mazzanti and DeFelice, 1987; Wellis et al., 1990). To measure these two components of Na current with accuracy requires analyzing the capacitance and the leak conductance for each patch. In the present study we use the simpler, blank subtraction method. Fig. 6 shows an example of the total Na current (after blank subtraction) from a patch in which a long opening occurred. Though this method does not resolve the fast Na current well, we conclude from such experiments that when a slow opening occurs the fast component is still present. The outward Na current, on the other hand, is absent. Since the outward current requires elevated Na levels at the inner face of the membrane (Wellis et al., 1990), we may postulate some connection between the absence of Na accumulation and the presence of a long opening. Such a correlation, however, must remain tentative until a more careful analysis is done of the slow Na currents during the period just after the upstroke of the action potential.

How do the slow currents contribute to the action potential? We have approached this question by evaluating the following expression:

$$V(t) - K\int_0^t i_{Na}(t)\,dt,$$

where $V(t)$ is the action potential and K is a proportionality constant. Using this approximation (see Methods) we showed that adding TTX to the bath (Fig. 6) and subtracting a voltage due to the slow Na current both have the same qualitative effect on the action potential plateau. This result is expected for TTX-sensitive, long-opening time Na channels, and it was suggested from voltage-clamp studies by Kunze et al. (1985), Grant and Starmer (1987), and Fozzard et al. (1987). The present experiments are the first to demonstrate this effect by direct measurement.

The constant of proportionality, K, that we used to give qualitative agreement shown in Fig. 6 between bath applied TTX and the contribution of the long opening to the action potential shape was 125 mV/pC. This constant provides a measure of the number of channels participating in the long openings. However, since we used a single long opening in these calculations, rather than an average long opening, the exact correspondence between K and channel number is unknown. This device was necessary due to the low probability and the imprecise definition of "long opening." Nevertheless, it is useful to show the relation between the present data and our previous results. Let us assume that there are 100 channels in the patch ($\sim 20/\mu^2$ and an area of 5 μ^2) and that a long opening occurs once every 100 beats (Table 1). The probability of a long opening is therefore $p = 10^{-4}$. Thus,

$$K \cong pN/C,$$

may be used as a constancy check on the density of Na channels. Using these figures:

$$N = (125\ \text{mV/pC})(1\ \mu\text{F/cm}^2) \times 10^4 \cong 13\ \text{channels}/\mu^2,$$

which is close to our previous estimate obtained from the fast openings (23 channels/μ^2, Mazzanti and DeFelice, 1987). This calculation does not prove the interpretation we have made; it merely demonstrates consistency between the two sets of data, and it allows us to draw the following picture.

In a beating cell, less than 10% of the total number of Na channels (≅20,000) are open at any time (Mazzanti and DeFelice, 1987). Thus, ~2,000 Na channels per cell conduct the fast Na current and, by the above calculations, only two Na channels per cell conduct the slow Na current. In individual cells, therefore, we expect substantial fluctuations in action potential duration from beat to beat. Indeed, such fluctuations are observed in beating cells (Clay and DeHaan, 1979). In light of our present experiments, it would be interesting to perform interbeat interval fluctuation analysis while titrating TTX. Such experiments have not been done. Two channels per cell may seem an extraordinarily low number; however, one long opening is capable of generating, late in the action potential, a voltage of over 50 mV in a 750 μ^2 cell. From Fig. 6 (*middle*), the accumulated charge reaches the value of 0.4 pC, and therefore,

$$V = \frac{0.4\ \text{pC}}{7.5\ \text{pF}} = 53\ \text{mV}.$$

This voltage contribution of the long opening is in the depolarizing direction, and it increases the duration of the action potential by a considerable fraction (Fig. 6, *bottom*). In whole-cell experiments, and in all free-running cells, blocking one current changes the voltage, but the voltage changes many currents. Our calculations assume the primary effect in Fig. 6 (*top*) is the block of the long-lasting opening. This assumption effects principally the estimate of the number of channels that are involved. We disagree with the assertion that the noninactivating Na channel is without functional significance for cardiac excitation (Kohlhardt et al., 1987). The assertion may be approximately true for large numbers of well-coupled cells, but it misses a rather important point for small groups of cells isolated to a greater or lesser extent from surrounding tissues. Such fluctuations may, therefore, become noticeable in normal or ectopic pacemaker activity. We have shown that long openings do occur under physiological conditions, and that they are responsible not only for the generation of action potentials, but also for the maintenance of its plateau phase. However, we ought not expect that every heart cell will have the long Na channel openings. For example, Hume and Uehara (1985) have shown that the plateau and duration of the guinea-pig ventricle action potential are insensitive to concentrations of TTX as high as 30 μM. On the other hand, Coraboeuf et al. (1979) in dog Purkinje fibers, and Kiyosue and Arita (1989) in guinea-pig ventricle, find that TTX, in concentrations as low as 0.33 μM, effects action potential shape, and that late Na currents are the probable cause. A great deal may depend on Na channel density, local Na concentrations, beat frequency, and other factors that could influence the occurrence of long openings. Among these factors we may now include phosphorylation of the Na channel by protein kinase C (Numann et al., 1991).

We wish to thank B. J. Duke for preparing the solutions and the tissue cultures, and W. N. Goolsby for the electronics and computer support necessary for this work.

This work was supported by the National Institutes of Health HL-27385.

Received for publication 7 January 1992 and in final form 30 April 1992.

REFERENCES

Attwell, D., I. Cohen, D. Eisner, M. Ohba, and C. Ojeda. 1979. The steady-state TTX-sensitive ("window") Na current in cardiac Purkinje fibers. *Pfluegers Arch. Eur.* 379:137–142.

Beeler, G. W., and H. Reuter. 1977. Reconstruction of the action potential of ventricular myocardial fibers. *J. Physiol. (Lond.).* 268: 177–210.

Carmeliet, E. 1987. Slow inactivation of the Na current in rabbit cardiac Purkinje fibers. *Pfluegers Arch.* 408:18–26.

Cavalie, A., D. Pelzer, and W. Trautwein. 1986. Fast and slow gating behavior of single Ca channels in cardiac cells. *Pfluegers Arch.* 406:241–258.

Clay, J. R., and R. L. DeHaan. 1979. Fluctuations in interbeat internal in rhythmic heart-cell clusters. *Biophys. J.* 28:377–390.

Coraboeuf, E., E. Deroubaix, and A. Coulombe. 1979. Effect of TTX on action potentials of the conducting system in dog heart. *Am. J. Physiol.* 236:H561–567.

DeFelice, L. J. 1989. Excitability and channel kinetics in the free-running membrane. *In* Noise in Physiological Systems. E. Ambrozy, editor. Elsevier Science Publishers, The Netherlands. 303–312.

DeFelice, L. J., W. N. Goodsby, and M. Mazzanti. 1990. K channels and the repolarization of cardiac cells. *In* Embryonic Origins of Defective Heart. N.Y. Acad. Sci., New York. 588:174–184.

DeHaan, R. L. 1967. Regulation of spontaneous activity and growth of embryonic chick heart cells in tissue culture. *Dev. Biol.* 16:216–249.

DiFrancesco, D., and D. Noble. 1985. A model of cardiac electrical activity incorporating ionic pumps and concentration charges. *Phil. Trans. (Royal. Soc.)* B307:353–398.

Fischmeister, R., L. J. DeFelice, R. J. Ayer, R. Levi, and R. L. DeHaan. 1984. Channel currents in embryonic chick heart cells. *Biophys. J.* 46:267–272.

Fozzard, H. A., D. A. Hanck, J. C. Makielski, B. E. Scanley, and M. F. Sheets. 1987. Na channels in cardiac Purkinje cells. *Experimentia.* 43:1162–1168.

Fujii, S., R. K. Ayer, and R. L. DeHaan. 1988. Development of the fast Na current in early embryonic chick heart cells. *J. Membr. Biol.* 101:209–223.

Hess, P., J. B. Lansman, and R. W. Tsien. 1984. Different modes of Ca channel gating behavior favoured by dihydropyridine Ca agonists and antagonists. *Nature (Lond.).* 311:538–544.

Hodgkin, A. L., and Huxley, A. F. 1952. A qualitative description of

membrane current and its application to conduction in nerve. *J. Physiol. (Lond.)*. 117:440–544.

Hume, J. R., and A. Uehara. 1985. Ionic basis of the different action potential configurations of the guinea-pig. *J. Physiol. (Lond.)*. 366:525–544.

Kirsch, G. E., and A. M. Brown. 1989. Kinetic properties of single Na channels in rat heart and rat brain. *J. Gen. Physiol.* 93:85–100.

Kiyosue, T., and M. Arita. 1989. Late Na current and its contribution to action potential configuration in guinea-pig ventricular myocytes. *Cir. Res.* 64:389–397.

Kohlardt, M., U. Froebe, and J. W. Herzig. 1987. Properties of normal and noninactivating single cardiac Na channels. *Proc. R. Soc. Lond. B. Biol. Sci.* 232:71–93.

Kojima, M., and N. Sperelakis. 1983. Ca antagonistic drugs differ in ability to block the slow Na channels of young embryonic chick hearts. *Eur. J. Pharm.* 94:9–18.

Kojima, M., and N. Sperelakis. 1985. Development of slow Ca-Na channels during organ culture of young embryonic chick hearts. *J. Dev. Physiol.* 7:355–363.

Kunze, D. L., A. E. Lacerda, D. L. Wilson, and A. M. Brown. 1985. Cardiac Na currents and the inactivating, reopining, and waiting properties of single cardiac Na channels. *J. Gen. Physiol.* 86:691–719.

Mazzanti, M., and L. J. DeFelice. 1987. Na channel kinetics during the spontaneous heart beat in chick ventricle cells. *Biophys. J.* 52:95–100.

Mazzanti, M., and L. J. DeFelice. 1988. K channel kinetics during the spontaneous heart beat in embryonic chick ventricle cells. *Biophys. J.* 54:1139–1148.

Mazzanti, M., and L. J. DeFelice. 1990. Ca channel gating during cardiac action potentials. *Biophys. J.* 58:1059–1065.

Mazzanti, M., L. J. DeFelice, and Y.-M. Liu. 1991. Gating of L-type Ca channels in embryonic chick ventricle cells: dependence on voltage, current, and channel densities. *J. Physiol. (Lond.)*. 443:307–334.

McAllister, R. E., D. Noble, and R. W. Tsien. 1975. Reconstruction of electrical activity of cardiac Purkinje fibres. *J. Physiol. (Lond.)*. 251:1–59.

Nilius, B. 1987. Modal gating behavior of single Na channels from the guinea-pig heart. *Biomed. Biochem. Acta.* 46:S662–S667.

Nilius, B., J. Vereecke, and E. Carmeliet. 1989*a*. Properties of the bursting Na channel in the presence of DPI 201-106 in guinea-pig ventricle myocytes. *Pfluegers Arch. Eur. J. Physiol.* 413:234–241.

Nilius, B., J. Vereecke, and E. Carmeliet. 1989*b*. Different conductance states of the bursting Na channel guinea-pig ventricle myocytes. *Pfluegers Arch. Eur. J. Physiol.* 413:242–248.

Numann, R., W. A. Catterall, and T. Scheuer. 1991. Functional modulation of brain Na channels by protein kinase C phosphorylation. *Science (Wash. DC)*. 254:115–118.

Patlak, J. B., and M. Ortiz. 1985. Slow currents through single Na channels of the adult rat heart. *J. Gen. Physiol.* 86:89–104.

Patlak, J. B., and M. Ortiz. 1986. Two modes of gating during late Na channel currents in frog sartorius muscle. *J. Gen. Physiol.* 87:305–326.

Ten Eick, R., J. Yeh, and N. Matsuki. 1984. Two types of voltage-dependent Na channels suggested by differential sensitivity of single channels to TTX. *Biophys. J.* 45:70–73.

Vandenberg, C. A., and F. Bezanilla. 1991*a*. Single-channel, macroscopic, and gating currents from Na channels in the squid giant axon. *Biophys. J.* 60:1499–1510.

Vandenberg, C. A., and F. Bezanilla. 1991*b*. A Na channel gating model based on single-channel, macroscopic, and gating currents in the squid giant axon. *Biophys. J.* 60:1511–1533.

Wellis, D., L. J. DeFelice, and M. Mazaanti. 1990. Outward Na current in beating heart cells. *Biophys. J.* 57:41–48.