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Voltage-Dependent Calcium Channels from *Paramecium* Cilia Incorporated into Planar Lipid Bilayers

Abstract. *Two different divalent cation–selective channels from* Paramecium *cilia were incorporated into planar lipid bilayers. Both channels were much more permeable to divalent than univalent cations, and one of them discriminated significantly among the divalent cations. The selectivity and voltage dependence of the latter channel are comparable to those of voltage-dependent calcium channels found in a variety of cells. A combined biochemical, biophysical, and genetic study of calcium channels is now possible.*

Although Ca^{2+} currents mediate a large variety of cellular processes, Ca^{2+} channels have been difficult to study in cell membranes because of problems in controlling transmembrane voltage, in varying ionic composition, and in isolating Ca^{2+} currents (*1*). These cell-related problems can be avoided by incorporating Ca^{2+} channels into a planar lipid bilayer. We used vesicles made from *Paramecium* ciliary membranes because these cilia are an excellent source of Ca^{2+} channels that are well characterized electrophysiologically (*2*) and easily modified genetically and biochemically (*3*, *4*). We found that *Paramecium* ciliary membranes contain two types of channels that are divalent cation–selective. Although both channels are virtually impermeable to monovalent cations, the channels differ in conductance, divalent cation selectivity, and voltage dependence. The larger [30-picosiemens (pS)] channel selects poorly among divalent cations and is only weakly voltage-dependent. The smaller (1.5- to 2-pS) channel is very selective (Ca^{2+} and Ba^{2+} can be current carriers, but Mg^{2+} is virtually impermeant) and is strongly voltage-dependent (an *e*-fold change in membrane conductance for an ~9-mV change in membrane potential). The larger channel may be related to the mechanoreceptor of *Paramecium* (*5*) and the smaller channel is probably responsible for the Ca^{2+} current measured in the intact *Paramecium*. [A Ca^{2+}-activated K^+ channel has probably also been identified in ciliary membranes by incorporation into planar bilayers (*6*)].

Ciliary membrane vesicles were prepared as described by Adoutte *et al.* (*7*), except that 1 m*M* KCl was added to all solutions after deciliation. Experiments were done with six different preparations of vesicles; fresh or frozen vesicles yielded the same results. Membranes made from asolectin (90 percent) and cerebroside (10 percent) or from phosphatidylethanolamine (75 percent), phosphatidylserine (15 percent), and cerebroside (10 percent) were formed by painting a solution of lipid in decane across a 50- to 100-μm hole in a Teflon sheet that bisected a Lucite chamber (*8*). Cholesterol was added to the lipid solution in some experiments, but had no detectable systematic effects. Vesicles were always added to the *cis* compartment. All experiments were done at room temperature under voltage-clamp conditions. Values for the holding potentials refer to the potential of the *cis* compartment with respect to the *trans* compartment, which was held at virtual ground. Membrane currents were amplified, converted to

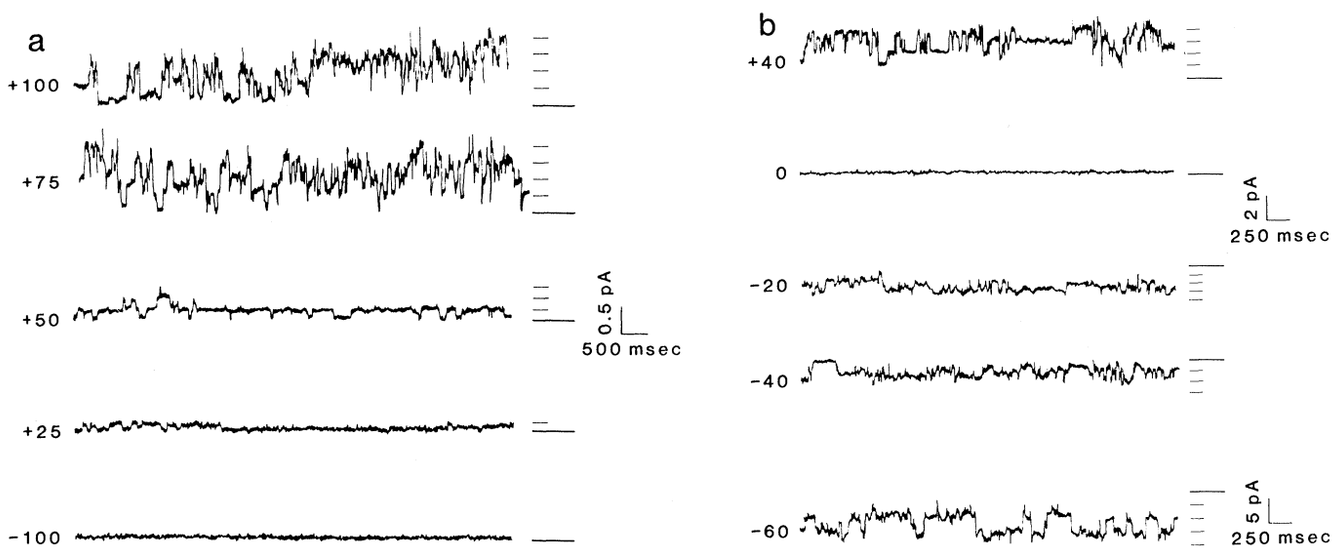

Fig. 1. Single-channel currents from *Paramecium* ciliary membrane vesicles incorporated into a planar bilayer. Long horizontal lines to the right of current traces indicate the zero current level and short lines indicate single-current steps; bare bilayer current has been subtracted in all records. Numbers on the left indicate the holding potential of the *cis* compartment with respect to the *trans* compartment, which is held at virtual ground. Upward deflections from the zero current level represent positive current from the *cis* to *trans* compartment. (a) Records from an experiment in which only the small channel was observed with 50 m*M* $BaCl_2$ (*cis*) and 50 m*M* $MgCl_2$ and 0.1 m*M* $BaCl_2$ (*trans*). Single-channel conductance is 1.6 pS. During the experiment, potential steps were randomized so that "off" periods cannot be interpreted as "no-channel" periods. This membrane contains four active channels. The probability of observing an open channel increases with more positive potentials (see Fig. 3). Records were filtered at 60 Hz. (b) Records from an experiment in which only the larger channel was observed with 10 m*M* $BaCl_2$ (*cis*) and 10 m*M* $MgCl_2$ and 0.1 m*M* $BaCl_2$ (*trans*). Single-channel conductance is 29 pS. This membrane contains four active channels that are on most of the time, independent of voltage. Records were filtered at 150 Hz.

voltages, and recorded with a Vetter tape recorder and a Physiograph chart recorder. The records were analyzed by hand.

After adding small quantities of vesicles (final concentration, 5 to 10 μg of protein per milliliter) to the *cis* compartment and waiting at least 10 minutes for vesicle-bilayer interactions to occur, we generally observed single-channel currents of two sizes (Fig. 1). These two sizes represent different ionic channels with single-channel conductances of 30 pS (Fig. 1b) and 1.5 to 2 pS (Fig. 1a) in 10 m*M* $BaCl_2$. To determine whether either channel was the voltage-dependent Ca^{2+} channel, we compared the characteristics of both to the known characteristics of Ca^{2+} channels.

Both channels were virtually impermeable to Cl^- and K^+ compared to Ba^{2+}. (The reversal potentials for single-channel currents in the presence of Ba^{2+}, K^+, and Cl^- gradients indicate ideal Ba^{2+} selectivity.) The most distinguishing characteristic of Ca^{2+} channels, however, is their discrimination among the divalent cations. The larger (30-pS) channel was slightly more permeable to Mg^{2+} than to Ba^{2+} (calculated from the biionic potential of 0 to 5 mV). Since this result is inconsistent with Ca^{2+} channel selectivity measured in vivo, it is highly unlikely that the 30-pS channel was the voltage-dependent Ca^{2+} channel. The divalent cation selectivity of this channel resembles that of mechanoreceptors in *Paramecium* (*5*).

The smaller channel, on the other hand, was an excellent discriminator between Ba^{2+} and Mg^{2+}. The current reversed at −45 mV when the membrane separated 50 m*M* Ba^{2+} (*cis*) from 50 m*M* Mg^{2+} and 0.1 m*M* Ba^{2+} (*trans*) (Fig. 2). Similar results were generated when 10 and 100 m*M* salts were used. The average of eight experiments showed that the ratio of Ba^{2+} and Mg^{2+} permeability coefficients was 100 to 1. In addition, in experiments in which many channels had been incorporated into the bilayer, the permeability coefficient for Ca^{2+} greatly exceeded that for Mg^{2+}. These results are consistent with the finding in vivo (*1*) that Mg^{2+} does not carry current through the Ca^{2+} channel.

Another general characteristic of Ca^{2+} channels is their strong voltage dependence. For the larger divalent cation–selective channel, the probability of being open did not vary with voltage from −60 to 60 mV (Fig. 1b). In contrast, the probability of finding the smaller divalent cation–selective channel open increased steeply with voltage (Figs. 1a and 3). The steepest slope gives an *e*-fold change in

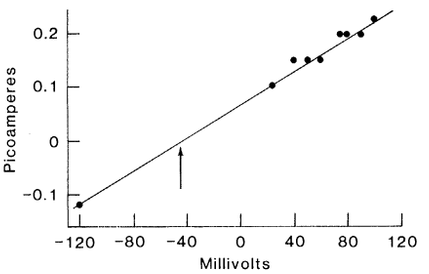

Fig. 2. Single-channel current-voltage relation for the small channel. Data are from the same experiment as in Fig. 1a. (The single-channel current at −120 mV was determined from only four channel openings.) Reversal potential occurs at −45 mV (arrow); for this experiment the ratio of Ba^{2+} and Mg^{2+} permeability coefficients was calculated to be 40 to 1 with the Goldman-Hodgkin-Katz equation.

probability of 9 ± 4 mV (mean ± standard deviation, $n = 5$). This result is similar to the 10 mV calculated from single-channel currents measured in cultured myocytes (*9*), 6 mV from Ca^{2+} currents in R15 cells of *Aplysia* (*10*), and 7 mV from Ca^{2+} currents in intact *Paramecium* (*11*). In addition, the mean open time appeared to be voltage-independent, as in the patch-clamped Ca^{2+} channel (*9, 12–14*). Single-channel conductances were the same in 10, 50, and 100 m*M* $BaCl_2$, suggesting saturation at 10 m*M*. This lends additional support to our

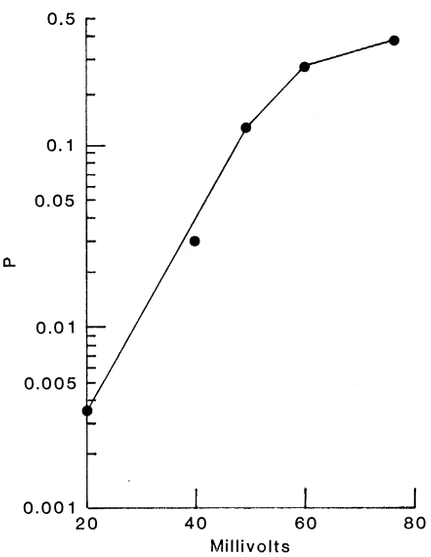

Fig. 3. Voltage dependence of the smaller channel in the same solutions as used in the experiment represented in Fig. 1a. In this experiment two active channels were observed. The probability of a channel being open (P) was calculated from $P = I/Ni$, where I is the time-averaged current, N is the number of active channels, and i is the single-channel current. I is determined from 15 to 24 seconds of current recordings at each voltage. From the steepest slope we see that the membrane potential changes 8 mV for an *e*-fold change in P.

hypothesis that the smaller channel is the Ca^{2+} channel, since in intact *Paramecium* Ca^{2+} currents are maximal by 1 m*M* (*11*).

An interesting difference between bilayer incorporated channels on the one hand and cell patch and perfused cell Ca^{2+} channels on the other is that the latter seem to disappear within minutes (*13, 15*). This apparent difference may be explained by the lack of cellular proteases in vesicles made from pure cilia or by species differences. Alternatively, since we were adding ~10^{10} Ca^{2+} channels per experiment, more than 99 percent of the Ca^{2+} channels could have lost their activity and we would still have had an enormous pool of active Ca^{2+} channels available for incorporation into a planar bilayer (*16*).

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