

# MULTI-ION CONDUCTION AND SELECTIVITY IN THE HIGH-CONDUCTANCE $\text{Ca}^{++}$ -ACTIVATED $\text{K}^+$ CHANNEL FROM SKELETAL MUSCLE

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**ABSTRACT** Open-channel ion permeation properties were investigated for  $\text{Ca}^{++}$ -activated  $\text{K}^+$  (CaK) channels in solutions of  $\text{K}^+$  and its analogues  $\text{Tl}^+$ ,  $\text{Rb}^+$ , and  $\text{NH}_4^+$ . Single CaK channels were inserted into planar lipid bilayers composed of neutral phospholipids, and open-channel current-voltage (I-V) relations were measured in symmetrical and asymmetrical solutions of each of these individual ions. For all concentrations studied, the zero-voltage conductance falls in the sequence  $\text{K}^+ > \text{Tl}^+ > \text{NH}_4^+ > \text{Rb}^+$ . The shape of the I-V curve in symmetrical solutions of a single permeant ion is non-ohmic and is species-dependent. The I-V shape is sublinear for  $\text{K}^+$  and  $\text{Tl}^+$  and superlinear for  $\text{Rb}^+$  and  $\text{NH}_4^+$ . As judged by reversal potentials under bi-ionic conditions with  $\text{K}^+$  on one side of the bilayer and the test cation on the other, the permeability sequence is  $\text{Tl}^+ > \text{K}^+ > \text{Rb}^+ > \text{NH}_4^+$  at 300 mM, which differs from the conductance sequence. Symmetrical mixtures of  $\text{K}^+$  or  $\text{NH}_4^+$  with  $\text{Rb}^+$  show a striking anomalous mole fraction behavior, i.e., a minimum in single-channel conductance when the composition of a two-ion mixture is varied at constant total ion concentration. This result is incompatible with present models that consider the CaK channel a single-ion pore. In total, the results show that the CaK channel finely discriminates among  $\text{K}^+$ -like ions, exhibiting different energy profiles among these species, and that several such ions can reside simultaneously within the conduction pathway.

## INTRODUCTION

Many cells exhibit a  $\text{K}^+$  permeability which is activated by an increase in cytoplasmic  $\text{Ca}^{++}$  concentration (Meech, 1978). It has been shown that this  $\text{Ca}^{++}$ -activated  $\text{K}^+$  (CaK) permeability is mediated by several types of ion channels, one of which has been characterized in some detail. This CaK channel is voltage-dependent, with opening being enhanced at depolarized potentials. The main effect of cytoplasmic  $\text{Ca}^{++}$  is to shift the voltage activation curve along the voltage axis, and thus "activate" the channel. Whereas the  $\text{Ca}^{++}$ - and voltage-dependent gating processes of this channel have been intensely studied for several years (Marty, 1981; Pallota and Magleby, 1981; Methfessel and Boheim, 1982; Latorre et al., 1982; Magleby and Pallota 1983a,b; Moczydlowski and Latorre, 1983a,b; Pallotta, 1985), less attention has been paid to the open-channel conduction properties. One of the most striking characteristics of this channel is its very high  $\text{K}^+$  conductance (300 pS at 150 mM  $\text{K}^+$ ), combined with high  $\text{K}^+$  selectivity. Neither  $\text{Na}^+$ ,  $\text{Li}^+$ , nor  $\text{Cs}^+$  shows any measurable permeation (Vergara, 1983; Blatz and Magleby, 1984; Yellen, 1984a), and large differences in permeation properties are seen among cations of sizes very similar to that of  $\text{K}^+$ , such as  $\text{Rb}^+$  and  $\text{NH}_4^+$ . Despite the demonstrable permeability to  $\text{Rb}^+$ ,  $\text{Tl}^+$ , and  $\text{NH}_4^+$  (Yell-

en, 1984a, b; Blatz and Magleby, 1984), single-channel conductances in symmetrical solution of ions other than  $\text{K}^+$  have not yet been measured. Here we study the single-channel conduction properties of the close  $\text{K}^+$  analogues  $\text{Rb}^+$ ,  $\text{Tl}^+$ , and  $\text{NH}_4^+$ , the only other permeant ions for this channel.

This paper adds the following three new pieces of information about permeation by small monovalent cations. First, it shows that single channel conductances and I-V shapes can be measured in symmetrical single salt solutions of several monovalent cations besides  $\text{K}^+$ . Second, it demonstrates that the current-voltage (I-V) curves differ in shape for these different permeant species. Third, it establishes that a striking conductance minimum exists in varying mixtures of permeant ions at constant ionic strength. This third finding, often referred to as an "anomalous mole fraction effect," is one of the most unambiguous indications presently known for multiple ion occupancy (Neher, 1975; Sandblom et al., 1977; Hille and Schwarz, 1978; Hess and Tsien, 1984).

## METHODS

### Biochemical

Transverse tubule membranes from rat skeletal muscle were prepared by density gradient centrifugation as described (Moczydlowski and Latorre,

1983b), and were stored as aliquotted suspensions in 0.4 M sucrose at  $-80^{\circ}\text{C}$ . Lipids used were 1-palmitoyl, 2-oleoyl-phosphatidylethanolamine (POPE), or the corresponding phosphatidylcholine (POPC), and were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Ultra-pure  $\text{Rb}^+$  (Cl) or  $\text{Ti}^+$  (acetate or  $\text{NO}_3$ ) salts were obtained from Alpha Inorganics (Danvers, MA). Thallous salts were further purified by treatment of 3 M aqueous solutions with activated charcoal, followed by filtration through Whatman No. 1 paper (Whatman Inc., Clifton, NJ). Lower grades of these salts could not be used because they are contaminated by enough  $\text{Ba}^{++}$  (verified by atomic absorption analysis) to block this channel (Vergara and Latorre, 1983).

## Planar Bilayers

Planar bilayers were formed from n-decane solutions of POPE (21 mM)/POPC (9 mM) according to methods described earlier (Labarca et al., 1980). The electronics were essentially as described (Hanke and Miller, 1983), except that a single time constant capacitance compensation circuit (Sigworth, 1983) was included to cancel most of the bilayer capacitive current. Bilayer capacitances were 150–300 pF. Single CaK channels were transferred from the native plasma membrane into the planar bilayers using the “fusion” method (Latorre et al., 1982). Insertion was always asymmetric, with the  $\text{Ca}^{++}$ -activation side of the channel facing towards the side of the membrane to which vesicles were added, which we call the “inside” solution. In all experiments, we use the physiological voltage convention, with electrical ground defined on the opposite outward-facing side of the channel.

Aqueous solutions used all contained the test cation at an appropriate concentration, and 10 mM morpholino propane sulfonic acid neutralized to pH 7.3 with the hydroxide of the test cation. The internal solution contained  $\text{CaCl}_2$  (5–50  $\mu\text{M}$ ), and the external solution contained 0.1 mM EGTA.

**Criteria for Recognizing the CaK Channel.** Because the behavior of CaK channels in  $\text{K}^+$ -containing solutions has been intensively studied (Pallota and Magleby, 1981; Latorre et al., 1982; Marty, 1981; Yellen, 1984a, b; Blatz and Magleby, 1984), it is easy to recognize this channel when it appears in the planar bilayer. For measurements in pure symmetrical single-cation solutions of other conducting species, however, it was necessary to establish criteria for identifying the correct channel. We did this in several ways when testing a new cation. First, we incorporated the channel in  $\text{K}^+$  solutions, and then changed solutions to introduce the test cation ( $\text{Ti}^+$ ,  $\text{Rb}^+$ , or  $\text{NH}_4^+$ ), by extensive perfusion of the bilayer chamber. This procedure established an approximate conductance to expect for solutions containing the test cation. We then performed channel insertion in symmetrical solutions of the test cation, and checked that the channel observed displayed the following characteristics: activation at depolarized potentials, activation by increased internal  $\text{Ca}^{++}$  (10–100  $\mu\text{M}$ ), and induction of slow ( $\sim 10$  s) blocking events by internal  $\text{Ba}^{++}$  (1–5  $\mu\text{M}$ ) and external charybdotoxin (3 nM). Channels reported here satisfied these criteria. In some cases, after a measurement in the test cation, we re-introduced  $\text{K}^+$  medium to one or both sides to confirm that the familiar properties of the CaK channel were preserved.

**Procedures for Measuring I-V Curves.** Open channel I-V curves were determined on membranes containing a single channel in one of two ways: by voltage ramps or by on-line amplitude histograms. With the former method, single-channel current was collected during a linear applied voltage ramp; in this way, a full I-V curve can be taken in a single sweep. In practice, at least 64 such sweeps were collected and averaged to produce the final I-V curve. The on-line histogram procedure was useful for collecting single-channel currents at low voltages. A constant voltage was applied, and the open-channel current was measured from the sharp peaks in the amplitude histogram constructed from 100–300 opening events. The signals were filtered by an eight pole bessel filter at 1 kHz cutoff frequency.

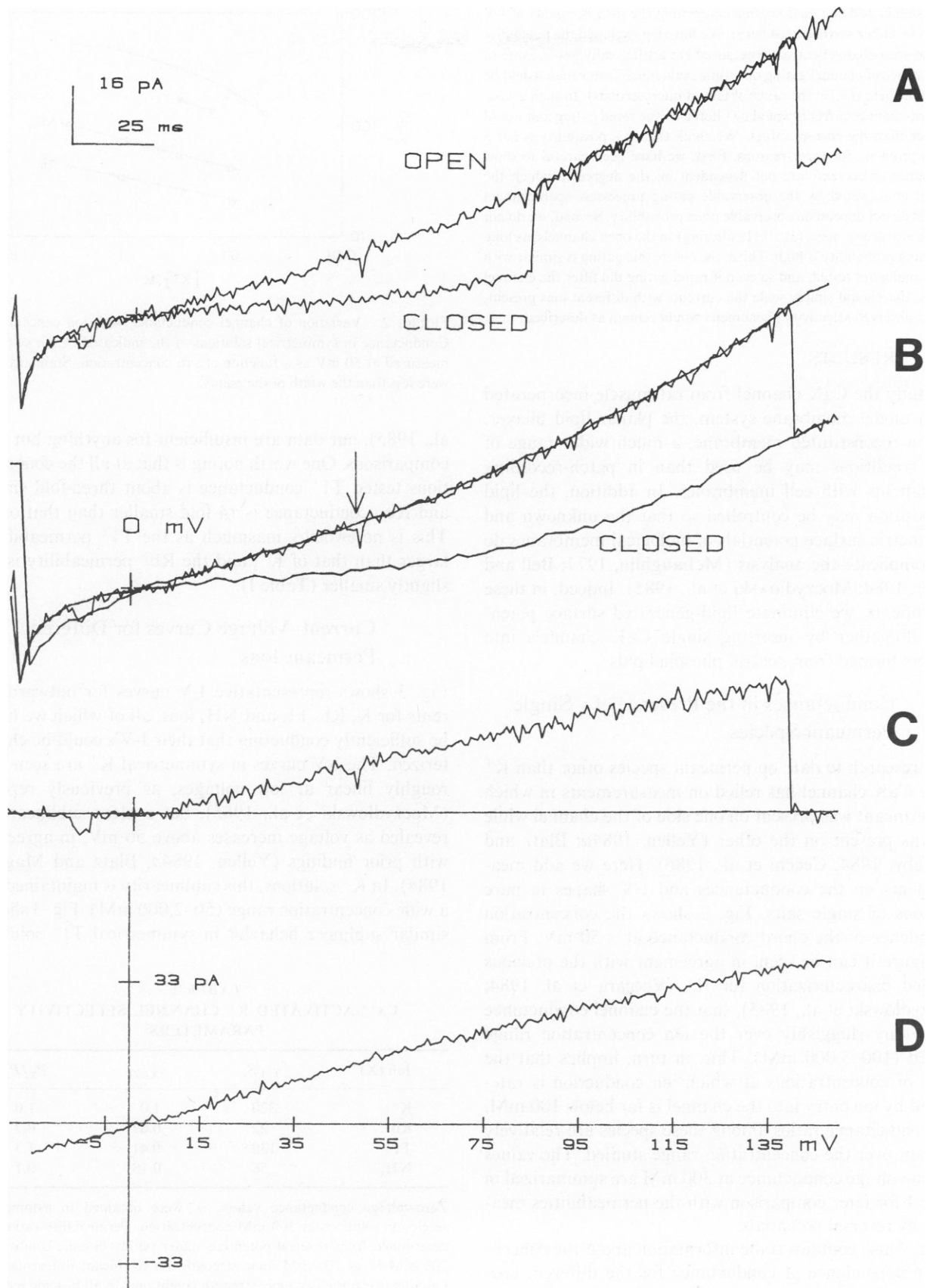
An example of our I-V curve acquisition procedure is shown in Fig. 1. In this experiment, a single channel in symmetrical 300 mM  $\text{K}^+$  was subjected to a series of 256-ms voltage ramps from  $-25$  to  $+150$  mV. *A* shows superimposed current responses to two such ramps. Differences between open and closed levels are apparent, as labeled. *B* shows one of these responses superimposed on the cumulative averages (indicated by fine lines) for the open and closed states obtained from all 64 records. The good agreement between the open/closed levels in a single sweep and those of the cumulative averages further demonstrates the reliability of this method. *C* shows the current for the record of *B* after subtraction of the closed state current. In this sweep, the channel opens briefly at  $\sim -10$  mV, then closes, then opens again at  $\sim +10$  mV and remains open until  $+135$  mV. *D* presents the final average I-V for this experiment, which is simply the difference between the open and closed state averages (fine lines) from *B*. The I-V obtained from the single trace in *C* agrees well with the less noisy average in *D*.

As noted by Yellen (1984a) there are three sources of artifacts that must be guarded against in determining channel I-V curves from ramp data. One of these is capacitive current. Because we measure the open-channel I-V's as the difference between two experimental records with exactly the same capacitive artifacts, only partial capacity compensation, to avoid saturating the amplifiers, is required. The difference between closed and open records gives the extra current that flows during a single channel opening. A second artifact arises from opening or closing events too fast to be fully resolved by the amplifier. To avoid biasing the averaged I-V curves, we excluded (by examination of individual sweeps) brief events like the one indicated at the arrow in *B*. A third source of possible artifact, heterogeneity among individual channels of the same general type (Methfessel and Boheim, 1982), is not a problem in this system. Values of open-channel current were surprisingly constant from bilayer to bilayer for different channels, giving standard deviations of  $<5\%$  of the mean value. Control experiments showed that the open-channel I-V curves were unaffected by the particular procedure used to obtain the data—the ramp speed (0.1–10 V/s), the repetition frequency (0.1–40 Hz), or the ramp polarity (upward or downward going). In addition, the I-V curves measured in this way agreed quantitatively with those measured in the steady-state by the amplitude histogram method (see Fig. 4).

In the present study a single I-V curve was obtained by applying at least 64 repeated voltage ramps to a membrane containing a single channel, and collecting the current records on a laboratory computer (Indec, Sunnyvale, CA).  $\text{Ca}^{++}$  concentration was adjusted so that channels were open more than 60% of the time, and low concentrations of  $\text{Ba}^{++}$  (1–3  $\mu\text{M}$ ) or charybdotoxin (1–5 nM) were included to induce long-lived “blocking” events (Vergara and Latorre, 1983; Miller et al., 1985). This ensured that several “blank” records, i.e., sweeps with no channel opening events, would be collected. Neither blocking agent affected the open-channel properties at these concentrations.

To increase the likelihood of channel opening at negative potentials, we sometimes increased the internal  $\text{Ca}^{++}$  concentration (occasionally up to as high as 1.9 mM). Because internal  $\text{Ca}^{++}$  is known to alter the I-V shape in a voltage-dependent way, through a fast blocking which is intensified at positive voltages (unpublished data), we checked that the amounts of internal  $\text{Ca}^{++}$  used to prolong channel open times did not influence the I-V curve shape at the voltage of interest by measuring curves at varying Ca concentrations. To avoid possible distortion of the I-V shape by  $\text{Ca}^{++}$  we confined most of the studies reported here to  $\text{Ca}^{++}$  levels  $< 75 \mu\text{M}$  (unless otherwise noted) and usually did not try to obtain data at potentials more negative than  $-40$  mV under these conditions.

Because the effect on I-V shape of internal  $\text{Ca}^{++}$  is important only at high positive potentials, a satisfactory procedure to obtain a full I-V curve is to obtain the currents for the highest positive voltage values at low  $\text{Ca}^{++}$  levels (10–30  $\mu\text{M}$ ), then obtain the currents for the range 0–80 mV with moderate  $\text{Ca}^{++}$  levels (up to 300  $\mu\text{M}$ ), and finally obtain the currents at negative voltages using  $\text{Ca}^{++}$  levels as high as 1–2 mM. For each range of voltages we have established that the currents are independent of the  $\text{Ca}^{++}$  concentration over at least a 10-fold range.



**FIGURE 1.** Example of ramp procedure. A bilayer containing a single  $\text{Ca}^{++}$ -activated  $\text{K}^+$  channel was held at  $-25$  mV, and then a voltage ramp was applied over 256 ms to 150 mV. Current was sampled at one point per second. Voltage was then returned to  $-25$  mV. **A** shows raw data from two successive sweeps. **B** displays a single sweep superimposed on the open and closed state averages (fine lines) of 64 sweeps. The arrow marks a short closing which was manually excluded from the open-state average. In **C**, a single sweep is shown after subtraction of the closed-state average. **D** illustrates the average open-channel I-V curve calculated from all the sweeps.

We should add one qualification concerning the measurements of I-V curves (by either method used here). We have not excluded the possibility that the open-channel currents measured are artifactually low because of the existence of channel gating on a time scale much faster than would be observable here (i.e., in the range of tens of microseconds). In such a case, our open-channel currents would be filtered by the rapid gating and would be lower than the correct values. We think that this possibility is not a serious problem, for three reasons. First, we have been careful to show that values of currents are not dependent on the degree to which the channel is activated by the observable gating processes; open-channel currents do not depend on observable open probability. Second, we do not observe any excess noise (at 2 kHz filtering) in the open channels, as long as opening probability is high. Third, the observable gating is similar with all K<sup>+</sup> analogues tested, and so even if rapid gating did filter the channel records, this should simply scale the currents with different ions present, and our observed selectivity phenomena would remain as described.

## RESULTS

We study the CaK channel from rat muscle incorporated into a model membrane system, the planar lipid bilayer. With a reconstituted membrane, a much wider range of ionic conditions may be used than in patch-recording experiments with cell membranes. In addition, the lipid composition may be controlled so that the unknown and asymmetric surface potentials of biological membranes do not complicate the analysis (McLaughlin, 1977; Bell and Miller, 1984; Moczydlowski et al., 1985). Indeed, in these experiments, we eliminate lipid-generated surface potentials altogether by inserting single CaK channels into bilayers formed from neutral phospholipids.

### Conductances in the Presence of a Single Permeant Species

Most research to date on permeant species other than K<sup>+</sup> in the CaK channel has relied on measurements in which the permeant was present on one side of the channel while K<sup>+</sup> was present on the other (Yellen, 1984a; Blatz and Magleby, 1984; Cecchi et al., 1986). Here we add measurements on the conductances and I-V shapes in pure solutions of single salts. Fig. 2 shows the concentration dependence of the chord conductance at +50 mV. From this figure it can be seen, in agreement with the previous detailed characterization for K<sup>+</sup> (Vergara et al., 1984; Moczydlowski et al., 1985), that the channel conductance varies only sluggishly over the ion concentration range studied (100–3,000 mM). This, in turn, implies that the range of concentrations at which ion conduction is rate-limited by ion entry into the channel is far below 100 mM. The conductance ratios among these species are relatively constant over the concentration range studied. The values of zero-voltage conductance at 300 mM are summarized in Table I for later comparison with the permeabilities measured by reversal potentials.

Fig. 2 also contains some information about the concentration dependence of conductance for the different species. Because the concentration dependence of K<sup>+</sup> conductance has been shown to be more complex than simple single-site Michaelis–Menten behavior (Moczydlowski et

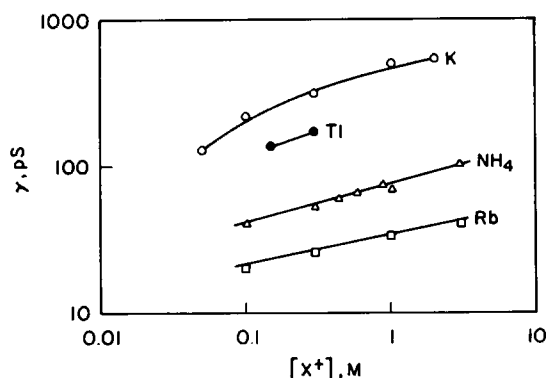


FIGURE 2. Variation of channel conductance with ion concentration. Conductance in symmetrical solutions of the indicated cation salts were measured at 50 mV as a function of salt concentration. Standard errors were less than the width of the points.

al., 1985), our data are insufficient for anything but gross comparisons. One worth noting is that at all the concentrations tested Tl<sup>+</sup> conductance is about three-fold smaller and Rb<sup>+</sup> conductance is 14-fold smaller than that of K<sup>+</sup>. This is noteworthy inasmuch as the Tl<sup>+</sup> permeability is larger than that of K<sup>+</sup>, and the Rb<sup>+</sup> permeability is only slightly smaller (Table I).

### Current–Voltage Curves for Different Permeant Ions

Fig. 3 shows representative I-V curves for outward currents for K, Rb, Tl, and NH<sub>4</sub> ions, all of which we find to be sufficiently conducting that their I-V's could be characterized. The I-V curves in symmetrical K<sup>+</sup> are seen to be roughly linear at low voltages, as previously reported (Moczydlowski et al., 1985), but a clear sublinearity is revealed as voltage increases above 50 mV, in agreement with prior findings (Yellen, 1984a; Blatz and Magleby, 1984). In K<sup>+</sup> solutions, this sublinearity is maintained over a wide concentration range (50–2,000 mM). Fig. 3 shows a similar sublinear behavior in symmetrical Tl<sup>+</sup> solutions.

TABLE I  
CA<sup>++</sup>-ACTIVATED K<sup>+</sup> CHANNEL SELECTIVITY  
PARAMETERS

Ion (X)	γ, pS	γ <sub>X</sub> /γ <sub>K</sub>	P <sub>X</sub> /P <sub>K</sub>
K <sup>+</sup>	320	1.0	1.0
Rb <sup>+</sup>	22	0.07	0.7
Tl <sup>+</sup>	130	0.41	1.3
NH <sub>4</sub> <sup>+</sup>	56	0.18	0.1

Zero-voltage conductance values, γ, were obtained in symmetrical, single-ion solutions at 300 mM concentration. Permeability ratios were determined from reversal potentials under purely bi-ionic conditions at 300 mM or at 100 mM ionic strength; no significant differences were found between the two ionic strength conditions. In all bi-ionic measurements, K<sup>+</sup> was applied on the external side of the channel, and the test cation on the internal side. Permeability ratio is defined as  $P_X/P_K = \exp(-FV_r/RT)$ , where  $V_r$  is the measured reversal potential.

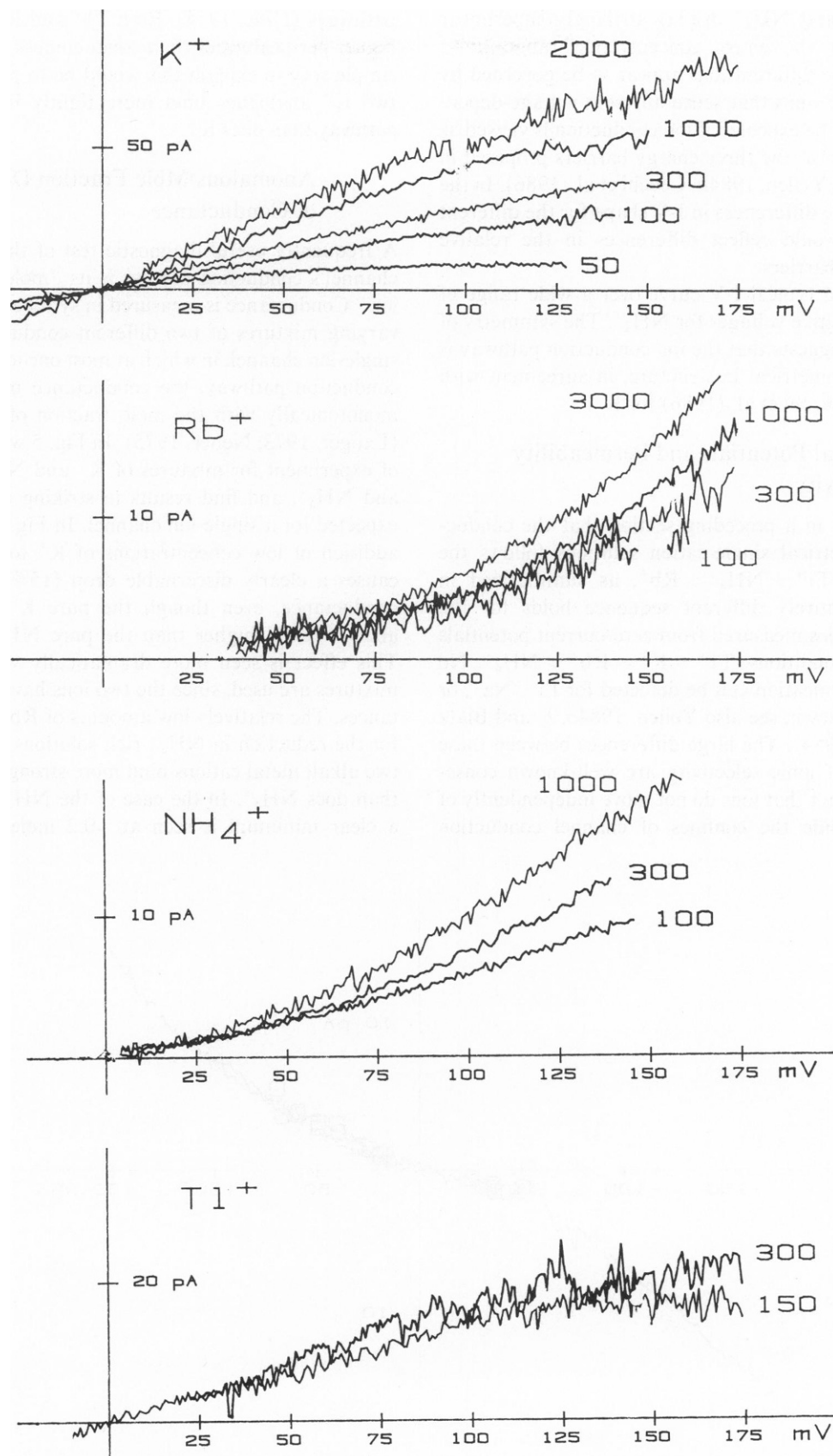


FIGURE 3. Current-voltage behavior for  $K^+$  and its analogues. Open-channel I-V curves obtained by the ramp procedure are shown in symmetrical salts of the indicated cations, at the concentrations shown (in millimolars). Depending on salt concentration,  $Ca^{++}$  needed to activate the channels ranged from 10 to 600  $\mu M$ , but under no conditions did  $Ca^{++}$  block contribute to the shapes of the I-V curves.

However,  $\text{Rb}^+$  and  $\text{NH}_4^+$  display strikingly superlinear I-V curves over the entire concentration range. It is apparent that the different ions appear to be governed by rate-determining steps that sense different voltage-dependences, as would be expected if ion conduction is viewed as a series of hops over the three energy barriers proposed in present theories (Yellen, 1984a; Cecchi et al., 1986). In the simplest view, the differences in I-V shape for the different  $\text{K}^+$  analogues would reflect differences in the relative heights of these barriers.

Fig. 4 shows a typical I-V curve over a wide range of negative and positive voltages for  $\text{NH}_4^+$ . The symmetry of this I-V curve suggests that the ion conduction pathway is not grossly asymmetrical in structure, in agreement with the findings of Cecchi et al. (1986) for  $\text{K}^+$ .

### Reversal Potentials and Permeability Selectivity

We have shown in a preceding section that the conductance in symmetrical single-cation solutions follows the sequence  $\text{K}^+ > \text{Tl}^+ > \text{NH}_4^+ > \text{Rb}^+$ , as summarized in Table I. An entirely different sequence holds for the permeability ratios measured from zero-current potentials under bi-ionic conditions:  $\text{Tl}^+ > \text{K}^+ > \text{Rb}^+ > \text{NH}_4^+$ . No measureable permeation can be detected for  $\text{Li}^+$ ,  $\text{Na}^+$ , or  $\text{Cs}^+$  (data not shown; see also Yellen, 1984a, b, and Blatz and Magleby, 1984). The large differences between these two measures of ionic selectivity are well-known consequences of the fact that ions do not move independently of one another inside the confines of channel conduction

pathways (Hille, 1975). Both  $\text{Tl}^+$  and  $\text{Rb}^+$  display much higher permeabilities than conductances, relative to  $\text{K}^+$ ; a simple way to explain this would be to propose that these two  $\text{K}^+$  analogues bind more tightly in the conduction pathway than does  $\text{K}^+$ .

### Anomalous Mole Fraction Dependence of Conductance

A frequently useful diagnostic test of the simplicity of a channel's conduction process is its "mole fraction behavior." Conductance is measured in symmetrical solutions of varying mixtures of two different conducting ions. For a single-ion channel, in which at most one ion can occupy the conduction pathway, the conductance must always vary monotonically with the mole fraction of the test cations (Lauger, 1973; Neher, 1975). In Fig. 5 we report this type of experiment for mixtures of  $\text{K}^+$  and  $\text{NH}_4^+$  and of  $\text{Rb}^+$  and  $\text{NH}_4^+$ , and find results in striking contrast to those expected for a single-ion channel. In Fig. 5A, we see that addition of low concentrations of  $\text{K}^+$  to  $\text{NH}_4^+$  solutions causes a clearly discernable drop (15%) in the channel conductance, even though the pure  $\text{K}^+$  conductance is about fivefold higher than the pure  $\text{NH}_4^+$  conductance. This effect is seen more dramatically when  $\text{Rb}^+$ - $\text{NH}_4^+$  mixtures are used, since the two ions have similar conductances. The relatively low amounts of  $\text{Rb}^+$  or  $\text{K}^+$  required for the reduction in  $\text{NH}_4^+$ -rich solutions shows that these two alkali metal cations bind more strongly to the channel than does  $\text{NH}_4^+$ . In the case of the  $\text{NH}_4^+$ - $\text{Rb}^+$  mixtures a clear minimum is seen at ~0.2 mole fraction of  $\text{Rb}^+$ ,

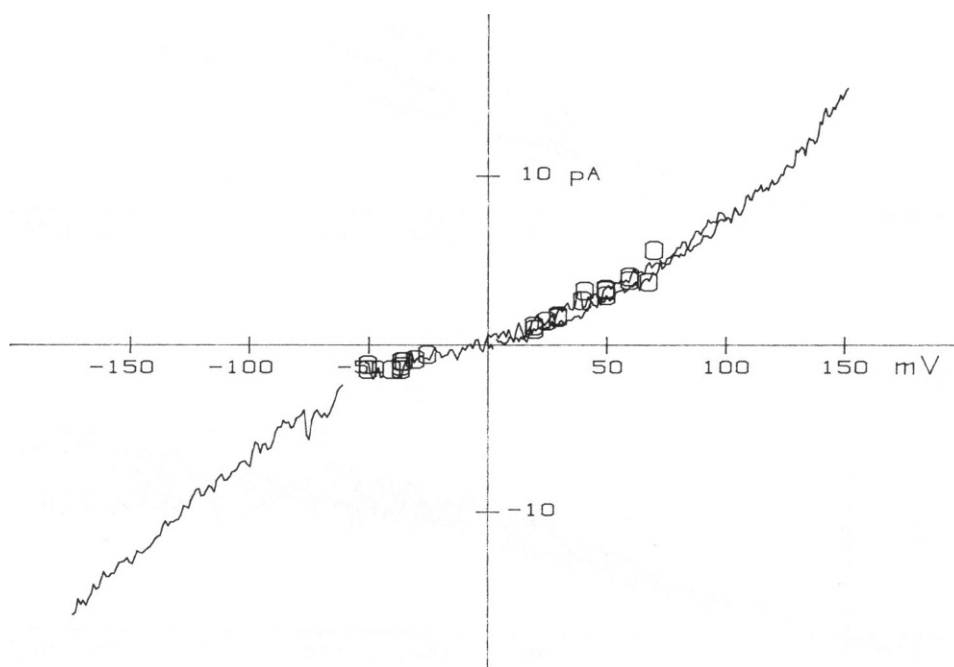


FIGURE 4. Symmetry of I-V curve for  $\text{NH}_4^+$ . The open-channel I-V curve was measured over the entire voltage range for symmetrical  $\text{NH}_4^+$  solutions at a concentration of 300 mM. Continuous curves, ramp method in two different bilayers. Open boxes, current histogram method at indicated holding voltages, taken from three different bilayers. Internal  $\text{Ca}^{++}$  concentration was 300  $\mu\text{M}$ .

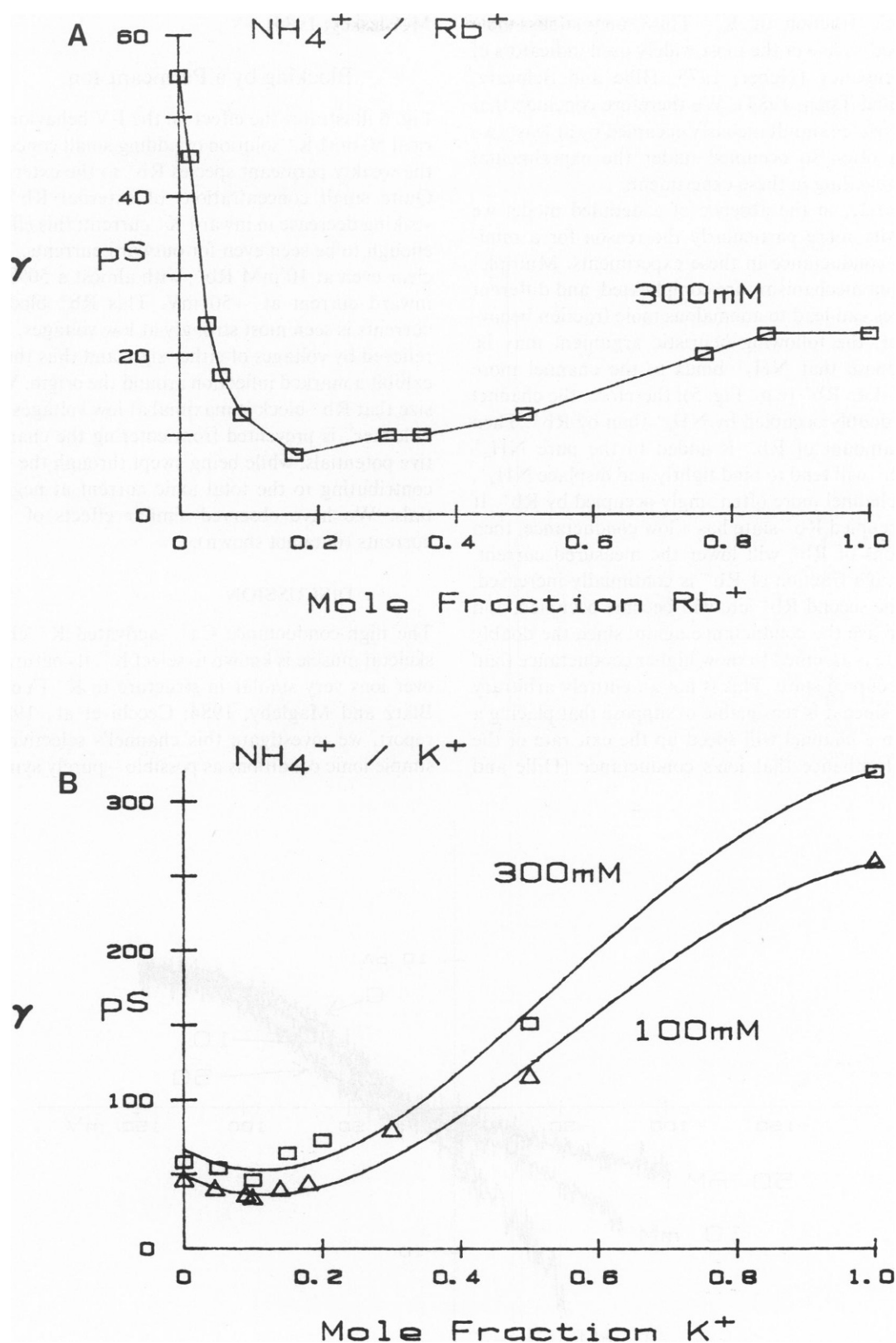


FIGURE 5. Anomalous mole fraction behavior. Zero-voltage conductance was measured by the steady-state histogram method for channels facing symmetrical solutions of varying mixtures of two cations, as indicated. The total concentration of salt is indicated on the figure. Clear minima are seen in mixtures of  $\text{NH}_4^+ / \text{Rb}^+$ , and of  $\text{NH}_4^+ / \text{K}^+$ . Solid curves are drawn by hand and carry no theoretical connotations.

whereas for the  $\text{NH}_4^+$ - $\text{K}^+$  mixtures a minimum is seen at  $\sim 0.1$  mole fraction of  $\text{K}^+$ . This "anomalous mole fraction effect" is one of the most widely used indicators of multiple occupancy (Neher, 1975; Hille and Schwarz, 1978; Hess and Tsien, 1984). We therefore conclude that this channel can be simultaneously occupied by at least two ions, and is often so occupied under the experimental conditions prevailing in these experiments.

Unfortunately, in the absence of a detailed model we cannot identify more particularly the reason for a minimum in the conductance in these experiments. Multiple-ion conduction mechanisms are complicated, and different circumstances can lead to anomalous mole fraction behavior. However, the following heuristic argument may be helpful. Suppose that  $\text{NH}_4^+$  binds to the channel more weakly than does  $\text{Rb}^+$  (e.g., Fig. 5); therefore, the channel is less often doubly occupied by  $\text{NH}_4^+$  than by  $\text{Rb}^+$ . Then as a small amount of  $\text{Rb}^+$  is added to the pure  $\text{NH}_4^+$  situation,  $\text{Rb}^+$  will tend to bind tightly and displace  $\text{NH}_4^+$ , leaving the channel more often singly occupied by  $\text{Rb}^+$ . If the singly occupied  $\text{Rb}^+$  state has a low conductance, then these additions of  $\text{Rb}^+$  will lower the measured current. But as the mole fraction of  $\text{Rb}^+$  is continually increased, eventually the second  $\text{Rb}^+$  site will become occupied, and this will increase the conductance again, since the doubly occupied state is assumed to show higher conductance than the singly occupied state. This is not an entirely arbitrary assumption, since it is reasonable to suppose that placing a second ion in a channel will speed up the exit rate of the first ion and enhance that ion's conductance (Hille and

Schwarz, 1978; Hess and Tsien, 1984; Almers and McCleskey, 1984).

### Blocking by a Permeant Ion

Fig. 6 illustrates the effect on the I-V behavior of symmetrical 50 mM  $\text{K}^+$  solution of adding small concentrations of the weakly permeant species  $\text{Rb}^+$  to the external solution. Quite small concentrations of external  $\text{Rb}^+$  produce a striking decrease in inward  $\text{K}^+$  current; this effect is strong enough to be seen even for outward currents. The effect is clear even at 10 mM  $\text{Rb}^+$ , with almost a 50% decrease in inward current at  $-50$  mV. This  $\text{Rb}^+$  blocking of  $\text{K}^+$  currents is seen most strongly at low voltages. The block is relieved by voltages of either sign, and thus the I-V curves exhibit a marked inflection around the origin. We hypothesize that  $\text{Rb}^+$  block is maximal at low voltages because the "blocker" is prevented from entering the channel at positive potentials, while being swept through the channel and contributing to the total ionic current at negative potentials. We have observed similar effects of  $\text{Tl}^+$  on  $\text{K}^+$  currents (data not shown).

### DISCUSSION

The high-conductance  $\text{Ca}^{++}$ -activated  $\text{K}^+$  channel from skeletal muscle is known to select  $\text{K}^+$ , its natural substrate, over ions very similar in structure to  $\text{K}^+$  (Yellen, 1984a; Blatz and Magleby, 1984; Cecchi et al., 1986). In this report, we investigate this channel's selectivity under as simple ionic conditions as possible—purely symmetrical or

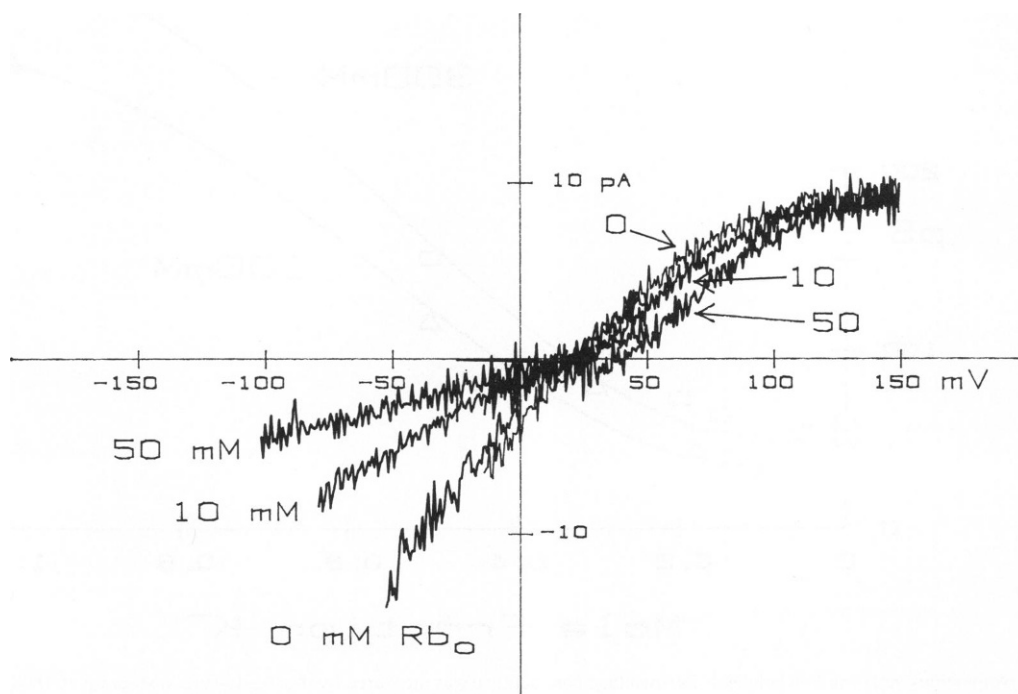


FIGURE 6. Blocking of  $\text{K}^+$  currents by  $\text{Rb}^+$ . Open-channel I-V curves are shown in 50 mM symmetrical  $\text{K}^+$  solutions, to which varying concentrations of  $\text{Rb}^+$  have been added on the external side. Internal  $\text{Ca}^{++}$  concentration was 1.5 mM.



purely bi-ionic solutions of  $K^+$  and its close analogues  $Rb^+$ ,  $NH_4^+$ , and  $Tl^+$ . We further confirm that this channel is exquisitely designed for  $K^+$ ; in symmetrical, single-salt solutions,  $Rb^+$ , an ion closely similar to  $K^+$  in size and thermodynamics of hydration (Cox and Parker, 1973), shows a 14-fold lower conductance than  $K^+$ . Ammonium ion and  $K^+$  are of almost precisely the same unhydrated size, but  $NH_4^+$  conducts at fivefold lower rates than does  $K^+$ ; even  $Tl^+$ , a "soft" cation that can adjust the structure of its valence electrons with much more flexibility than the alkali metal cations, does not permeate the channel as well as  $K^+$ ; this result is particularly noteworthy, since many biological  $K^+$ -liganding groups actually prefer  $Tl^+$  over  $K^+$ , and since  $Tl^+$  permeates several  $K^+$  channels more rapidly than does  $K^+$  (Latorre and Miller, 1983).

A complete conduction study would require the determination of the concentration dependence of channel currents over a wide range of ion concentrations, as has been done for  $K^+$  currents in this channel (Moczydlowski et al., 1985). Our results show that the channel conductance varies only weakly with cation concentration in the range 100–2,000 mM for all the ions tested, and the channel is close to being saturated with the conducting ion already by 100 mM. This result means that all the  $K^+$  analogues bind to the channel with relatively high affinities ( $K_D < 50$  mM). We find that the ion selectivity of conductance does not agree with the selectivity measured by the bi-ionic reversal potential. This discrepancy is not surprising, since these two indicators of selectivity reflect different rate processes inside the conduction pathway (Hille, 1975).

Shortly after the discovery of this channel, and of its unusual combination of high  $K^+$  conductance and high ionic selectivity, it was proposed that this and other "maxi- $K^+$ " channels operate by a single-ion rule, in which at most one conducting ion is allowed to occupy the pore at a given time (Latorre and Miller, 1983). Blatz and Magleby (1984) provided evidence to support this notion by showing that the bi-ionic reversal potentials for several  $K^+$  analogues do not vary with absolute ion concentration. During the past three years, however, the single-ion hypothesis has been weakened by evidence that this channel allows several ions to interact simultaneously within the conduction pathway. Yellen (1984b) found that  $K^+$ -like ions can relieve the block of this channel by  $Na^+$  by speeding the exit rate of the blocking ion. In addition, the blocking by  $Cs^+$  is far more voltage dependent than can be accommodated within a single-ion scheme (Cecchi, X., D. Wolff, O. Alvarez, and R. Latorre, manuscript submitted for publication). The anomalous mole fraction effects presented here provide a third and very strong violation of single-ion behavior. The minimum in channel conductance at varying mole fractions of ionic mixtures requires that several ions interact within the conduction pathway. These three indicators of multiple occupancy are not inconsistent with the invariance of bi-ionic reversal potential with absolute ion concentration mentioned above, since this

latter characteristic, while demanded of a single ion channel, is easily accommodated in many classes of multi-ion models.

It is not our intention here to provide a quantitative ion conduction model for this high-conductance CaK channel. Instead, we have endeavored to outline the basic ion selectivity characteristics that any future conduction models will have to explain. We view the tight ion selectivity of this channel as a consequence of a liganding structure within the pore, a "selectivity filter" which is small and inflexible enough to permit the passage only of ions very close to  $K^+$  in physical dimensions (Latorre and Miller, 1983). The conduction mechanism's multi-ion nature, which we now consider to be firmly established, suggests that a satisfactory theory for  $K^+$  conduction in this channel will be complex. Future work in this area will therefore require that experiments be carried out over a wide range of ionic conditions in as simple a system as possible. A reconstituted membrane of some kind will almost certainly be needed in such studies.

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