Conduction Properties of the Cloned *Shaker* K^+ Channel

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ABSTRACT The conduction properties of the cloned *Shaker* K^+ channel were studied using electrophysiological techniques. Single channel conductance increases in a sublinear manner with symmetric increases in K^+ activity, reaching saturation by 0.6 M K^+. The *Shaker* K^+ channel is highly selective among monovalent cations; under bi-ionic conditions, its selectivity sequence is $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+$, whereas, by relative conductance in symmetric solutions, it is $K^+ > NH_4^+ > Rb^+ > Cs^+$. In Cs^+ solutions, single channel currents were too small to be measured directly, so nonstationary fluctuation analysis was used to determine the unitary Cs^+ conductance. The single channel conductance displays an anomalous mole-fraction effect in symmetric mixtures of K^+ and NH_4^+, suggesting that the conducting pore is occupied by multiple ions simultaneously.

INTRODUCTION

Potassium channels all share a set of highly characteristic open channel properties. They are extremely selective among monovalent cations. Although clearly permeable to Rb^+, NH_4^+, and Tl^+, they effectively exclude the smaller Na^+ ion (Hille, 1973; Yellen, 1984). In addition, several independent lines of evidence suggest that K^+ channels have multi-ion pores. First, flux-ratio exponents greater than unity have been measured in a number of K^+ channels (Hodgkin and Keynes, 1955; Begenisich and DeWeer, 1980; Vestergaard-Bogind et al., 1985). Second, the affinities of several different K^+ binding sites have been measured directly in the large-conductance Ca^{2+}-activated K^+ channel (Neyton and Miller, 1988a; Neyton and Miller, 1988b). And third, K^+ channels demonstrate the anomalous mole-fraction effect typically associated with multiple occupancy (Hagiwara et al., 1977; Eisenman et al., 1986). Although K^+ channels display very diverse gating mechanisms, these two permeation properties, high selectivity, and multiple occupancy, appear nearly universal.

The following experiments describe several ion conduction properties of a cloned *Shaker* K^+ channel. This channel is highly selective for K^+ over other alkali metal cations: Na^+ is not measurably permeant. In contrast, the channel clearly conducts Cs^+. In fact, because of the high expression levels achieved using heterologous expression, Cs^+ permeation could be studied in some detail. The *Shaker* channel also displays a characteristic feature of multi-ion channels: an anomalous mole-fraction effect. These results illustrate that the conduction properties of the cloned *Shaker* K^+ channel are characteristic of those traditionally found in other K^+ channels.

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METHODS

Channel construct and expression

The *Shaker* H4 channel, with amino-terminal inactivation removed, was used throughout these experiments. The construction of this channel, in the pBluescript KS^+ vector (Stratagene, La Jolla, CA), has been described previously (Hoshi et al., 1990; Yellen et al., 1991). DNA was linearized with a *Hin*dIII (New England Biolabs, Beverly, MA) digest, and cRNA transcribed using T7 RNA polymerase (Promega Corp., Madison, WI). *Xenopus* oocytes (*Xenopus* One, Ann Arbor, MI) were injected with varying amounts of cRNA, and incubated at 18°C for a period ranging from 1 to 10 days prior to recording.

Solutions

A standard set of solutions was used in these experiments. The extracellular (pipette) solution contained (in millimolar): 95 *X*Cl (*X* = Na^+, K^+, Rb^+, Cs^+, NH_4^+), 1.8 $CaCl_2$, 1.0 $MgCl_2$, and 10 HEPES, pH 7.1. The internal solution contained 105 XCl (X= Na^+, K^+, Rb^+, Cs^+, NH_4^+), 1.0 $MgCl_2$, 5.0 EGTA, and 10 4-(2-hydroxyethyl)1-piperazineethanesulfonic acid (HEPES), pH 7.1. In each case, pH was adjusted to 7.1 with the hydroxide of the monovalent cation.

In the conductance-concentration and anomalous mole-fraction experiments, KCl concentrations were increased symmetrically. Other than the concentration of monovalent cation, the external solution was the same as above. In addition to KCl, the internal solution contained (in millimolar) 10 *N*-methyl-D-glucamine, 5 EGTA, 1 $MgCl_2$, and 10 HEPES, pH 7.1. The anomalous mole-fraction effect was measured at the single channel level in symmetric solutions that contained a total of 300 mM K^+ and NH_4^+. Other than the concentration of monovalent cation, the solutions were the same as those used in conductance-concentration experiments.

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA), with

the exception of CsCl, which was from Fluka Chemical Co. (Ronkonkoma, NY).

Electrophysiological recording

Except where specifically noted, all recordings were from excised, inside-out patches. In most cases, the electrode offset was trimmed to zero in the test solution prior to obtaining a seal. However, it was difficult to obtain seals in the presence of high bath [K^+] (above 300 mM), since the oocytes shrank rather extensively. For these experiments only, most electrodes were set to zero in bath solutions containing 300 mM KCl, and seals were obtained. High [K^+] ($\geq$ 500 mM) solutions were then washed into the bath after the patch was excised. Because of the large single channel conductances of the *Shaker* channel under these conditions, the reversal potential could still be fairly accurately estimated. However, to serve as a control at each ion concentration, a single patch was studied in which the electrode had been zeroed in the test solution.

Even in oocytes expressing large amounts of current at normal K^+ concentrations, there is a low probability of having a patch with any gating *Shaker* channels when the solutions contained high [K^+]. The probability of observing an active channel decreased as the K^+ concentration increased; by 1 M K^+, only one patch in approximately 40 had any open *Shaker* channels. Also, it was difficult to obtain more than one seal from oocytes which had been exposed to K^+ concentrations above 300 mM; therefore, in these experiments each patch was from a separate oocyte.

Single channel recordings were filtered at 1 kHz and sampled at rates of 2.5 kHz. Unless otherwise noted, macroscopic current recordings were filtered at 2 kHz and sampled at 14–25 kHz. In experiments using fluctuation analysis, current recordings were filtered at 5 kHz and sampled at 14 kHz.

Junction potentials at the bath-electrode surface associated with solution changes were insignificant, since the chloride concentration was constant in the different bath solutions.

All measurements were made at 22°C.

Data analysis

When constructing macroscopic *I-V* curves, a linear leak subtraction was used prior to determining reversal potentials. The leak current measured between −100 and −110 mV was scaled and subtracted from each trace. In the current trace in Fig. 6 *A*, both stationary leak and capacitive current, measured during a pulse from −80 to −90 mV, were subtracted.

Nonstationary fluctuation analysis was performed much as originally described by Sigworth (1980). To calculate the variance at each timepoint, each trace was compared to a mean of three sweeps (itself and two surrounding it), thereby minimizing the effects of drift in the macroscopic current during the timecourse of the experiment. A single pulse protocol was repeated 60 times, and the variance was determined on 58 of these sweeps.

In the experiments with Cs^+, the RMS noise (due to both channel gating and system noise) had a maximum of 4.6 pA, approaching the size of the A-D converting bit unit (2.48 pA for measurements at −120 and −150 mV). The slight error that this level of granularity will introduce is within the scatter of our data, and will not affect our estimations of i and N.

RESULTS

Concentration dependence of single channel conductance

This study begins by focusing on a very basic question: how is the channel conductance affected by the K^+ concentration? Single channel currents were recorded as K^+ concentrations were varied symmetrically on both sides of the membrane (Fig. 1 *A*). These recordings show that, at a given voltage, the single channel amplitude increases as the activity

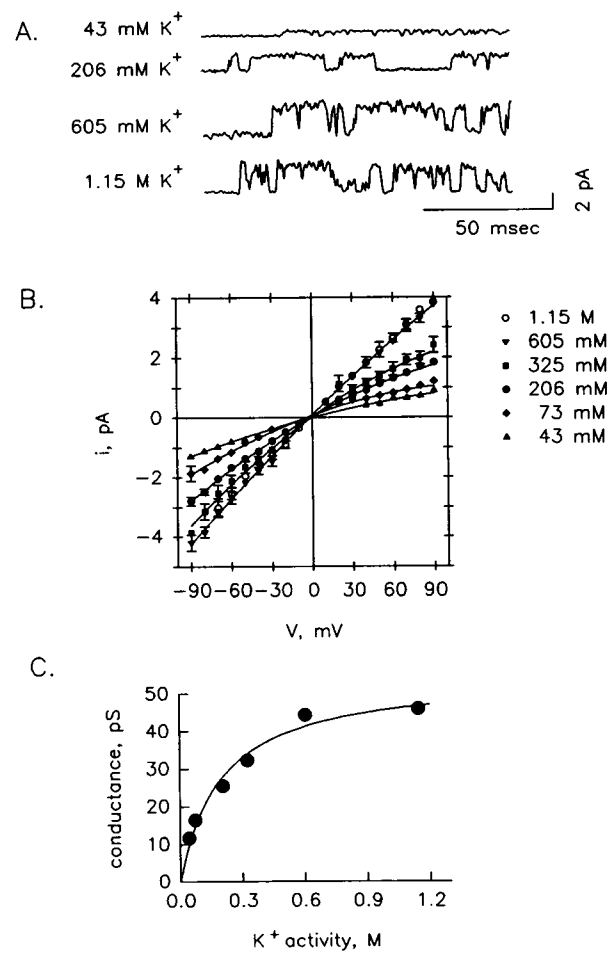

FIGURE 1 The effect of K^+ activity on single channel conductance. (*A*) Single channel recordings were made at +80 mV in symmetric K^+ solutions. (*B*) Single channel *i-V* relationships, in symmetric solutions of the indicated K^+ activities. Each point represents the mean ± SE of three to six measurements from independent patches. Curves were fit to the function $i = a + bV + cV^2$ (of no theoretical significance), to provide an estimate of the zero-voltage conductance. (*C*) Zero-voltage conductances, determined from the curves shown in *B*, as a function of K^+ activity.

of K^+ is raised up to 0.6 M K^+ (Fig. 1 *A*). However, the single channel amplitude is the same in 0.6 and 1.2 M K^+. The single channel *i-V* relationships show that there is a qualitatively similar result over the entire voltage range studied (Fig. 1 *B*).

At each K^+ concentration, the conductance was measured from the slope of the *i-V* curve at 0 mV. Fig. 1 *C* shows how the conductance varies as a function of K^+ activity (Fig. 1 *C*). Conductance increases in a sublinear fashion over the lower K^+ concentrations, as if the pore is becoming saturated. In fact, saturation is reached by high K^+ activities: there is no appreciable difference between conductances measured in 0.6 and 1.2 M K^+.

The finding of conductance saturation is consistent with the idea that there is a saturable step in ion conduction. The shape of the conductance-concentration curve suggests that this process has an "affinity" of approximately 300 mM. This result is similar to what has been reported in a high conductance Ca^{2+}-activated K^+ channel (Eisenman et al., 1986).

At low K^+ concentrations (Fig. 1 *B*), the *Shaker* channel displays a slight inward rectification. Here we see that as the K^+ concentration is raised, the *i-V* relationship becomes more linear. In some K^+ channels, inward rectification results from blockade of the outward current by internal Mg^{2+}. A previous study has reported that this is not the cause of rectification in the *Shaker* channel over the voltage range studied here; the channel still rectifies in the absence of Mg^{2+} (MacKinnon and Yellen, 1990).

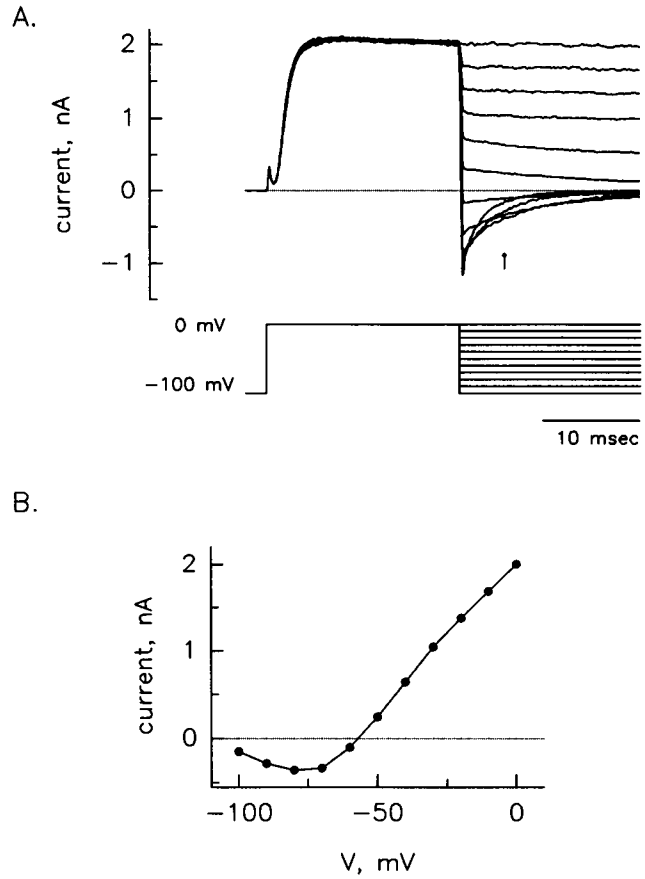

FIGURE 2 Example of macroscopic reversal potential measurements. (*A*) Macroscopic current recording from an inside-out patch, with 105 K^+ in the bath (internal), and 95 mM NH_4^+ in the pipette (external). Membrane current was recorded during the illustrated voltage protocol. After an initial depolarizing pulse to 0 mV, the membrane was clamped at different test potentials. (*B*) An isochronal *I-V* curve was generated from the data shown in *A*. Current was measured at a time after the capacitive current had decayed (illustrated by the *arrow* in *A*), and was corrected for any linear membrane leak, as described in Methods. The reversal potential was determined as the point at which straight lines between the measured values crossed the abscissa.

Ionic selectivity

This section investigates the ability of the *Shaker* K^+ channel to discriminate among monovalent cations. Traditionally, two kinds of measurements have been used to characterize ionic selectivity. The first is the permeability ratio, determined from the reversal potential when a test ion is placed on one side of the membrane and a comparison ion (K^+ in the experiments described here) on the other side. The second measure of selectivity is the relative conductance: comparing conductance of different ions measured under symmetric conditions.

Permeability ratios were measured using inside-out patches. Channels were activated by an initial pulse to 0 mV, after which the patch was stepped to different test potentials (Fig. 2 *A*). In the example shown, NH_4^+ (95 mM) was present outside the patch, and K^+ (105 mM) was present on the inside. Isochronal current-voltage (*I-V*) curves were generated by measuring the current as a function of the test potential (Fig. 2 *B*). The reversal potential of −57 mV indicates that K^+ is considerably more permeant than NH_4^+ under these conditions.

Fig. 3 shows isochronal *I-V* relationships measured with K^+ on the outside of the patch and the indicated test ions on the inside. Outward currents are clearly visible with either K^+, Rb^+, NH_4^+, or Cs^+; the channel is permeable to each of these cations. In contrast, we were unable to measure significant Na^+ conduction. The small outward current visible in the Na^+ *I-V* at extreme depolarized potentials is indistinguishable from background oocyte current. Based on the reversal potentials, the *Shaker* channel displays a relative permeability sequence of $K^+ > Rb^+ > NH_4^+ > Cs^+ \gg Na^+$. This same permeability sequence has been found for several other K^+ channels, including those in the frog node of Ranvier (Hille, 1973), lymphocytes (Shapiro and Decoursey, 1991), and a Ca^{2+}-activated K^+ channel (Blatz and Magleby, 1984).

The relative permeability (P_A/P_B) of the two monovalent cations A and B is operationally defined by the relationship obtained from Nernst-Planck electrodiffusion

$$\frac{P_A}{P_B} = \frac{[B]_o}{[A]_i} e^{-(FV_{rev}/RT)}, \quad (1)$$

where *A* and *B* are on the inside and outside, respectively,

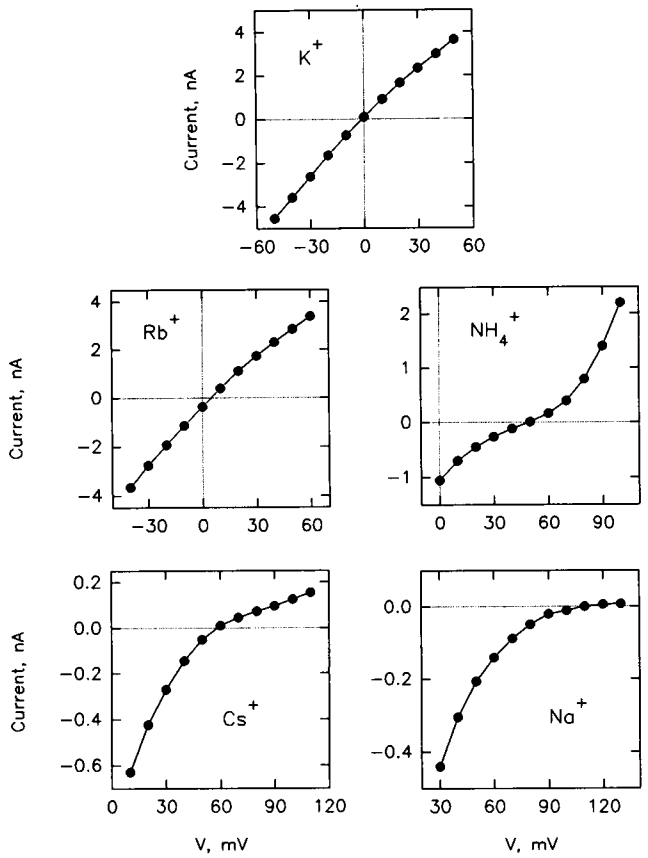

FIGURE 3 Isochronal *I-V* relationships measured under bi-ionic conditions. In each case, the pipette solution contained 95 mM K^+, while the bath solution contained 105 mM of the indicated test ion. Reversal potentials under these conditions were: K^+, −2 mV; Rb^+, +5 mV; NH_4^+, +50 mV; Cs^+, +59 mV; Na^+, > +90 mV.

V_{rev} is the reversal potential, and F, R, and T have their usual meanings (Goldman, 1943; Hodgkin and Katz, 1949). The permeability ratios of the different ions are compared in Table 1. The *Shaker* channel displays the same overall permeability sequence when the orientation of the ions is reversed, i.e., when the permeability of an external test ion is measured relative to internal K^+. In fact, even the exact permeability ratios depend only slightly on orientation (Table 1).

TABLE 1 Selectivity of the *Shaker* channel

Ion (X)	P_X/P_K		g_X/g_K
	K^+_{in}	K^+_{out}	
K^+	1.0	1.0	1.0
Rb^+	0.66	0.88	0.50
NH_4^+	0.09	0.16	0.75
Cs^+	0.06	0.10	0.01
Na^+		<0.03	

Reversal potentials were measured under bi-ionic conditions, with 95 and 105 mM monovalent cation in the external and internal solutions, respectively. The permeability of each ion X^+ relative to K^+ was calculated according to Eq. 1. Single channel conductances (g) were determined from either direct single channel recordings (K^+, NH_4^+, and Rb^+, Fig. 4) or from fluctuation analysis (Cs^+, Fig. 6).

In order to compare relative conductances, single channel *i-V* relationships were measured in symmetric solutions containing a single permeant ion (Fig. 4 *A*). Both inward and outward currents were clearly discernible in either symmetric K^+ or Rb^+ (Fig. 4 *B*). However, in NH_4^+, channel openings were extremely flickery at negative membrane potentials, making the amplitude of the open state impossible to determine. As illustrated in Table 1, the *Shaker* channel shows a relative conductance sequence of $K^+ > NH_4^+ > Rb^+$. We were not able to record Cs^+ or Na^+ current through single channels under these conditions. The selectivity measured by conductance ratios is identical to what has been reported previously in the Ca^{2+}-activated K^+ channel (Eisenman et al., 1986).

It is worth noting that selectivity of the channel as determined by conductance ratios is different than that determined by relative permeability. As these are two independent measures of selectivity, the difference is not unexpected. Interestingly, the Ca^{2+}-activated K^+ channel shows the same relationship between selectivity measured in these two ways (Eisenman et al., 1986) as does the *Shaker* K^+ channel.

Cs^+ conduction through a potassium channel

In Fig. 3 we saw that Cs^+ supported measurable macroscopic current through a membrane patch containing many *Shaker*

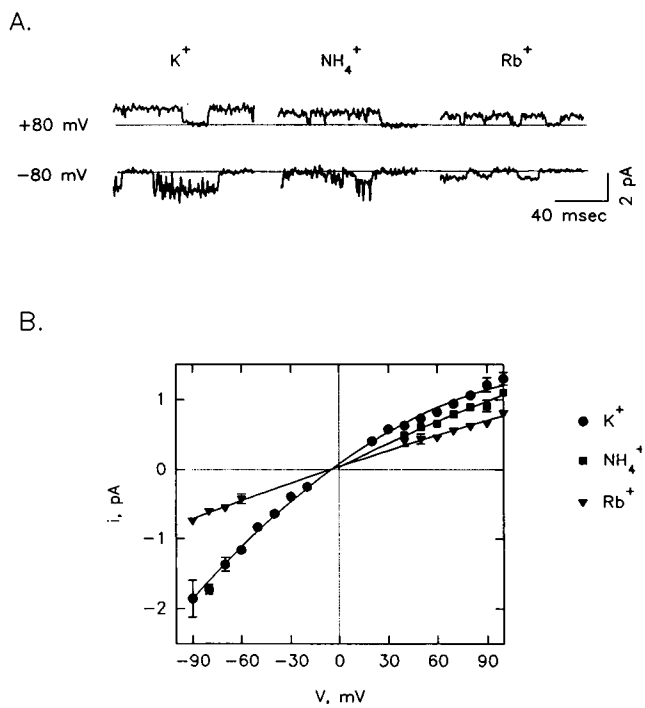

FIGURE 4 Single channel conductances in symmetric solutions containing a single permeant ion. (*A*) Single channel recordings made in either K^+ or Rb^+ showed clear openings and closings at both +80 and −80 mV. In contrast, single channel amplitude could only be determined at positive potentials in NH_4^+ solutions. (*B*) Single channel *i-V* relationships for different cations. Each point represents the mean ± SE for three to six measurements in separate patches. Again, curves were fit to the function $i = a + bV + cV^2$ (of no theoretical significance) to provide an estimate of the zero-voltage conductance. In each case, the pipette and bath solutions contained 95 and 105 mM cation, respectively.

K^+ channels. The single channel Cs^+ current is very small, but its amplitude can be determined by studying the noise produced by Cs^+ conducting channels, using fluctuation analysis of macroscopic currents. The macroscopic current traces shown in Fig. 5 provide a qualitative indication of how big the unitary Cs^+ conductance must be. Currents recorded in symmetric Cs^+ are compared to those carried by K^+ under similar conditions. The two patches contain roughly the same amount of total current. But there is one striking difference between the Cs^+ and K^+ currents: the traces display very different amounts of noise. In K^+ solutions, random noise is clearly visible on individual current sweeps, particularly those recorded at the most extreme potentials. In contrast, the recordings made in symmetric Cs^+ have substantially less noise. Because the macroscopic currents have similar amplitudes, the difference in noise suggests that the patch in Cs^+ contains many more channels than the patch in K^+, and that these channels have a much lower single channel conductance. Furthermore, patches that displayed macroscopic current in symmetric Cs^+ could only be obtained from oocytes that expressed immeasurably high levels of K^+ current.

To determine the conductance of the *Shaker* channel in symmetric Cs^+, fluctuation analysis was performed on macroscopic currents such as those shown in Fig. 5. After depolarizing the membrane patch to maximally activate the channels, the voltage was stepped to a test potential. Analysis was carried out on "tail" currents during the hyperpolarizing pulse, since the rate of deactivation is slower than the rate of activation. Thus it was possible to sample many points over the entire range of open probability, and at the same time make measurements at potentials far from the reversal potential.

The data obtained from one such experiment are illustrated in Fig. 6. In this case, an activating pulse to 30 mV was followed by a hyperpolarizing step to −120 mV. The average current, a mean of 58 individual traces, is shown (Fig. 6 *A*) along with the variance at each time point (Fig. 6 *B*). Within the tail pulse, the variance is clearly smallest at the end of the pulse, when the macroscopic current is small (when P_o nears 0). For a population of identical, independently gating channels, the relationship between variance and mean current was given by Sigworth (1980),

$$\sigma^2 = i * I - \frac{I^2}{N}, \qquad (2)$$

where I and σ^2 are the mean and variance of the macroscopic current, N is the total number of channels, and i is the single channel current. Plotting variance as a function of mean current (Fig. 6 *C*), the single channel current (i) is found to be 18 fA and the number of channels (N) is about 160,000.

The high level of expression suggested by the above calculation (N = 160,000 channels) is clearly apparent upon careful inspection of the current traces in Fig. 6 *A*. The initial bump in the pulse to +30 mV is gating current; the linear capacitive current has been fully subtracted in this figure. The large ratio of gating current to ionic current illustrated here is compatible with a previous estimate of gating charge in the *Shaker* channel (Schoppa et al., 1992).

The estimate of a low conductance, together with a large number of channels in a single patch, is consistent with the lack of noise seen in the macroscopic Cs^+ current (Fig. 5). As a control we also used fluctuation analysis to estimate the single channel conductance in symmetric K^+. Under these conditions, the variance of the macroscopic current is much greater than in symmetric Cs^+ (Fig. 6 *D*). For this patch, i is approximately 1.8 pA, with N = 3800. This measurement gives a single channel conductance of 15 pS, similar to our direct measurement of single *Shaker* channels in the same K^+ solutions (see Fig. 1).

Outward Cs^+ conduction depends on the species of external ion

Cs^+ has long been used to block K^+ channels in whole cell experiments (e.g., Sigworth, 1980), yet we have seen that Cs^+ carries current under the conditions described above. Does Cs^+ always carry current through the *Shaker* channel, or is it impermeant under some conditions?

We looked to see if the ionic composition of the external solution affected the outward Cs^+ currents. The results of this experiment are illustrated in Fig. 7. Using outside-out patches, current was elicited by a step to +50 mV (near the reversal potential), with subsequent tail potentials ranging from −40 to +100 mV. With potassium in the external solution (Fig. 7 *A*), both inward K^+ currents and outward Cs^+ currents are clearly visible during the tail pulses. In the same patch, outward Cs^+ currents are much smaller when the external solution is switched to Na^+ (Fig. 7 *B*). This explains why Cs^+ can be used to block K^+ currents in whole cell experiments: in the presence of external Na^+, there is little outward Cs^+ current.

Anomalous mole fraction effect

One striking characteristic of some multi-ion channels is the presence of an anomalous mole-fraction effect. The *Shaker* channel clearly demonstrates this effect at the single channel level. Single channel currents were recorded in symmetric

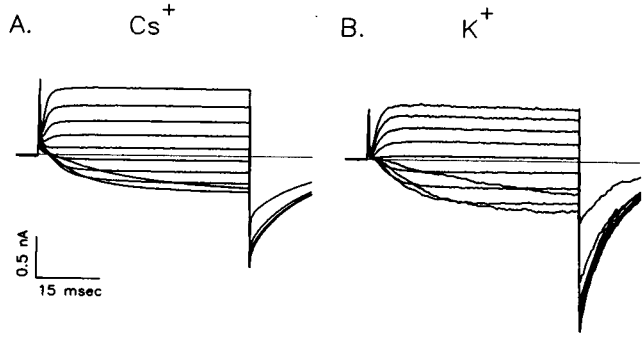

FIGURE 5 Macroscopic current recordings from patches in solutions containing either Cs^+ (*A*) or K^+ (*B*). In both cases, currents were generated in response to voltage steps from −50 to +40 mV, in 10-mV increments, from an initial holding potential of −80 mV. The subsequent hyperpolarizing pulse is to −100 mV. These measurements were made with 105 mM monovalent cation in the bath, and 95 mM in the pipette.

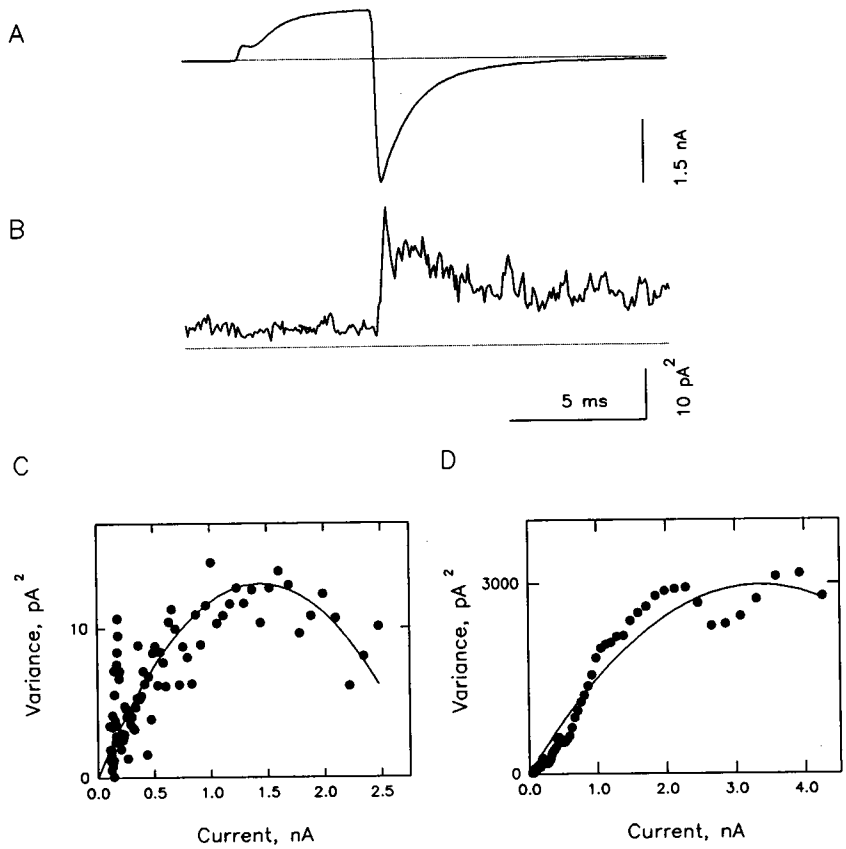

FIGURE 6 Stationary fluctuation analysis of macroscopic Cs^+ currents. Mean (*A*) and variance (*B*) of the current from 58 recordings from a single patch. Current was elicited by an initial step to +30 mV, from a holding potential of −80 mV; the subsequent test (hyperpolarizing) potential was −120 mV. In *A* and *B*, the dotted lines correspond to 0 current and variance, respectively. (*C*) Variance as a function of macroscopic current, from the same experiment shown in *A* and *B*. The continuous curve is calculated from the relationship $\sigma^2 = i*I - I^2/N$ (see text), with $i = 0.018$ pA, and $N = 1.6 \times 10^5$ channels. A constant component of noise (of 7.1 pA^2, the mean of the variances measured between 17 and 24 ms in the tail step) has been subtracted. (*D*) Same as *C*, but of a patch in symmetric K^+ solutions. In this case, the curve is based on $i = 1.8$ pA, and $N = 3800$. These measurements were made with 105 mM monovalent cation in the bath, and 95 mM in the pipette.

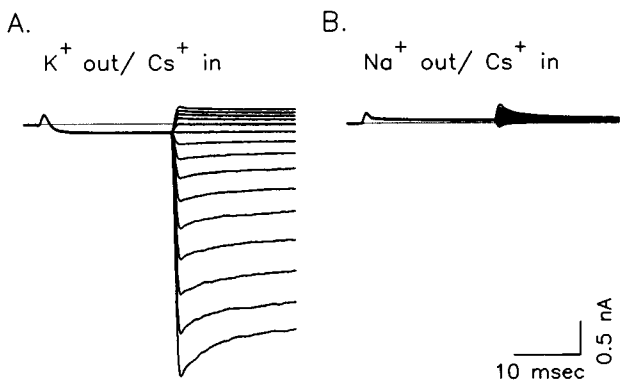

FIGURE 7 Cs^+ currents in the presence of different external cations. With 105 mM Cs^+ in the pipette, a single outside-out macropatch was exposed to either 95 mM K^+ (*A*) or 95 mM Na^+ (*B*) in the bath. In both cases, the membrane was depolarized by a step to +50 mV (near the reversal in external K^+) and then stepped to different tail potentials, ranging from −40 to +100, in 10-mV increments. These are raw current recordings: the current which remains with external Na^+ may be capacitive, leak, rapidly-inactivating current through the *Shaker* channel, or a combination of these.

solutions containing either 300 mM NH_4^+ (Fig. 8 *A*, *top*), 300 mM K^+ (*bottom*), or a 1:1 mixture of the two (*middle*). Current amplitude is nearly the same in either K^+ or NH_4^+ solutions; however, it is noticeably reduced in the mixture. As the mole-fraction of NH_4^+ in the solution is gradually increased, the single channel current goes through a minimum (Fig. 8 *B*). This anomalous effect is observed at all positive voltages, but it is more dramatic at low membrane potentials than during extreme depolarizations (Fig. 8 *C*).

In some K^+ channels, the reversal potential, measured under asymmetric ionic conditions, also exhibits an anomalous mole-fraction effect (Hagiwara et al., 1977). However, this is not seen in the *Shaker* channel. As the fractions of K^+ and NH_4^+ are varied on one side of the membrane, the macroscopic reversal potential changes in a strictly monotonic fashion (Fig. 8 *D*). Analogous experiments, varying the mole-fractions of Cs^+ and K^+ in the bath solutions, showed a qualitatively similar outcome.

DISCUSSION

This work describes some of the basic permeation properties of the *Shaker* K^+ channel. Working with a cloned channel made it possible to study both single channel and macroscopic currents under identical conditions. In addition, because the channel can be expressed at very high levels, we have been able to study Cs^+ conduction through this K^+ channel in some detail.

The conductance of the *Shaker* channel saturates at high $[K^+]$, similar to what is described in other K^+ channels (Coronado et al., 1980; Eisenman et al., 1986). It is interesting that saturation of the *Shaker* channel occurs over the same K^+ range as it does in the high-conductance Ca^{2+}-activated K^+ channel, despite the nearly 20-fold difference in absolute conductance between the channels. Although similar interactions may underlie this measured "affinity" in

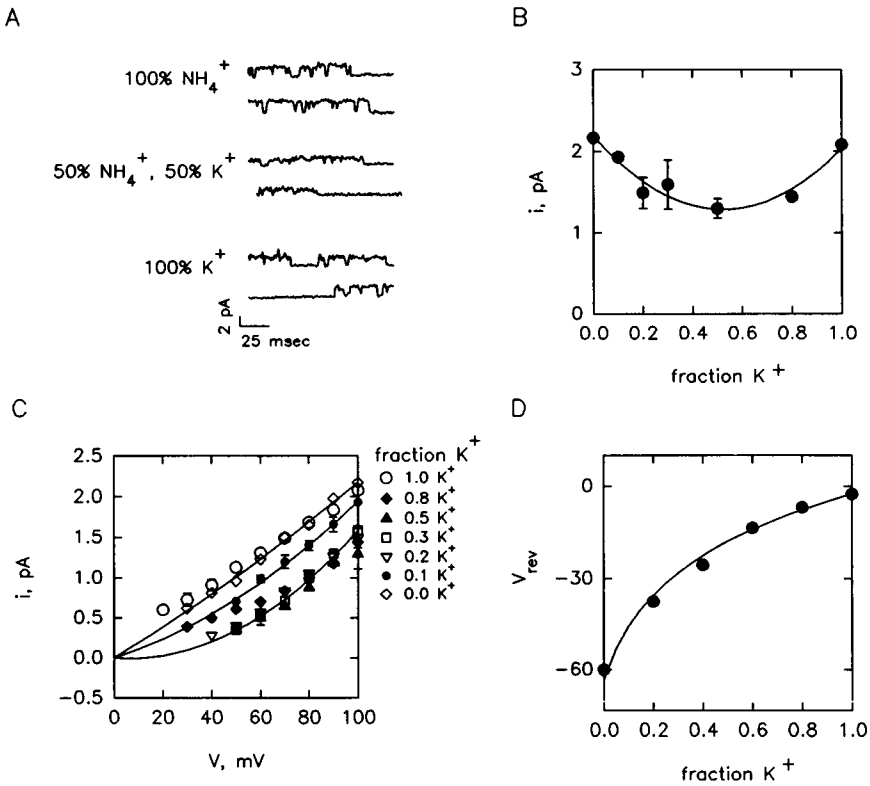

FIGURE 8 Anomalous mole-fraction effect in mixtures of NH_4^+ and K^+. In the experiments shown in *A–C*, single channel recordings were made in symmetric solutions containing mixtures of NH_4^+ and K^+, where $[NH_4^+] + [K^+]$ was 300 mM. (*A*) Single channel recordings, at +100 mV, in solutions containing K^+, NH_4^+, or a 1:1 mixture of the two cations. (*B*) Effect of mole-fraction on single channel current measured at 100 mV. (*C*) Single channel *i-V* relationships in solutions with different mole-fractions of NH_4^+. The illustrated curves are fits of the 0, 0.1, and 0.3 fraction K^+ data to the equation $i = aV + bV^2$ (no theoretical significance). (*D*) Effect of mole-fraction on macroscopic reversal potential. Reversal potentials were determined with 105 mM K^+ on the inside of a patch, and the illustrated solutions on the outside ($[NH_4^+] + [K^+] = 95$ mM). In this case, the curve is the prediction of the Goldman-Hodgkin-Katz equations with $P_{NH4}/P_K = 0.09$.

the two channels, the determinants of absolute conductance must differ. Despite other evidence that the *Shaker* K^+ channel contains a multi-ion pore, the conductance-concentration curve provides no suggestion of multiple occupancy. The anticipated decrease in conductance at extremely high ion concentrations has been reported in a few channels (Hladky and Hayden, 1972; Villarroel et al., 1988): perhaps a similar decrease would also be seen in the *Shaker* channel at higher concentrations of K^+.

The *Shaker* channel is highly selective for K^+ over other monovalent cations. Na^+, with a radius of 0.95 Å, does not carry measurable current through the channel. Cations larger than K^+ are permeable, although in general, the larger the ion, the less permeable it is. Cs^+ is the largest ion found to conduct current; Cs^+ current is visible at a macroscopic level under both bi-ionic conditions and in symmetric Cs^+ solutions. The finding of Cs^+ permeation places a lower limit of 3.34 Å (the diameter of a Cs^+ ion) on the diameter of the conduction pore.

Our ability to measure Cs^+ currents was a direct result of high levels of expression using heterologous expression. The *Shaker* channel is not unique among K^+ channels in displaying Cs^+ permeability; other voltage-activated K^+ channels have similar Cs^+ permeabilities under bi-ionic conditions (Shapiro and Decoursey, 1991; Reuter and Stevens, 1980). In contrast, Cs^+ permeation has not yet been reported in the high conductance Ca^{2+}-activated K^+ channel. In these channels, Cs^+ currents are not visible either under bi-ionic conditions (Yellen, 1984; Cecchi et al., 1987) or in symmetric Cs^+ (Cecchi et al., 1987) at a single channel level; instead, Cs^+ appears to block conduction. However, Cs^+ blockade is relieved at extreme membrane potentials, suggesting that Cs^+ does permeate these channels, although poorly (Cecchi et al., 1987). With the recent cloning of the Ca^{2+}-activated K^+ channels (Atkinson et al., 1991; Adelman et al., 1992; Butler et al., 1993), it may soon be possible to measure Cs^+ current through the high-conductance Ca^{2+}-activated K^+ channels using macroscopic current recordings.

The *Shaker* channel displays the permeability sequence $K^+ > Rb^+ > NH_4^+ > Cs^+$. The same selectivity is found in many other K^+ channels. Despite their differences in gating properties, delayed rectifiers (Hille, 1973; Wagoner and Oxford, 1987; Shapiro and Decoursey, 1991) and Ca^{2+}-activated K^+ channels (Yellen, 1984; Blatz and Magleby, 1984; Eisenman et al., 1986) are all similar to the *Shaker* channel with respect to selectivity measured under bi-ionic conditions.

It is interesting to compare the selectivity of the *Shaker* channel with that of the high conductance Ca^{2+}-activated K^+ channel (Table 2). These two channels are distantly related at a molecular level, and show extensive homology in one region that is thought to form part of the pore. These channels are equally selective when measured under bi-ionic conditions, and yet they have very different relative conductances g_{Rb^+}/g_{K^+} and g_{NH4^+}/g_{K^+} (Table 2). It is as if the determinants of bi-ionic selectivity, but not conductance, have been conserved in these two channels.

The *Shaker* channel displays a feature that is characteristic of multi-ion pores: an anomalous mole-fraction effect. It also resembles many other K^+ channels in this regard.

TABLE 2 Comparison of the selectivities of the high-conductance Ca^{2+}-activated K^+ channel and the *Shaker* K^+ channel

	Ion (X)	BK	*Shaker*
P_X/P_K	Tl^+	1.3	
	K^+	1.0	1.0
	Rb^+	0.7	0.66
	NH_4^+	0.1	0.09
	Cs^+		0.06
g_X/g_K	K^{s+}	1.0	1.0
	NH_4^+	0.18	0.75
	Rb^+	0.07	0.50
	Cs^+		0.01
	Tl^+	0.41	

The selectivity of the *Shaker* K^+, from Table 1, is compared to that of the high-conductance Ca^{2+}-activated K^+ channel (BK: Yellen, 1984; Blatz and Magleby, 1984; Eisenman et al., 1986).

Concentration-dependent permeability ratios (Wagoner and Oxford, 1987) have provided evidence that other voltage-activated K^+ channels can have multi-ion pores. The most direct confirmation of multiple occupancy comes from determinations of the flux-ratio exponent in voltage-dependent K^+ channels from cuttlefish (Hodgkin and Keynes, 1955) and squid giant axon (Begenisich and DeWeer, 1980) and the Ca^{2+}-activated K^+ channel from red cells (Vestergaard-Bogind et al., 1985). In addition, the affinities of multiple K^+ binding sites have been measured directly in the high-conductance Ca^{2+}-activated K^+ channel (Neyton and Miller, 1988a, 1988b).

These experiments illustrate that the conduction properties of the *Shaker* K^+ channel are similar to those previously described in a wide variety of K^+ channels. The *Shaker* channel is both highly selective among monovalent cations and displays an anomolous mole-fraction effect consistent with it being a multi-ion channel.

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REFERENCES

Adelman, J. P, K.-Z. Shen, M. P. Kavanaugh, R. A. Warren, Y.-N. Wu, A. Lagrutta, C. T. Bond, and R. A. North. 1992. Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron.* 9:209–216.

Atkinson, N. S., G. A. Robertson, and B. Ganetzky. 1991. A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science (Wash. DC).* 253:551–555.

Begenisich, T. B., and P. DeWeer. 1980. Potassium flux ratio in voltage-clamped squid giant axons. *J. Gen. Physiol.* 76:83–98.

Blatz, A., and K. Magleby. 1984. Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. *J. Gen. Physiol.* 84:1–23.

Butler, A., S. Tsunoda, D. P. McCobb, A. Wei, and L. Salkoff. 1993. *mSlo*, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science (Wash. DC).* 261:221–224.

Cecchi, X., D. Wolff, O. Alvarez, and R. Latorre. 1987. Mechanisms of Cs^+ blockade in a Ca^{2+}-activated K^+ channel from smooth muscle. *Biophys. J.* 52:707–716.

Coronado, R., R. L. Rosenberg, and C. Miller. 1980. Ionic selectivity, saturation, and block in a K^+-selective channel from sarcoplasmic reticulum. *J. Gen. Physiol.* 76:425–453.

Eisenman, G., R. Latorre, and C. Miller. 1986. Multi-ion conduction in the high-conductance Ca^{2+}-activated K^+ channel from skeletal muscle. *Biophys. J.* 50:1025–1034.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27:37–60.

Hagiwara, S., S. Miyazaki, S. Krasne, and S. Ciani. 1977. Anomalous permeabilities of the egg cell membrane of a starfish in K^+-Tl^+ mixtures. *J. Gen. Physiol.* 70:269–281.

Hille, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* 61:669–686.

Hladky, S. B., and D. A. Hayden. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. I. Studies of the unit conductance channel. *Biochim. Biophys. Acta.* 274:294–312.

Hodgkin, A. L., and B. Katz. 1949. The effect of Na ions on the electrical activity of the giant axon of the squid. *J. Physiol.* 108:37–77.

Hodgkin, A. L., and R. D. Keynes. 1955. The potassium permeability of a giant nerve fibre. *J. Physiol.* 128:61–88.

Hoshi, T., W. N. Zagotta, and R. W. Aldrich. 1990. Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science (Wash. DC).* 250:533–538.

MacKinnon, R., and G. Yellen. 1990. Mutations affecting TEA blockade and ion permeation in voltage-activated K^+ channels. *Science (Wash. DC).* 250:276–279.

Neyton, J., and C. Miller. 1988a. Potassium blocks barium permeation through calcium-activated potassium channels. *J. Gen. Physiol.* 92: 549–567.

Neyton, J., and C. Miller. 1988b. Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+}-activated K^+ channel. *J. Gen. Physiol.* 92:569–586.

Reuter, H., and C. F. Stevens. 1980. Ion conductance and ion selectivity of potassium channels in snail neurones. *J. Membr. Biol.* 57:103–118.

Schoppa, N. E., K. McCormack, M. A. Tanouye, and F. J. Sigworth. 1992. The size of gating charge in wild-type and mutant *Shaker* potassium channels. *Science (Wash. DC).* 255:1712–1715.

Shapiro, M. S., and T. E. Decoursey. 1991. Selectivity and gating of the type L potassium channel in mouse lymphocytes. *J. Gen. Physiol.* 97: 1227–1250.

Sigworth, F. J. 1980. The variance of sodium current fluctuations at the node of Ranvier. *J. Physiol.* 307:97–129.

Vestergaard-Bogind, B., P. Stampe, and P. Christophersen. 1985. Single-file diffusion through the Ca^{2+}-activated K^+ channel of human red cells. *J. Membr. Biol.* 88:67–75.

Villarroel, A., O. Alvarez, and G. Eisenman. 1988. A maximum in conductance occurs for the large Ca-activated K channel at high Rb concentration. *Biophys. J.* 53:259*a*. (Abstr.)

Wagoner, K., and G. S. Oxford. 1987. Cation permeation through the voltage-dependent potassium channel in the squid axon. *J. Gen. Physiol.* 90:261–290.

Yellen, G. 1984. Relief of Na^+ block of Ca^{2+}-activated K^+ channels by external cations. *J. Gen. Physiol.* 84:187–199.

Yellen, G., M. Jurman, T. Abramson, and R. MacKinnon. 1991. Mutations affecting internal TEA blockade identify the probable pore-forming region of a K^+ channel. *Science (Wash. DC).* 251:939–941.