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Barium- and Calcium-Permeable Channels Open at Negative Membrane Potentials in Rat Ventricular Myocytes

Alain Coulombe, Isabel Ann Lefèvre, Isabelle Baro, and Edouard Coraboeuf
Laboratoire de Physiologie Comparée et Laboratoire des Biomembranes et des Ensembles Neuronaux (CNRS URA 1121), Université Paris-XI, Bât. 443, 91405 Orsay Cedex

Summary. Ca^{2+}- and Ba^{2+}-permeable channel activity from adult rat ventricular myocytes, spontaneously appeared in the three single-channel recording configurations: cell-attached, and excised inside-out or outside-out membrane patches. Single-channel activity was recorded at steady-state applied membrane potentials including the entire range of physiologic values, and displayed no "rundown" in excised patches. This activity occurred in irregular bursts separated by quiescent periods of 5 to 20 min in cell-attached membrane patches, whereas in excised patch experiments, this period was reduced to 2 to 10 min. During activity, a variety of kinetic behaviors could be observed with more or less complex gating patterns. Three conductance levels: 22, 45 and 78 pS were routinely observed in the same excised membrane patch, sometimes combining to give a larger level. These channels were significantly permeable to divalent cations and showed little or no permeability to potassium or sodium ions. The inorganic blockers of voltage-gated Ca channels, cobalt (2 mM), cadmium (0.5 mM) or nickel (3 mM), had no apparent effect on these spontaneous unitary currents carried by barium ions. Under 10^{-5} M Bay K 8644 or nitrendipine, the activity was clearly increased in about half of the tested excised inside-out membrane patches. Both dihydropyridines enhanced openings of the larger conductance level, which was only very occasionally seen under control conditions. When the single-channel activity became sustained under 5×10^{-6} M Bay K 8644, it was possible to calculate the mean unitary current at different membrane potentials and show that the mean current value increased with membrane potential.

Key Words cardiac myocytes · single-channel recordings · calcium background channels · dihydropyridines · voltage dependence

Introduction

The mechanisms underlying transmembrane calcium movements in excitable and nonexcitable cells have received considerable interest, much of which has focused on membrane calcium channels. Three types of voltage-operated Ca channels, T, L and N, have been described in neurons (Nowycky, Fox & Tsien, 1985), two of which, T and L, are present in cardiac membranes (Nilius et al., 1985) where they are involved in a fast and a slow Ca current, respectively (Bean, 1985; Mitra & Morad, 1986). In addition to voltage-gated channels, other types of Ca channels have been identified. Receptor-operated (ATP-activated) channels (Benham & Tsien, 1987*a*) and "leak" channels (Benham & Tsien, 1987*b*) have been found in mammalian smooth muscle cells; a stretch-activated Ca-permeable channel has also been observed in amphibian smooth muscle cells (Kirber, Walsh & Singer, 1988). Divalent cation-permeable channels activated under steady-state conditions in a large range of negative membrane potentials, and poorly sensitive to Ni and other inorganic Ca blockers, have been described in *Aplysia* neurons (Chesnoy-Marchais, 1985). The open probability of these channels increases with depolarization. By contrast, *Aplysia* neuron membranes incorporated into planar lipid bilayers, express voltage-independent Ca-channel activity, with multiple conductance levels, up to strongly negative membrane potentials (Coyne, Dagan & Levitan, 1987). In mammalian heart, evidence for background Ca channels (B-type channels) has also been provided after reincorporation of calf sarcolemmal membranes in planar bilayers but not in intact cells (Rosenberg, Hess & Tsien, 1988).

The existence of a sizeable resting ^{45}Ca influx in cardiac cells has been known for a long time (Niedergerke, 1963) and has been included as a background calcium conductance in recent models of cardiac electrical activity (Fischmeister & Vassort, 1981; DiFrancesco & Noble, 1985). However, the mechanism responsible for such an entry of calcium into resting cells is far from being clearly understood. Because of the key role played by calcium ions in contractility and the excitation-contraction process in the heart, it was of interest to try to bring some information about this mechanism.

The purpose of the present study was, therefore, to search for Ca-permeable channels activated at the resting potential in enzymatically dissociated adult rat ventricular myocytes, using standard patch-clamp techniques. This report shows for the first time that single-channel activity with Ca or Ba as charge carriers can be detected in intact cardiac cells at negative membrane potentials where voltage-gated cardiac calcium channels are not activated. Some of the present results have already appeared in preliminary form (Coulombe & Lefèvre, 1988).

Materials and Methods

Adult rat ventricular myocytes were isolated according to the procedures of Powell, Terrar and Twist (1980) and Irisawa and Kokubun (1983). The digested ventricles were maintained in "KB" medium (Isenberg & Klöckner, 1980) and stored at 4°C before use. Cells were mechanically dispersed by gently shaking a small piece of tissue cut from either right or left ventricular myocardium in a plastic petri dish containing K^+-rich solution. The dish was then placed upon the stage of an inverted microscope. All experiments were conducted at room temperature (19–22°C).

Single-channel activity was measured using the methods of Hamill et al. (1981). Patch electrodes were pulled from Pyrex capillaries (Corning 7740) and were not fire-polished before use. A Dagan 8900 patch-clamp amplifier with a 10 GΩ feedback resistor was used. Three patch-clamp configurations were used: cell-attached, inside-out and outside-out. In excised membrane patch experiments, a flow of solution from one of a series of five piped outlets continuously perfused the patch from which a recording was being made. The flow rate of the perfusion solutions was 50–100 μl/min. Unitary currents were recorded and stored, either with a beta video cassette recorder (Sony) after 16-bit digitization at 22 kHz with a pulse code modulator (Sony PCM-701-ES), or with an FM tape recorder (EMI 7000) at 3 3/4 in/sec (bandwidth DC to 1.25 kHz). Records were subsequently filtered through an 8-pole Bessel low-pass filter 920 LPF (Frequency Devices) at either 550 or 1000 Hz (−3 dB point) for data representation and data analysis, respectively. Filtered data were occasionally retrieved directly on paper using a Gould 2400 pen recorder or, more frequently, after having been digitized (rate 1 or 5 kHz corresponding to five times the previous filtering frequency) by PDP 11/23 (DEC), on an HP 7475A plotter.

Unitary currents were measured using two methods. When traces were stable enough and transition levels well defined, we directly used the computer for forming histograms of baseline and open-level data points. These histograms were fitted to the sum of Gaussian functions using the non-linear least-squares gradient-expansion algorithm of Marquardt (1963), to determine the amplitude, the mean and the dispersion of each peak. Mean unitary currents correspond to mean of fitted peaks. As it was not always possible to obtain reliable computer-built histograms because of the complexity of the current traces, the amplitudes of every detectable transition between current levels were also measured by hand and histograms were then obtained by pooling measured data. In both cases, the mean unitary currents were calculated as the difference between the means of two peaks, with each pair of peaks corresponding to a single observed transition. Distributions of open and closed times were obtained from records where only a single open level was seen. The open/closed current levels were determined by setting the level for threshold detection at 50% of the mean single-channel current level determined from the amplitude histograms. Open- and closed-time histograms were fitted by the above-mentioned numerical method, using one or the sum of two exponential functions. F-tests were used to evaluate whether significant improvements of the fits were obtained with two *vs.* one exponential.

The contents of all the pipette, bath and perfusion solutions are listed in the figure legends.

Results

Steady-State Unitary Barium Currents

Cardiac myocytes were maintained in a K-rich medium in order to zero the cell membrane resting potential, and the patch membrane was voltage clamped at various steady-state potentials, no voltage pulse protocol being used. When the patch pipette contained 110 mM Ba^{2+}, spontaneous inwardly directed unitary currents could be recorded at different holding potentials (Fig. 1*A*). Channel activity occurred in irregular bursts, which could be separated by quiescent periods of 5 to 20 min (12 ± 9 min, mean ± SD, n = 15 different patches). In some cases, after one burst, activity never reappeared. Figure 1*A* shows four current traces recorded during such bursts of activity, illustrating the variety of kinetic behaviors and current levels observed. Several complex gating patterns were observed: the gating was variable from one membrane potential to another (*compare* trace at 0 mV with trace at −100 mV) and sometimes also changed with time at a given membrane potential (*see* traces at −60 and −100 mV). The traces show at least two amplitudes of single-channel current corresponding to either two different channels or two substate conductances of the same channel. Openings and closings seemed to occur more frequently at negative membrane potentials (*compare* traces at 0 and −60 or −100 mV) and this was a consistent observation. Single-channel activity was observed in 19 patches out of 68; the remaining patches were silent at all tested voltages. Figure 1*B* shows the current-voltage relationships (*I/V*) for the two single-channel current amplitudes shown in Fig. 1*A*. The *I/V* curves are not linear, at least in part due to asymmetrical barium concentrations. The linear regression lines, which were fitted to the membrane potential values more negative than −90 mV, closely intercepted the zero point of the *I/V* plane (which would be the expected interception point for experiments in isotonic symmetrical medium) and gave slope conductances of 21 and 38 pS. The zero cur-

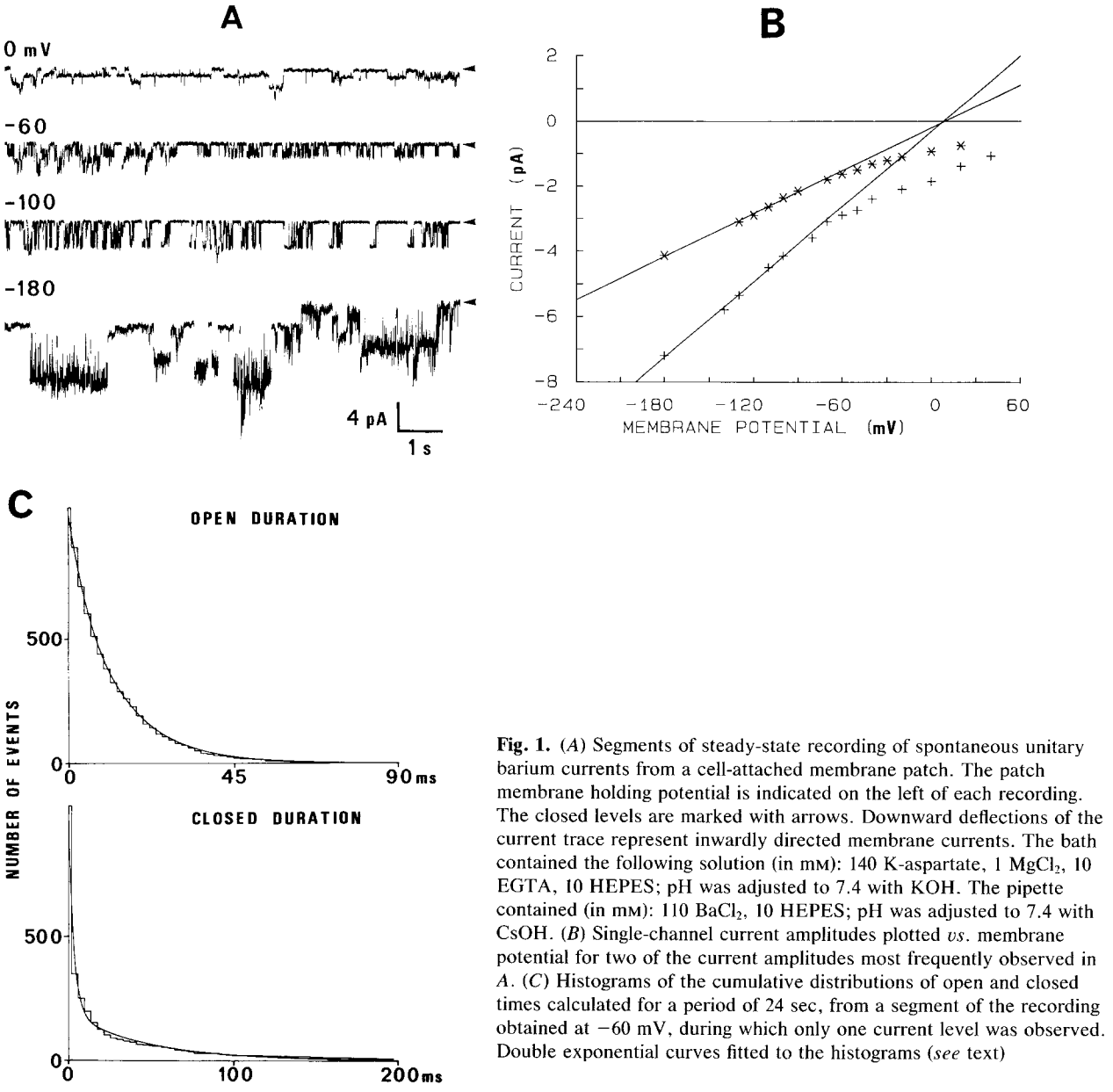

Fig. 1. (*A*) Segments of steady-state recording of spontaneous unitary barium currents from a cell-attached membrane patch. The patch membrane holding potential is indicated on the left of each recording. The closed levels are marked with arrows. Downward deflections of the current trace represent inwardly directed membrane currents. The bath contained the following solution (in mM): 140 K-aspartate, 1 $MgCl_2$, 10 EGTA, 10 HEPES; pH was adjusted to 7.4 with KOH. The pipette contained (in mM): 110 $BaCl_2$, 10 HEPES; pH was adjusted to 7.4 with CsOH. (*B*) Single-channel current amplitudes plotted *vs.* membrane potential for two of the current amplitudes most frequently observed in *A*. (*C*) Histograms of the cumulative distributions of open and closed times calculated for a period of 24 sec, from a segment of the recording obtained at −60 mV, during which only one current level was observed. Double exponential curves fitted to the histograms (*see* text)

rent points of both *I/V* curves appear to be at very positive potential values, suggesting that, according to the Goldman-Hodgkin-Katz equation, the channels are mainly permeable to barium. Kinetic analysis was performed on a 24-sec segment of recording obtained at −60 mV (partly shown in Fig. 1*A*). Within this segment, the activity of only one open-level current corresponding to the 21 pS conductance channel was observed. Such a long period of single-level activity occurred only once in all our cell-attached recordings. The distributions of open and closed times, shown in Fig. 1*C*, were best fitted with the sum of two exponential functions, giving two mean open times of 5.7 and 13.3 msec and two mean closed times of 3.2 and 47.8 msec.

We investigated whether similar spontaneous single-channel activity could be observed in excised inside-out patches with Ba^{2+} ions as the charge carrier. We used K-aspartate bathing solutions to avoid the contribution of possible inwardly directed chloride currents. Figure 2*A* shows unitary barium currents recorded from an inside-out patch maintained at different holding potentials. As in cell-attached patches, activity appeared in irregular bursts, which were, however, markedly different in their kinetics (*compare* Fig. 2*A* with 1*A*), and sepa-

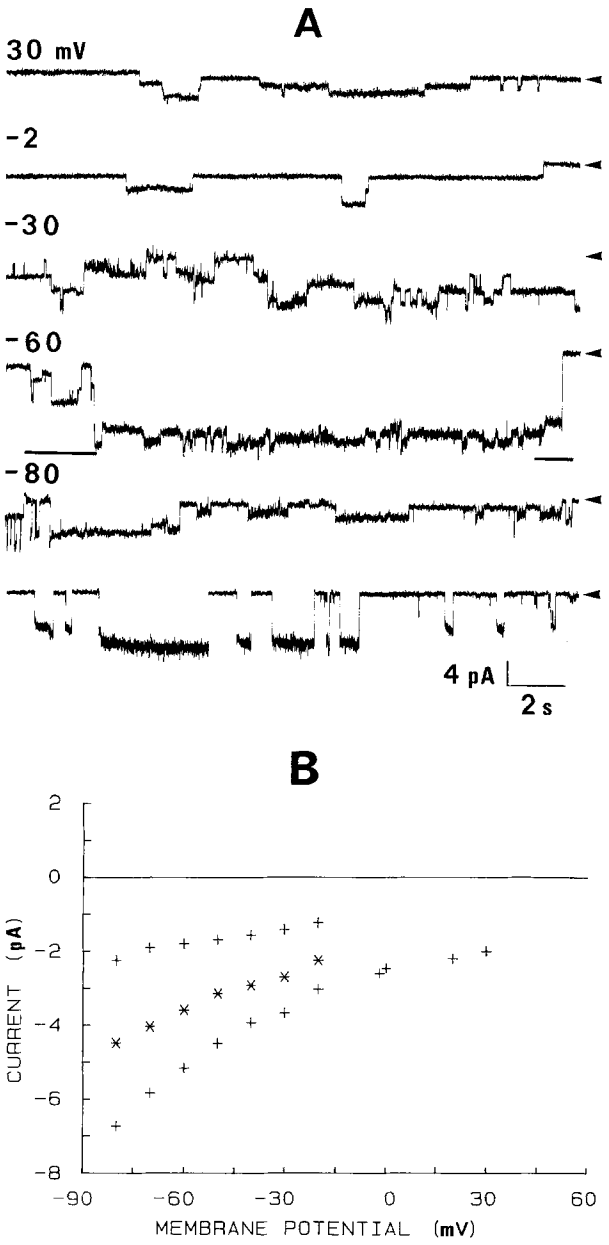

Fig. 2. (*A*) Spontaneous unitary inward barium currents recorded from an excised inside-out membrane patch. Records were collected at different holding potentials indicated on the left of each recording (for −80 mV, two consecutive current traces are shown). The closed levels are marked with arrows. Downward deflections of the current trace represent inwardly directed membrane currents. The bath contained the following solution (in mM): 125 K-aspartate, 1 $MgCl_2$, 5 EGTA, 10 HEPES; pH was adjusted to 7.4 with KOH. The pipette contained (in mM): 96 $BaCl_2$, 10 HEPES; pH was adjusted to 7.4 with $Ba(OH)_2$. (*B*) Single-channel current amplitudes plotted *vs.* membrane potential for the three most frequently observed current amplitudes

rated by shorter (2 to 10 min) silent periods. This kind of activity was observed in 52 patches out of 167. Replacing K-aspartate with Na-aspartate gave the same results. Despite the absence of enzymes or nucleotides in the bath solution, no apparent run-down of channel activity was observed, and periods of activity were recorded in this experiment over more than 45 min. At least three different amplitudes of open-channel current contribute to the complex patterns of activity shown. Two or three successive openings were sometimes followed by apparently simultaneous closures (*see* trace at −60 mV), or sometimes a series of two or more substates combined to give large openings and closures (*see* −80-mV trace). This concerted gating of several conductance levels was a major feature of the activity described here; only rarely, and for short periods of time compared with the channel kinetics, did one current level appear independently and this makes detailed kinetic analysis of these events very difficult. We calculated the mean single-channel current flowing through the patch for a long period of time for each different membrane potential, but no simple correlation with the potential could be established. This is very likely due to the episodic behavior of the channels. In Fig. 2*B*, we have plotted the current-voltage (*I/V*) relationships of the three open single-channel current levels most frequently observed. This excluded the very large and relatively rare events observable on traces at −60 and −80 mV. Three conductance levels, 22, 45 and 78 pS, can be calculated between −80 and −60 mV, using the same approximation as in Fig. 1*B*.

Steady-State Recordings of Unitary Calcium Currents

In order to test the permeability of these channels to calcium ions, experiments were carried out with Ca^{2+} as the charge carrier. Figure 3*A* shows that complex gating patterns were observed, similar to those obtained with barium as the charge carrier (Fig. 2*A*). Twenty-four excised inside-out membrane patches gave similar results. Figure 3*B* shows activity of only one channel recorded from a cell-attached patch. A conductance of 48 pS was estimated from the recordings between −120 and −60 mV. This conductance is close to the conductance of 45 pS obtained in similar conditions with barium as the charge carrier. In this example, at each membrane potential, short periods of activity were interspersed with long periods of inactivity. In 15 other cell-attached patches, the single-channel activity showed more complex gating behavior, similar to that shown in Fig. 1*A* when Ba^{2+} was the charge carrier.

Are These Divalent Cation-Permeable Channels Also Permeable to Na and K Ions?

The single-channel activity described above is clearly different from the available data concerning calcium channels in cardiac myocytes. At least a

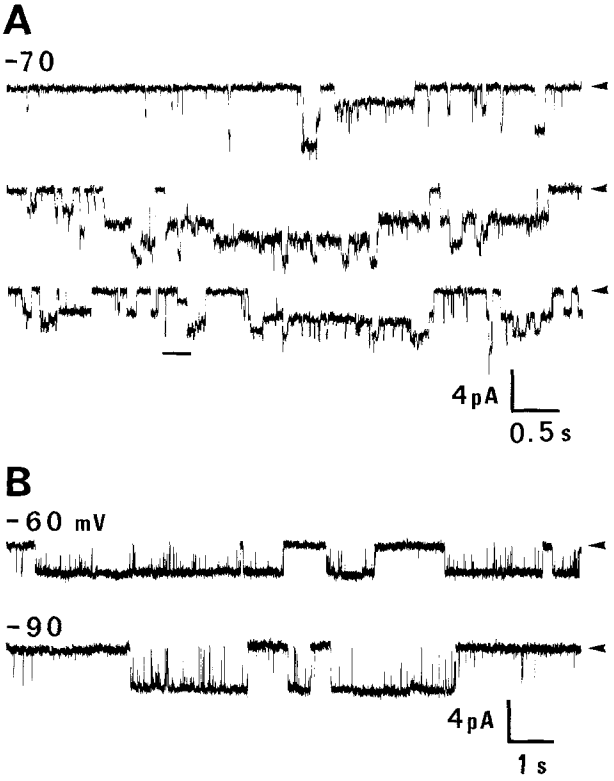

Fig. 3. Steady-state recordings of spontaneous single-channel activity with calcium ions as the charge carrier. (*A*) Three consecutive traces recorded from an excised inside-out membrane patch at a holding potential of −70 mV. The bath solution contained (in mM): 125 K-aspartate, 1 $MgCl_2$, 5 EGTA, 10 HEPES; pH was adjusted to 7.4 with KOH. The pipette contained (in mM): 96 Ca-lactate, 10 HEPES, 1 $MgCl_2$; pH was adjusted to 7.4 with $Ca(OH)_2$. (*B*) Unitary calcium currents recorded from a cell-attached membrane patch, at two different steady-state patch membrane potentials, as indicated. The bath solution was the same as in *A*. The pipette contained (in mM): 96 $CaCl_2$, 10 HEPES; pH was adjusted to 7.4 with CsOH

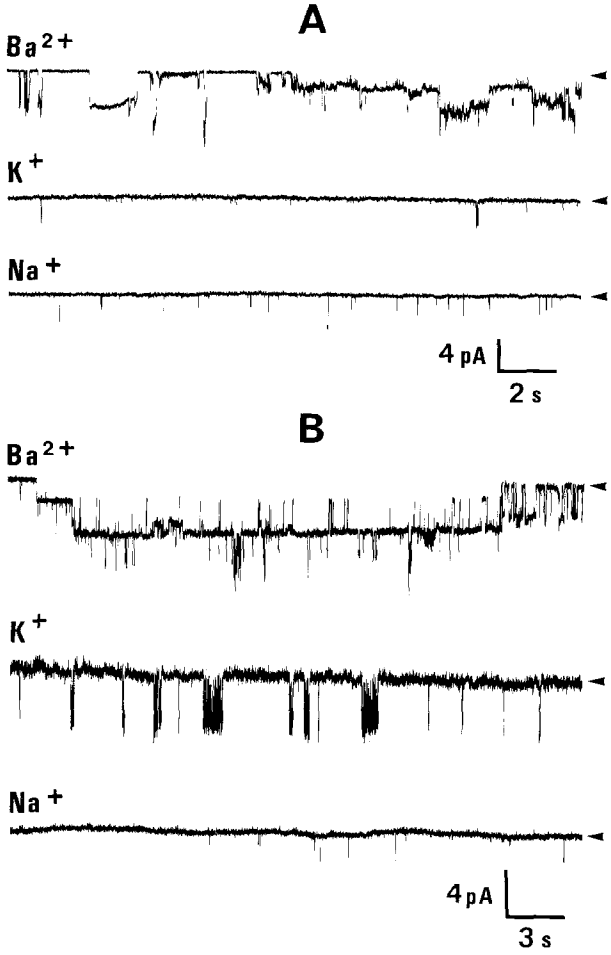

Fig. 4. Sequences of single-channel activity recorded from two different excised outside-out membrane patches (*A* and *B*) at a holding potential of −80 mV. The external surface of each patch was successively perfused with isotonic solutions of $BaCl_2$, KCl or NaCl as indicated on each trace. No more than 3 sec separate the three traces shown here. The pipette contained (in mM): 100 K-aspartate, 25 Cs-aspartate, 2 $MgCl_2$, 2 $BaCl_2$, 5 EGTA, 10 HEPES; pH was adjusted to 7.4 with CsOH. The perfusion mediums were (in mM): for Ba^{2+}-labeled traces: 96 $BaCl_2$, 10 HEPES; pH was adjusted to 7.4 with $Ba(OH)_2$; for K^+-labeled traces: 140 KCl, 3.6 $CaCl_2$, pH 7.4 with KOH; and for Na^+-labeled traces: 140 NaCl, 3.6 $CaCl_2$, pH 7.4 with NaOH

part (the 22-pS conductance level) of the spontaneous activity observed with calcium or barium as charge carriers could involve a nonselective cationic channel rather than calcium channels, since a nonselective cationic channel of 30 pS has been described in cardiac cells (Colquhoun et al., 1981) and in other cells (Maruyama & Petersen, 1982; Yellen, 1982). Figures 1 and 2 already showed that there was no significant potassium efflux through the channels studied here, since the reversal potential was clearly very positive. It remained to be tested whether inward currents carried by Na or K ions could be detected in patches in which spontaneous unitary inward barium or calcium currents were measured.

Figure 4 shows records from two excised outside-out membrane patches; in each case, the external surface of the patch membrane was first perfused with an isotonic $BaCl_2$ solution until single-channel activity appeared. After a short period during which Ba^{2+} currents were recorded, the perfusion solution was switched to an isotonic KCl solution, then to an isotonic NaCl solution. Two types of results were obtained with this experimental protocol. The first case is illustrated in Fig. 4*A*, where barium currents were clearly observable, whereas in isotonic KCl or NaCl solutions only a few very short channel openings were seen; this behavior was observed in two other outside-out patches. In the second case exemplified in Fig. 4*B*, after barium currents were observed, a clearly different channel activity appeared in KCl isotonic solution and no sizeable current in NaCl solution; this result was observed in three other patches. In one other mem-

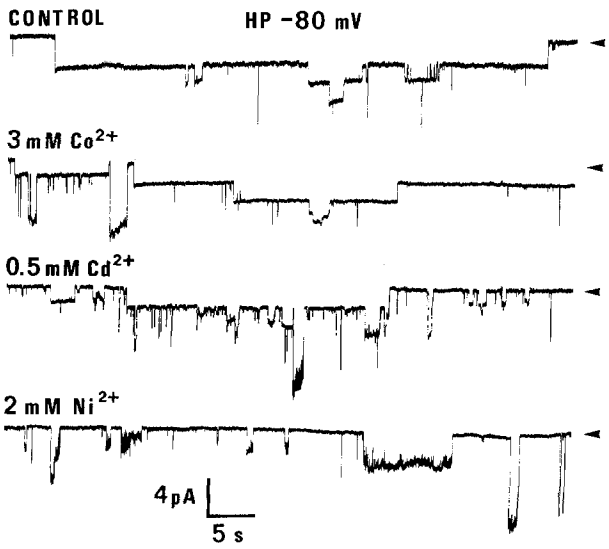

Fig. 5. Low sensitivity of unitary barium currents recorded from an excised outside-out membrane patch to externally applied Co^{2+}, Cd^{2+} or Ni^{2+}. The patch membrane potential was held at −80 mV. In control condition, the bath and the perfusion medium contained (in mM): 96 $BaCl_2$, 10 HEPES; pH was adjusted to 7.4 with $Ba(OH)_2$; Co, Cd and Ni were added to the perfusion medium at the concentrations indicated on the left of each trace. The pipette contained (in mM): 100 K-aspartate, 25 Cs-aspartate, 2 $MgCl_2$, 2 $BaCl_2$, 5 EGTA, 10 HEPES; pH was adjusted to 7.4 with CsOH

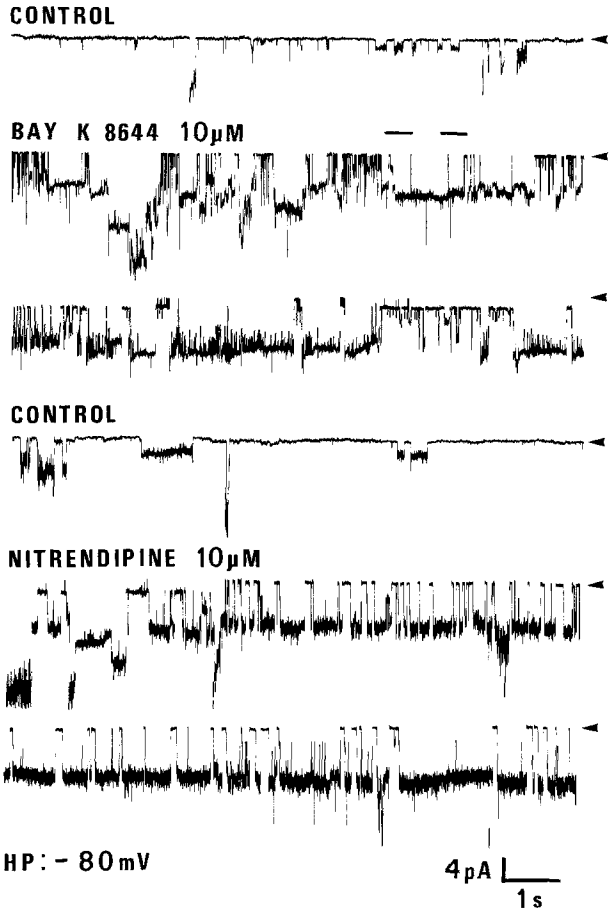

Fig. 6. Effects of internally applied dihydropyridines on unitary barium currents. These sequences of current recording were obtained from excised inside-out membrane patches, before (control traces) and during the application of 10^{-5} M of either Bay K 8644 or nitrendipine to the exposed internal surface of the patch membrane. The membrane potential was maintained at −80 mV. The three upper traces were obtained from one membrane patch, the three lower ones from another patch. The bath and the perfusion medium contained (in mM): 100 K-aspartate, 25 Cs-aspartate, 2 $MgCl_2$, 2 $BaCl_2$, 5 EGTA, 10 HEPES; pH was adjusted to 7.4 with CsOH. Bay K and nitrendipine were added in the perfusion medium. The pipette contained (in mM): 96 $BaCl_2$, 10 HEPES; pH was adjusted to 7.4 with $Ba(OH)_2$

brane patch, K channels were recorded in isotonic KCl solution and no channels were recorded in isotonic NaCl or $BaCl_2$. As divalent cation-permeable channel activity can be observed in the almost complete absence of K or Na channel activity (Fig. 4*A*), and as the pattern of activity observable in K solutions, when present, was markedly different from that observed, in the same patch, with Ba solutions (Fig. 4*B*), and can be attributed to resting K channels well known in cardiac membranes (essentially the background K channel, I_{K_1}), it seems reasonable to conclude that these divalent cation-permeable channels are weakly or not permeable to Na or K ions.

Pharmacological Investigations

As the channels studied here seem to show a significant permeability to divalent cations, we examined the effects of various calcium channel blockers applied to the external surface of excised outside-out membrane patches. The upper trace of Fig. 5 shows a control recording of channel currents carried by Ba^{2+} ions, whereas the other three traces show the effect of externally applied Co^{2+}, Cd^{2+} and Ni^{2+}, respectively. No clear effects of these blockers were observed in this example or in 11 other experiments. In seven inside-out membrane patches, the organic calcium channel blocker D-600 (5×10^{-6} M) was applied to the internal surface of the membrane without any evident inhibition of the previously existing activity. On the other hand, internally applied dihydropyridines exerted clear effects in some excised membrane patches. Figure 6 shows the effect of Bay K 8644 (Bay K) and nitrendipine on unitary barium currents recorded from excised inside-out membrane patches at −80 mV. In the upper three traces of Fig. 6, after a control period (first trace), Bay K was applied to the internal surface of the membrane (second and third traces); after 20 to 50

sec, channel activity markedly increased. Nitrendipine induced a similar increase of activity (lower three traces of Fig. 6). At the concentration used, both Bay K and nitrendipine enhanced openings of the larger conductance state of the channel, which was only occasionally (Fig. 2*A*, lower trace at −80 mV) observed under control conditions. It should be noted, however, that these activating effects were clearly observed in 10 out of 17 patches for Bay K and two out of six patches for nitrendipine. In the other cases, neither Bay K nor nitrendipine had any clear effect upon the ongoing channel activity. When activation was observed, it was never clearly reversible during the remaining life of the patch. Other substances such as ATP (5 mM), cAMP (2 mM), inositol (1,4,5)-trisphosphate (2×10^{-5} M), caffeine (10 mM), ryanodine (10^{-6} M) and quinidine (0.5 mM) were without effect on channel activity when applied at the internal surface of the membrane channel. Channel activity was neither activated nor suppressed by changing internal calcium concentration from 0 (EGTA 10 mM or EDTA 25 mM) to 0.1 mM.

Voltage-Dependence

The episodic nature of the activity of these channels had made it impossible for us to determine whether or not they showed any dependence upon membrane voltage. But in those cases where Bay K 8644 stimulated channel activity, this episodicity was markedly reduced. Figure 7*A* shows recordings obtained after the exposure of the internal surface of one such membrane patch to 5×10^{-6} M Bay K for 50 sec. Sustained channel activity showing complex gating behavior was observable over more than 40 min, even though the patch was perfused with K-rich solution, which no longer contained Bay K, but again single openings could not be analyzed because they were always interspersed with more complex events. In an attempt to partially circumvent this problem and to study the relationship between channel opening and membrane potential, we used the same method as Martinac et al. (1987). At each membrane potential, for a period of time of 3 to 4 min, the integral of the unitary current was computed and divided by the integral of the maximum current, which would flow through the same channels if they were permanently open over the same period. Figure 7*B* shows the values obtained, representing normalized mean unitary current, plotted *vs.* patch membrane potential. In the case of non-identical amplitudes of transition levels, this method only provides an indication that a relation exists between the single-channel activity and the membrane potential. The amplitude histograms computed from segments of steady-state recordings at different holding potentials (Fig. 7*C*), indicate a decrease of time spent in closed states when membrane potential becomes more positive. It is, however, possible that this voltage dependence was induced by Bay K.

Whole Cell Recordings

We tested the possibility that the above-described calcium-permeable channels may induce currents detectable in the whole cell recording configuration. Figure 8 shows records of currents obtained in a cell clamped at a holding potential of −80 mV, during 350-msec depolarizing pulses to 0 mV at a frequency of 0.2 Hz. It can be seen that the addition of 10^{-5} M Bay K induces both an increase of the macroscopic inward calcium current (lower trace) and minute inwardly directed background current detectable at higher magnification (upper trace). Erratic periods of small background activity were also observed under control conditions whereas in several cases no increase of activity appeared during Bay K perfusion. However, in four out of 14 experiments, a clear correlation was observed between Bay K perfusion and background activity. In three experiments, the dihydropyridine solvent (17 mM ethanol) decreased the amplitude of the voltage-dependent calcium current without inducing background irregularities similar to those shown in Fig. 8. In these experiments, 10^{-6} M ryanodine was added to the external medium to eliminate the possibility of spontaneous cell oscillations (Lakatta et al., 1985). In the absence of ryanodine, the addition of 10^{-5} M Bay K often induced larger background irregularities than those shown in Fig. 8, and in five out of 12 experiments, irreversible contracture occurred.

Discussion

The results presented here show for the first time that a spontaneous single-channel activity, using barium or calcium ions as charge carriers, can be detected over a large range of potentials (including very negative holding potentials) in the membrane of freshly isolated rat ventricular cells. Although we are presently unable to provide valuable information about the physiological significance of these channels, their activity could account for the resting calcium conductance in cardiac myocytes. Their main characteristic is their erratic behavior.

The divalent cation-permeable channels described here resemble classical voltage-activated Ca channels in only a few points whereas they differ from them by several properties. They resemble voltage-activated Ca channels in their dual perme-

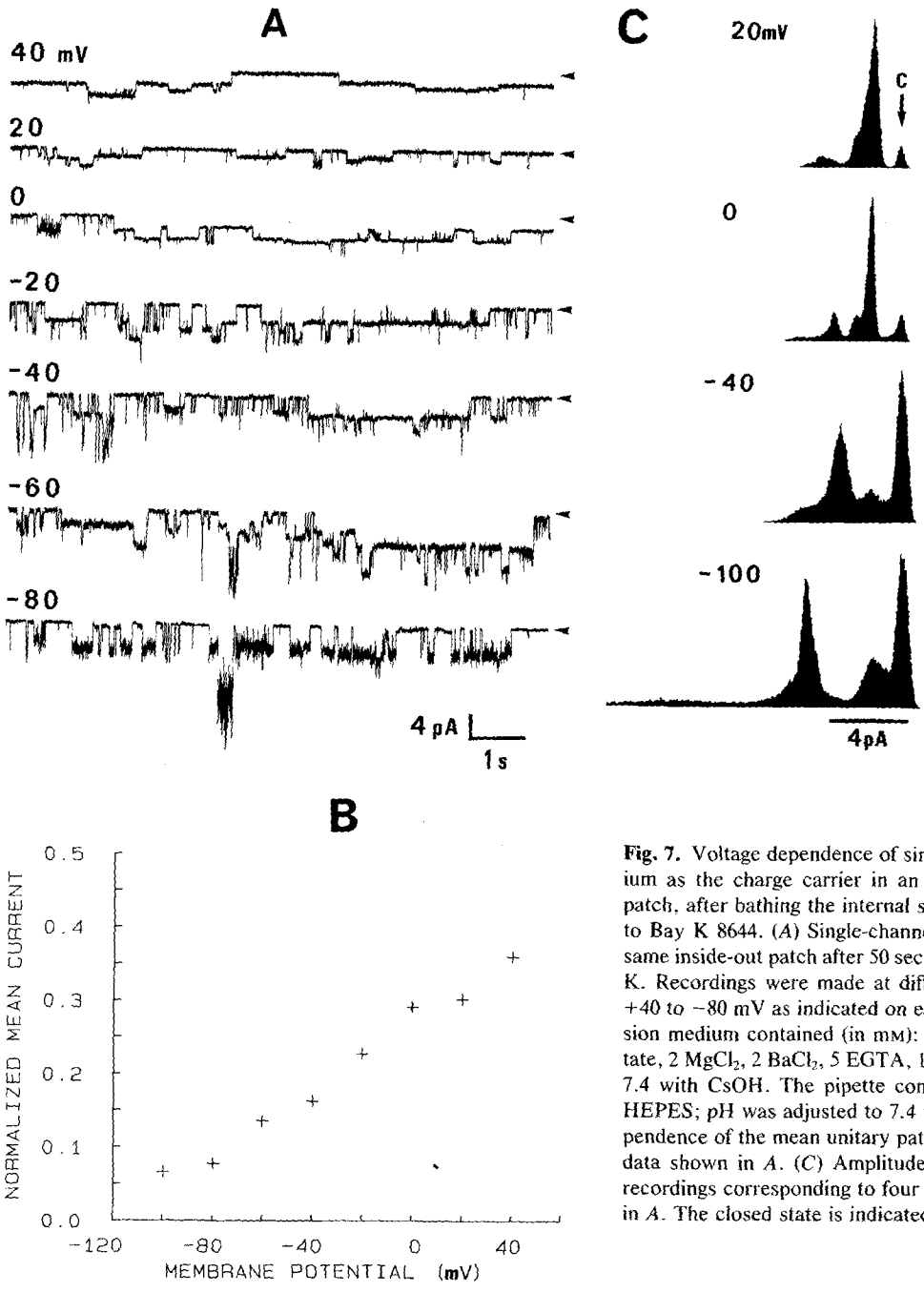

Fig. 7. Voltage dependence of single-channel activity, with barium as the charge carrier in an excised inside-out membrane patch, after bathing the internal surface of the patch membrane to Bay K 8644. (*A*) Single-channel current recordings from the same inside-out patch after 50 sec exposure to 5×10^{-6} M of Bay K. Recordings were made at different holding potentials from +40 to −80 mV as indicated on each trace. The bath and perfusion medium contained (in mM): 100 K-aspartate, 25 Cs-aspartate, 2 $MgCl_2$, 2 $BaCl_2$, 5 EGTA, 10 HEPES; pH was adjusted to 7.4 with CsOH. The pipette contained (in mM): 96 $BaCl_2$, 10 HEPES; pH was adjusted to 7.4 with $Ba(OH)_2$. (*B*) Voltage dependence of the mean unitary patch current calculated from the data shown in *A*. (*C*) Amplitude distribution histograms from recordings corresponding to four different membrane potentials in *A*. The closed state is indicated by an arrow and the letter *C*

ability to Ca and Ba, their activation by the calcium agonist, Bay K 8644, and the fact that one of their conductance values (≈20 pS) is close to that of the L-type Ca channel (25 pS in 96 mM external Ba, at 28°C in rat cardiac myocytes, Kokubun et al., 1986). They differ from both L- and T-type voltage-activated Ca channels by the following properties: (*i*) they open under steady-state conditions from at least −180 to +40 mV. In contrast, the L-type Ca channel (the only one described until now in the rat ventricle; Kokubun et al., 1986) activates only at membrane potentials more positive than −40 mV and then inactivates relatively slowly, whereas the T-type channel activates at potentials around −60 mV and inactivates rapidly (Nilius et al., 1985). (*ii*) They persist for hours after membrane isolation, a point which is not so distinct from T-type channel behavior but which is greatly different from L-type channel behavior, which rapidly disappears (run-down) in excised patches (Cavalié et al. 1983; Nilius

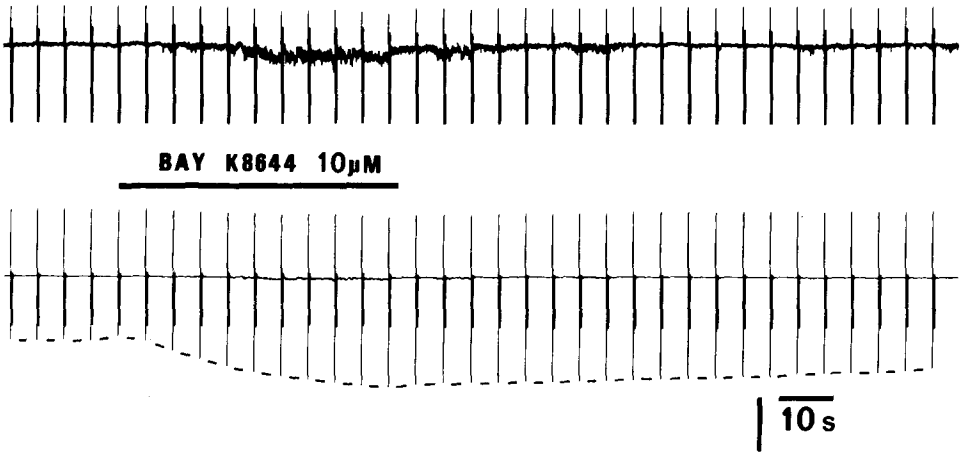

Fig. 8. Effect of Bay K 8644 on macroscopic voltage-dependent slow calcium current (lower trace) and background current (upper trace, recorded at higher magnification). Whole cell recording configuration. The bath contained (in mM): 135 NaCl, 5.4 KCl, 1 $MgCl_2$, 0.18 $CaCl_2$, 10 glucose, 1 ribose, 0.001 ryanodine, 10 HEPES; pH was adjusted to 7.4 with NaOH. The perfusion medium contained (in mM): 105 choline-Cl, 20 TEA-Cl, 1 $MgCl_2$, 2 $CaCl_2$, 3 4-aminopyridine, 10 glucose, 1 ribose, 0.001 ryanodine, 10 HEPES; pH was adjusted to 7.4 with CsOH. The pipette contained (in mM): 105 Cs-aspartate, 20 TEA-Cl, 4 ATP Mg^+-salt, 5 $MgCl_2$, 5 EGTA, 10 glucose, 2 KH_2PO_4, 10 HEPES; pH was adjusted to 7.2 with CsOH. Vertical bar: 100 pA in upper panel, 500 pA in lower panel

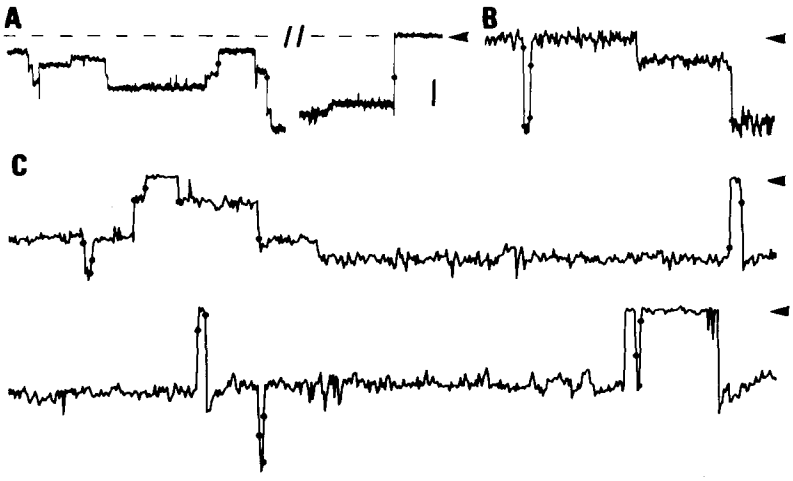

Fig. 9. Multiple conductance states of the channel permeable to divalent cations. Panels *A* and *B* represent traces recorded from excised membrane patches obtained in normal conditions, whereas panel *C* shows traces obtained under 10^{-5} M Bay K 8644. Traces of panel *A* were expanded from trace at −60 mV of Fig. 2*A*; trace of panel *B* was from Fig. 3*A*; traces of panel *C* were from Fig. 6. Dots indicate transitions poorly detectable or undetectable on the continuous records but clearly detectable on digitized records at 1 kHZ. Vertical bar in *A*: 4 pA. The expanded segments shown above are indicated by horizontal bars in Figs. 2*A* (−60 mV), 3*A* and 6 (Bay K 8644)

et al., 1985). (*iii*) They seem almost insensitive to usual inhibitory concentrations of inorganic calcium channel blockers, Cd, Ni and Co. (*iv*) Their gating properties are more complex than those which were described initially for the L-type by Hess, Lansman and Tsien (1984), although they are closer to the new gating pattern recently observed for these same channels (Chen & Hess, 1987). The results illustrated in Fig. 9, which correspond to expanded segments of Figs. 2, 3 and 6, show that several levels of activity can be controlled by a single gating mechanism. For example, in Fig. 9*A*, several levels of about 2 pA occur successively (corresponding to the small channel current level recorded at −60 mV in Fig. 2*B*), whereas in the right part of the figure the activity ceases by two closing steps of ≃4 and 6 pA. This suggests that the larger current levels correspond to simultaneous openings of multiple small conducting units in parallel operated by a main gate in addition to their own individual gate (Meves & Nagy, 1989).

The fact that the background Ca channels described here can be activated not only by Bay K 8644 but also by the Ca channel "antagonist," nitrendipine, is not a novel characteristic. Ca channel antagonists are known for demonstrating partial agonist properties (Hess et al., 1984; Bean, 1985).

The channels described in the present paper share some characteristics with other Ca-permeable channels. For example, a channel of 20 pS (in 60

mM Ca solution) has been observed under steady-state conditions in a large range of negative membrane potentials up to −120 mV in *Aplysia* neurons (Chesnoy-Marchias, 1985). The dihydropyridine-sensitive Ca channel from skeletal muscle transverse tubules incorporated into planar lipid bilayers (20 pS in 90–100 mM Ba; Affolter & Coronado, 1985; Flockerzi et al. 1986), also shows conductance heterogeneity (Talvenheimo, Worley & Nelson, 1987; Ma & Coronado, 1988). A complex voltage-independent channel system of three channels with conductances of 10, 25 and 36 pS, which are assumed to represent subconductance states of a single ion channel, permeable to calcium and barium and activated by Bay K 8644, has been observed from the *Aplysia* nervous system incorporated into phospholipid bilayers (Coyne et al., 1987). A background (B-type) voltage-dependent Ca channel has also been recorded in planar bilayers enriched in sarcolemmal vesicles from calf ventricular muscle (Rosenberg & Tsien, 1987; Rosenberg et al., 1988). This B-type channel seems particularly relevant to the present work in spite of the fact that it does not show complex gating properties and that Rosenberg et al. (1988) have not detected it in either cell-attached or excised patches of guinea-pig ventricular myocytes. When the B-type channel was recorded in 100 mM external Ba or Ca, its *I/V* relationship was similar to that of the smaller conductance level found here, 20 and 21 pS, respectively. The cardiac B channel could correspond to those described in the present paper and represent a simplified behavior of these same channels possibly as a result of the procedure of preparation and incorporation of the membrane vesicles into lipid bilayers.

A resting Ca influx is known to exist in cardiac cells (0.009 pmol $cm^{-1} \cdot sec^{-1}$ in the frog heart in 1 mM Ca-Ringer, Niedergerke, 1963). If one of the physiological roles of these background Ca channels is to provide this resting Ca influx, the relative abundance of such channels (compared with the paucity of voltage-activated Ca channels, almost 90% of the high resistance seals being deprived of single Ca channel activity, McDonald et al., 1986) may seem surprising. It is clear that in order to avoid resting Ca overload, such channels should be strictly controlled and maintained most of the time in their closed conformation. The fact that in our experiments and especially in cell-attached recordings, periods of activity were relatively rare and separated from each other by long and variable periods of quiescence could be explained if these channels were compatible with the relaxed diastolic state of cardiac cells, and this may be why they have not been systematically detected before. Unfortunately, their erratic behavior makes it difficult to estimate the quantity of calcium that could enter the cell through this pathway.

A possibility would be that these background Ca channels are part of a transmembrane-endocellular system involved in some way in the replenishment, or the emptying, of intracellular Ca stores (for example the terminal cisternae of the sarcoplasmic reticulum), which have been mechanically disconnected from internal structures (and thus artifactually opened) by the membrane distortion involved in the formation of a "giga-seal" between the pipette and the cell. The fact that activity is more frequent after patch isolation could be taken as an argument in favor of this hypothesis. In this case, these background Ca channels could be the equivalent, in cardiac cells, of the T-tubule channels isolated from skeletal muscle and reconstituted in lipid bilayers (Affolter & Coronado, 1985; Flockerzi et al., 1986; Talvenheimo et al., 1987; Ma & Coronado, 1988).

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