Permeation, Selectivity, and Blockade of the Ca^{2+}-activated Potassium Channel of the Guinea Pig Taenia Coli Myocyte

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ABSTRACT The permeation properties of the 147-pS Ca^{2+}-activated K^+ channel of the taenia coli myocytes are similar to those of the delayed rectifier channel in other excitable membranes. It has a selectivity sequence of K^+ 1.0 > Rb^+ 0.65 > NH_4^+ 0.50. Na^+, Cs^+, Li^+, and TEA^+ (tetraethylammonium) are impermeant. Internal Na^+ blocks K^+ channel in a strongly voltage-dependent manner with an equivalent valence (zd) of 1.20. Blockade by internal Cs^+ and TEA^+ is less voltage dependent, with d of 0.61 and 0.13, and half-blockage concentrations of 88 and 31 mM, respectively. External TEA^+ is about 100 times more effective in blocking the K^+ channel. All these findings suggest that the 147-pS Ca^{2+}-activated K^+ channel in the taenia myocytes, which functions physiologically like the delayed rectifier, is the single-channel basis of the repolarizing current in an action potential.

INTRODUCTION

By its voltage sensitivity, $[Ca^{2+}]_i$ sensitivity, and some kinetic properties in response to step depolarizations, the 150-pS K^+ channel in the taenia myocyte has been linked to the delayed rectifier in the whole cell (Yamamoto et al., 1989*b*). This paper presents further details on some permeation properties of this channel, and compares them with those of well-studied delayed rectifiers. Some of the results have been reported in abstract form (Hu et al., 1987).

MATERIALS AND METHODS

Details on the dispersion of myocytes, experimental set-up, and patch-clamping procedures have been presented before (Hu et al., 1989; Yamamoto et al., 1989*a*).

In experiments studying the relative permeability on inside-out patches, 135 mM KCl in the bath was replaced with an equimolar test ion in its chloride salt. In other experiments studying channel blockade, the blocking ions were added without changing the composition of the entire solution.

All experiments were performed at room temperature (22–24°C). The unit conductances of the channel reported in this paper denote conductances at 0 mV (see Hu et al., 1989).

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The relative permeability of test ion X^+ to K^+ (P_X/P_K) is calculated from the shift of the reversal potential upon changing the internal solution from 135 mM KCl to 135 mM XCl with constant external K^+ concentration (Goldman, 1943; Hodgkin and Katz, 1949):

$$E_X - E_K = RT/F \ln (P_K[K^+]_i/P_X[X^+]_i) \quad (1)$$

where RT/F = 25.4 mV at 22°C, and the other parameters have their usual meanings. The procedure of determining reversal potential has been described (Hu et al., 1989).

In experiments where channel blockade by internally applied ion B^+ was studied, some quantitative information of the blockade was calculated according to Woodhull's approach (1973), by using the relationship:

$$g_O/g_B = 1 + [B^+]_i \exp (zdEF/RT)/K_D(0) \quad (2)$$

where g_O/g_B is the ratio of single-channel conductance at 0 mV without or with blocking ion B^+, $[B^+]_i$ is the concentration of internal blocking ions, $K_D(0)$ is the dissociation constant at 0 mV, z is the blocking ion valence, and d is the electrical distance, representing the fraction of the total electrical potential drop, E, between inside and the blocking site (Blatz and Magleby, 1984). The value of d and $K_D(0)$ can be obtained from the slope and y-intercept of the plot of $\ln (g_O/g_B - 1)$ vs. E.

Data are given as the mean ± SEM, wherever it applies.

RESULTS

As shown before (Hu et al., 1989), the predominant K^+ channel in the taenia myocyte is a voltage-sensitive, $[Ca^{2+}]_i$-sensitive channel with a high selectivity for K^+ and the unit conductance of ~150 pS. In response to step depolarizations, the channel opens with a latency that shortens and a frequency that increases as the membrane potential becomes increasingly more positive. Averaged single-channel events show much similarity to the familiar delayed rectifier current in whole cells (Yamamoto et al., 1989*b*) as well as in tissues (Inomata and Kao, 1976). To confirm the suspicion that this 150-pS channel could be the molecular basis of delayed rectification, it is necessary to further test its permeation properties to some monovalent cations, and to see how it is blocked by some well-studied blocking agents.

Ionic Selectivity to Monovalent Cations

To compare the characteristics of the 150-pS channel with those of the delayed rectifier K^+ channel in other excitable membranes, we determined the selectivity sequence and examined some conductance properties of the channel. The relative selectivity of the channel to monovalent cations, such as Li^+, Na^+, Cs^+, TEA^+, Rb^+, and NH_4^+, were determined by reversal potential measurements (Chandler and Meves, 1965; Hille, 1971, 1973; see Methods).

Li^+, Na^+, Cs^+, and TEA^+ are impermeant. On inside-out patches, when internal K^+ was replaced by Li^+, no outward channel current was detected at membrane potentials as positive as 60 mV. Typical current records are shown in Fig. 1 *A*. Single-channel current vanished within 3 min upon replacement with Li^+. On washout of Li^+, current magnitude recovered as readily as it diminished. However, the open dwell time on recovery was significantly shorter than that in the control state (Fig. 1, *A* and *B*). Complete recovery of the mean open time was not observed even 20 min after readmission of K^+. Such a residual effect was not seen with mean closed

time. The reversal potential, if there would be some inward current, must be more positive than 60 mV, corresponding to an upper limit of P_{Li}/P_K of 0.004. Thus, Li^+ is either a nonconducting ion through the Ca^{2+}-activated K^+ channel or it modifies the channel-gating mechanism in such a manner that the channel does not open at all.

Results similar to those with Li^+ were also observed for replacement of K^+ by Cs^+, Na^+, or TEA^+. These ions did not carry measurable current over the range of potential studied (−50 to 50 mV). Hence, the relative permeabilities of these ions

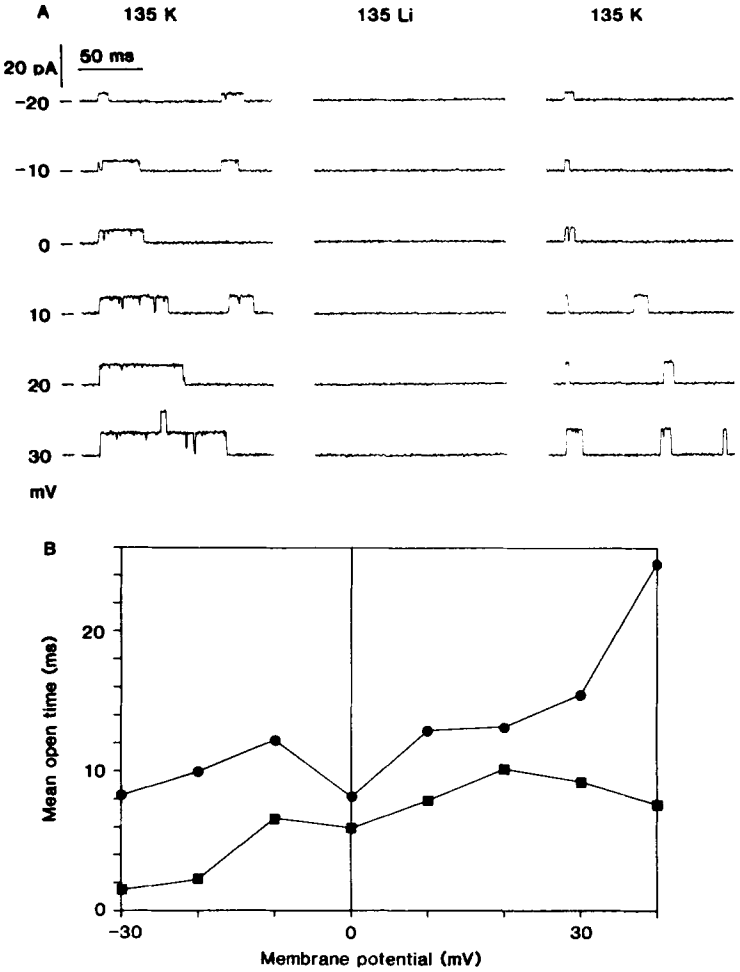

FIGURE 1. Impermeability of Ca^{2+}-activated K^+ channel to Li^+. (*A*) Single-channel currents at various clamped patch potentials (as indicated). Inside-out patch, internally exposed successively to 135 mM KCl (*left*), 135 mM LiCl (*middle*), and again 135 mM KCl (*right*). Short bars on left side indicate current level where channel was closed. Calibrations apply to all traces. Note the complete recovery of size of unitary current, but a shorter open time in K^+ after recovery than in control. (*B*) Mean open time vs. membrane potential for data shown above. Control, ●; recovery, ■.

to that of K^+ do not exceed 0.01. The channel mean open time mostly recovered after TEA^+ treatment, but it remained much briefer after applications of Cs^+ and Na^+.

Rb^+ and NH_4^+ are permeant. Rb^+ has been reported to carry current through Ca^{2+}-activated K^+ channel (Gorman et al., 1982; Blatz and Magleby, 1984; Yellen, 1984; Benham et al., 1986) as well as a variety of K^+ channels (Chandler and Meves, 1965; Bezanilla and Armstrong, 1972; Hille, 1973; Reuter and Stevens, 1980; Plant, 1986). Single-channel currents in 135 mM $[KCl]_i$ or $[RbCl]_i$ at several potentials are

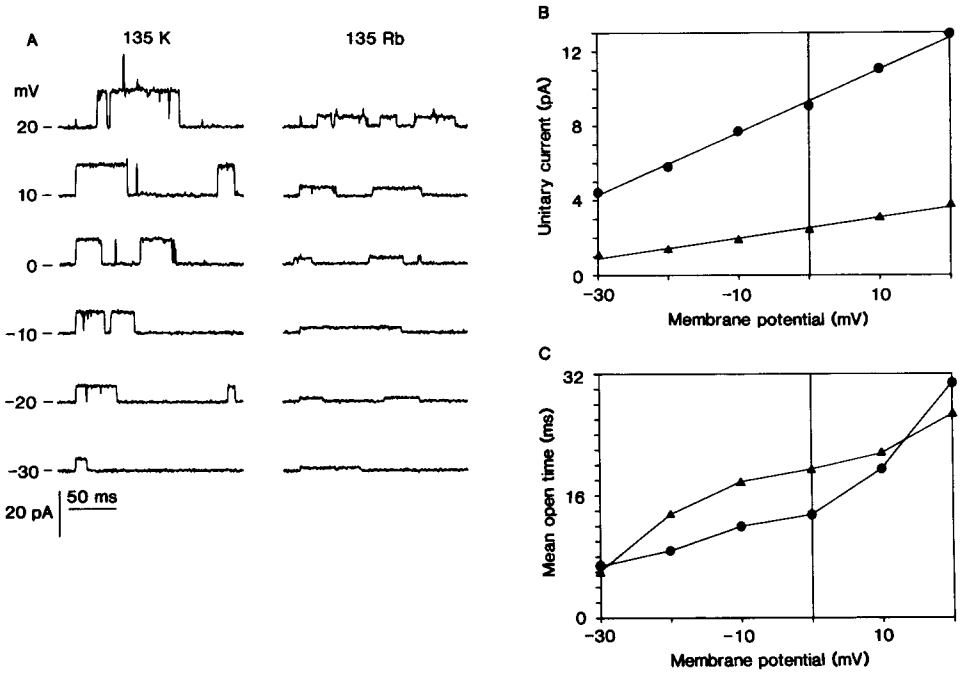

FIGURE 2. Reduced permeability of Rb^+ through Ca^{2+}-activated K^+ channel. (*A*) Single-channel currents at various potentials (as indicated). Inside-out patch internally bathed in a solution with 135 mM KCl (*left*) or 135 mM RbCl (*right*). At every potential the current in RbCl is reduced. Calibration bars valid for all current traces. (*B*) *i-V* plot for data recorded in *A*. Points are observed mean unitary currents in K^+ (●) or in Rb^+ (▲), continuous lines are linear regression fittings. (*C*) Mean open time vs. membrane potential in K^+ (●) or in Rb^+ (▲).

shown in Fig. 2 *A*. Outward current was readily detected, presumably because of Rb^+ movement through K^+ channel. The *i-V* relations from such observations (Fig. 2 *B*) give unit conductances of 169 pS for K^+ and 56 pS for Rb^+, and a positive shift of the reversal potential ($E_{Rb} - E_K$) of ~10 mV. In four experiments of the same type the average g_{Rb}/g_K is 0.31 ± 0.04, and a positive shift of reversal potential of 11.1 ± 1.8 mV. From Eq. 1 (see Methods) a relative permeability (P_{Rb}:P_K) of 0.65 is obtained.

Rb^+ caused some changes in channel gating. At voltages from −30 to 10 mV, the

channel mean open times in Rb^+ were longer than those in K^+ (Fig. 2 *C*), whereas the mean closed times were little affected (not shown). The probability of a channel being open at 0 mV in Rb^+ was about twice that in K^+ and declined markedly at potentials more positive than 20 mV, mainly because of a decline of the open time (not shown). There was no residual effect of Rb^+, as channel behavior recovered well after its removal.

Experiments similar to those with Rb^+ were also performed with NH_4^+ (Fig. 3). Outward single-channel currents in NH_4^+ were smaller than in K^+, but larger than in Rb^+ (Fig. 3 *A*). NH_4^+ induced a higher noise level in both open and closed states (see

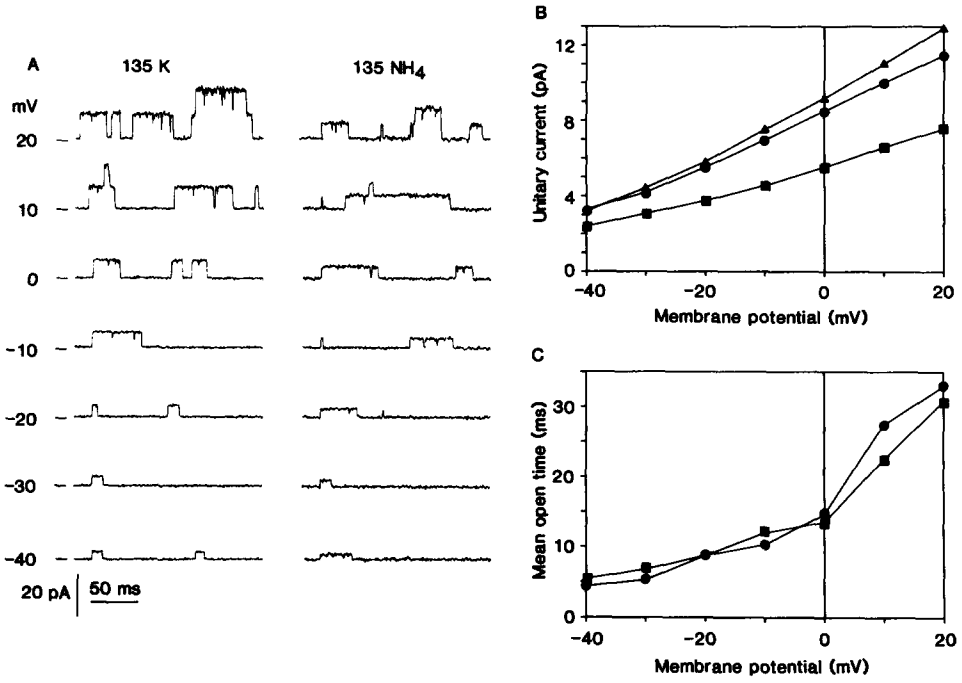

FIGURE 3. Permeability of Ca^{2+}-activated K^+ channel to NH_4^+. (*A*) Single-channel currents from inside-out patch in internal 135 mM KCl (*left*) or 135 mM NH_4Cl (*right*). Note noisier open and closed states and reduced currents in NH_4^+. (*B*) *i-V* plot from data shown in *A*. Symbols denote unitary currents in control (●), NH_4Cl (■), and recovery (▲) after NH_4Cl. Lines drawn by eye. Currents during recovery are often slightly larger than in control. (*C*) Mean open time vs. membrane potential in K^+ (●) and NH_4^+ (■).

traces in Fig. 3 *A*). Upon removal of NH_4^+, the recovery of the size of the unitary currents appeared to be biphasic: it readily overshot initially, then slowly returned to the prior level. This phenomenon was consistently observed in all experiments of this type. However, the traces tended to remain noisier than before, suggesting the persistence of some effect on channel-gating properties.

From the *i-V* relations (Fig. 3 *B*), conductances obtained ~0 mV are 149 for K^+ control, 77 pS for NH_4^+, and 169 pS for K^+ recovery. The conductance ratio (g_{NH_4}/g_K) averaged from four experiments is 0.50 ± 0.13. Assuming that the inde-

pendence principle could be applied to this channel for movements of K^+ and NH_4^+, calculations based on relative current amplitudes give a permeability ratio (P_{NH_4}/P_K) of 0.50, which is equal to the conductance ratio. We discarded the calculation of permeability ratio by reversal potential measurements, because the measured values in NH_4^+ were rather scattered, possibly owing to some uncontrolled pH variations resulting from the application of NH_4^+.

The overall channel activities in NH_4^+ were rather similar to those in K^+. Channel mean open time at various potentials in NH_4^+ was virtually the same as that in K^+ (Fig. 3 *C*).

Blockade of the Channel

The current through several types of K^+ channel can be blocked by a variety of cations. To further investigate the conductance property of the 150-pS Ca^{2+}-activated K^+ channel, we examined the effects of Cs^+, Na^+, and TEA^+.

Cs^+ and Na^+ block the channel from the inside. Cs^+ applied to the intracellular side of the membrane reversibly reduced single-channel current (Fig. 4 *A*). For these experiments, Cs^+ in concentrations of 10 or 20 mM were added. The effects are very similar to those seen when Cs^+ replaced all K^+ (such as shortened open time), but to a much less extent. The *i-V* relations from these records (Fig. 4 *B*) yield unit conductances of 179 pS before and 176 pS after Cs^+, and 137 (10 mM) and 100 (20 mM) in Cs^+. The blockade may be somewhat voltage dependent, because a weak outward rectification present in the control *i-V* curve was abolished in Cs^+.

Calculated from Eq. 2 (see Methods), the 0 mV dissociation constant $K_D(0)$ is 88 mM with $[Cs^+]_i$ = 20 mM. The electrical distance (d) of the blocking site from the inside surface is 0.61. As shown in the *p-V* relations (Fig. 4 *C*), Cs^+ reduced the open probability of the channel, more so at 20 mV than at 40 mV. Unlike the reversible reduction of the size of the unitary current, however, this effect on p is irreversible, as it persists even after the removal of Cs^+. This reduction in p is due mainly to an increase of long closed time and a lesser decrease of the mean duration of openings (details of analysis are not shown, but the trend can be seen from current traces in Fig. 4 *A*).

Single-channel currents, using intracellular Na^+ as a blocking ion, are shown in Fig. 5 *A*. Internal Na^+ induced a marked increase of open-channel noise. Channel openings seem to be interrupted very frequently, resulting in large numbers of fast ON-OFF transitions. The relation between the apparent unitary current (filtered at 1 kHz) and membrane potential are shown in Fig. 5 *B*, which gives a clear picture of the intensified blockade on increasing depolarization. This property is an important piece of evidence identifying the 150-pS channel with other Ca^{2+}-activated K^+ channels (rat muscle cell, Pallotta et al., 1981; bovine chromaffin cell, Marty, 1983; Yellen, 1984; toad stomach myocyte, Singer and Walsh, 1984), and with the delayed rectifier (node of Ranvier, Bergman, 1970; squid giant axon, Bezanilla and Armstrong, 1972; French and Wells, 1977). The effects of Na^+, especially those following large depolarizations are rarely reversed.

According to Eq. 2, an electrical distance of 1.20 was calculated as the site of blockade for the condition of $[Na^+]_i$ = 20 mM. Although the value exceeds 1 (see Discussion), it does suggest that Na^+ enters the channel and penetrates deeply into

the membrane electrical field. Owing to damage of the giga-seals, we could not raise the patch potential to very positive levels to see whether the blockade could be relieved with a resultant increase in single-channel current (see French and Wells, 1977).

In studying the probability of a channel being open, we regarded a burst as a single opening, because it was impossible to measure the duration of each short ON-OFF event in a burst. This practice led to an overestimation of p in the presence

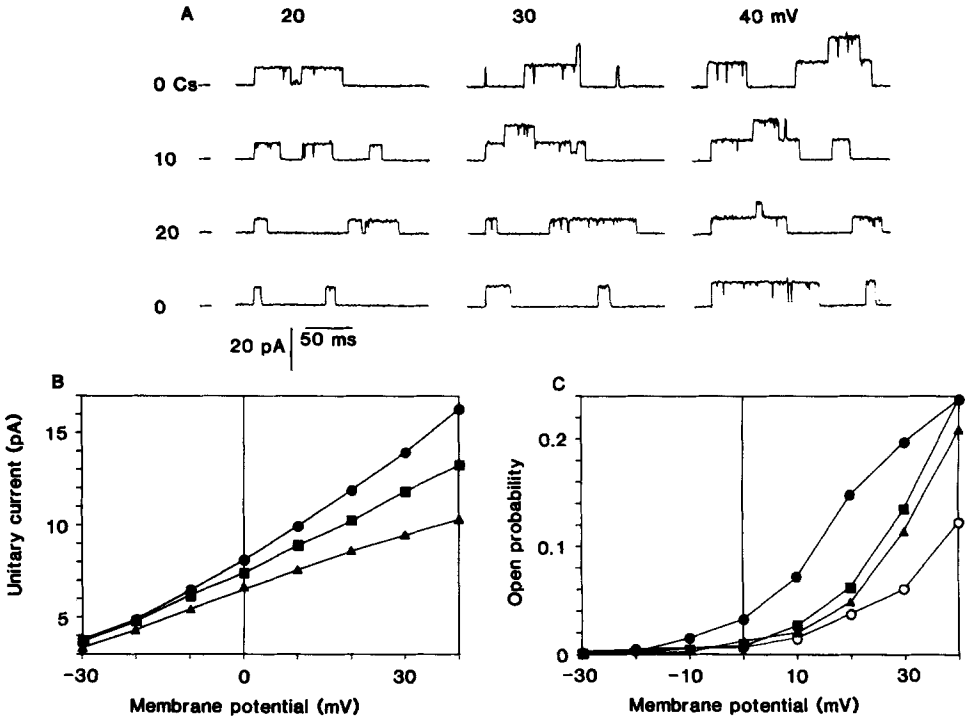

FIGURE 4. Internal Cs^+ reversibly decreases size of single-channel current and irreversibly reduces probability for channel being open. (*A*) Single-channel currents at membrane potentials of 20, 30, and 40 mV. Inside-out patch in 135 mM KCl containg 0 (first row), 10 (second row), 20 (third row), and again 0 mM Cs_i^+ (fourth row). Short bars on left side indicate the channel closed level. (*B*) *i-V* curve from data shown above. Symbols denote size of unitary currents in 0 (●), 10 (■), and 20 mM (▲) Cs_i^+. Lines drawn by eye. *i-V* curve for recovery (not shown) is superimposed on that of control. (C) *p-V* curve in control (●), 10 mM (■), 20 mM internal Cs^+ (▲), and recovery (○).

of internal Na^+ by a factor of i_O/i_B, the ratio of the unitary current in unblocked to blocked condition (Marty, 1983). From the *p-V* curves (after correction) in Fig. 5 *C*, it is evident that Na^+ reduces p in a voltage-dependent way, which results from a combination of a decline of mean open time and a prolongation of mean closed time (not shown).

TEA^+ blocks the channel from either side. TEA^+ reduces the K^+ conductance of delayed rectifier channel in many types of excitable membranes (for review see Stan-

field, 1983). In the present study, it was applied to either side of the membrane to see its effect on the Ca^{2+}-activated K^+ channel. When applied to the intracellular side of the membrane, the effect of TEA^+ appears to be a simple reduction of unitary current at all membrane potentials without modifying the gating kinetics (Fig. 6 *A*). The current traces from four such experiments showed no detectable alteration of the noise level of either open or closed state during the intracellular application of TEA^+.

Fig. 6 *B* shows the *i-V* relations in the presence or absence of TEA_i^+. The unit

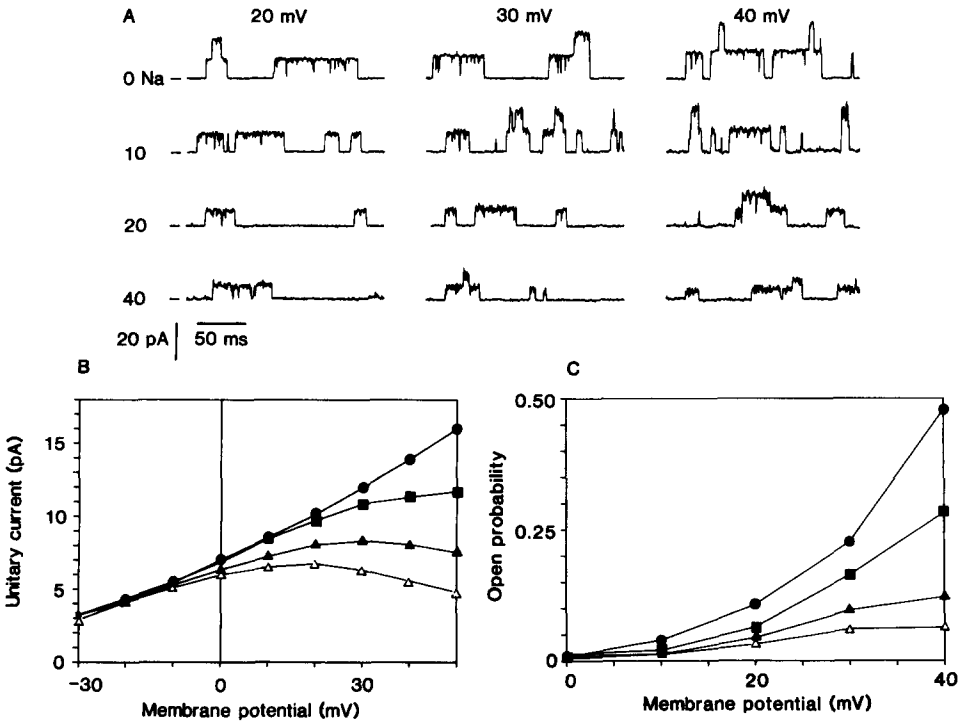

FIGURE 5. Internal Na^+ blocks Ca^{2+}-activated K^+ channel in voltage-dependent way. Inside-out patch. Records filtered at 1 kHz. (*A*) Single-channel recordings at 20, 30, and 40 mV without or with 10, 20, and 40 mM internal Na^+ (as indicated). Short bars on left side indicate zero-current level. Note Na_i^+ induces noisy and flickery open state. (*B*) *i-V* relationship from data shown above. Apparent unitary currents at various membrane potentials are represented by symbols: ●, control; ■, 10; ▲, 20; and △, 40 mM Na_i^+. (*C*) *p-V* plots. Points are observed values after correction (see text) at $[Na^+]_i$ of 0, ●; 10, ■; 20, ▲; and 40 mM, △.

conductances are 145, 117, 93, and 61 pS, respectively, in 0, 10, 20, and 40 mM of TEA_i^+. From the shape of the *i-V* curves, the blockade exhibits little voltage dependence. The electrical distance of the blocking site was calculated as 0.13, a value which falls between 0.26 reported for cultured rat muscle (Blatz and Magleby, 1984) and 0.1 for bovine chromaffin cells (Yellen, 1984).

Fig. 6 *C* gives the relation between normalized conductances (0 mV) and the concentrations of TEA_i^+. The observed data ($n = 4$) are best fitted by a bimolecular

reaction to its 1.4th power. From this dose-response relation, the concentration for a half-reduction of the unit conductance is 31 mM. This value can be compared with 11.8 mM in the guinea pig mesenteric artery myocyte (Benham et al., 1985), 25 mM in the canine airway smooth muscle cell (McCann and Welsh, 1986), 27 mM in the bovine chromaffin cell (Yellen, 1984), and 60 mM in the cultured rat muscle cell (Blatz and Magleby, 1984).

The effect of external TEA^+ was studied by incorporating TEA^+ in the patch

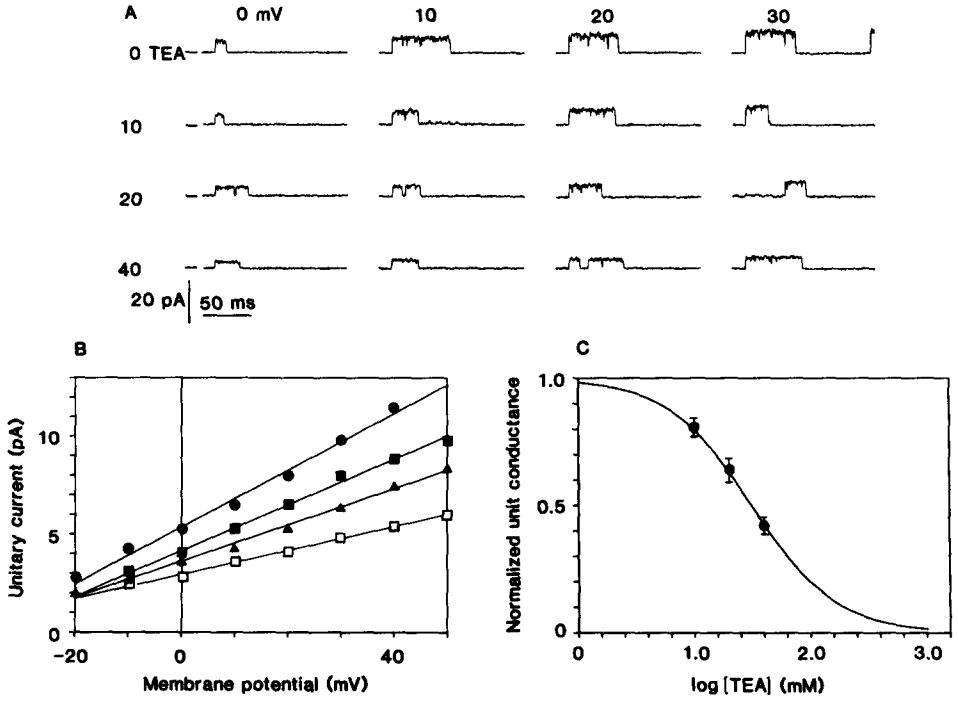

FIGURE 6. Internal TEA^+ blocks Ca^{2+}-activated K^+ channel by reducing the magnitude of unitary current with little voltage dependence. (*A*) Single-channel currents from inside-out patch internally treated with 0, 10, 20, and 40 mM TEA^+ at potentials of 0, 10, 20, and 30 mV. Bars on left side indicate closed level. (*B*) *i-V* curve from data presented above. Points denote observed data, $[TEA^+]_i$ is as follows: ●, 0 mM; ■, 10 mM; ▲, 20 mM; □, 40 mM. Continuous lines are linear regression fittings. Using Eq. 2, $K_D(0)$ is 41.8 mM for 10 mM TEA (as blocking ion), 35.8 mM for 20 mM TEA, and 29.0 mM for 40 mM TEA. (*C*) Concentration-effect relation. Abscissa: $[TEA^+]_i$ in a logarithmic scale; ordinate: unit conductance with TEA_i^+ normalized to that without TEA_i^+. Points are averages from four experiments. Continuous line is bimolecular reaction curve to 1.4th power.

pipette. Once a giga-seal was formed at the tip of a pipette that contained more than 2 mM TEA^+, channel openings of ordinary size were not observed over a wide range of patch potential. Only some small flickerings with long closed times in-between appeared at very positive potentials (middle and right panels of Fig. 7). Because the same patch could not be used for both control and TEA^+, comparisons had to be made on pairs of patches from the same cell. The concentration needed to

reduce unit conductance to 50% was estimated to be between 0.1 and 0.3 mM ($n = 5$ pairs). A more precise estimate was not made because of variations in the sensitivity of different patches and the lack of a real control. Thus, external TEA^+ could be 100 times more effective than internal TEA^+ in blocking the Ca^{2+}-activated K^+ channel. This finding is in agreement with those reported earlier (Hermann and Gorman, 1981; Adams et al., 1982; Latorre et al., 1982; Blatz and Magleby, 1984; Yellen, 1984; Inoue et al., 1985). The differences in the blocking pattern and in the

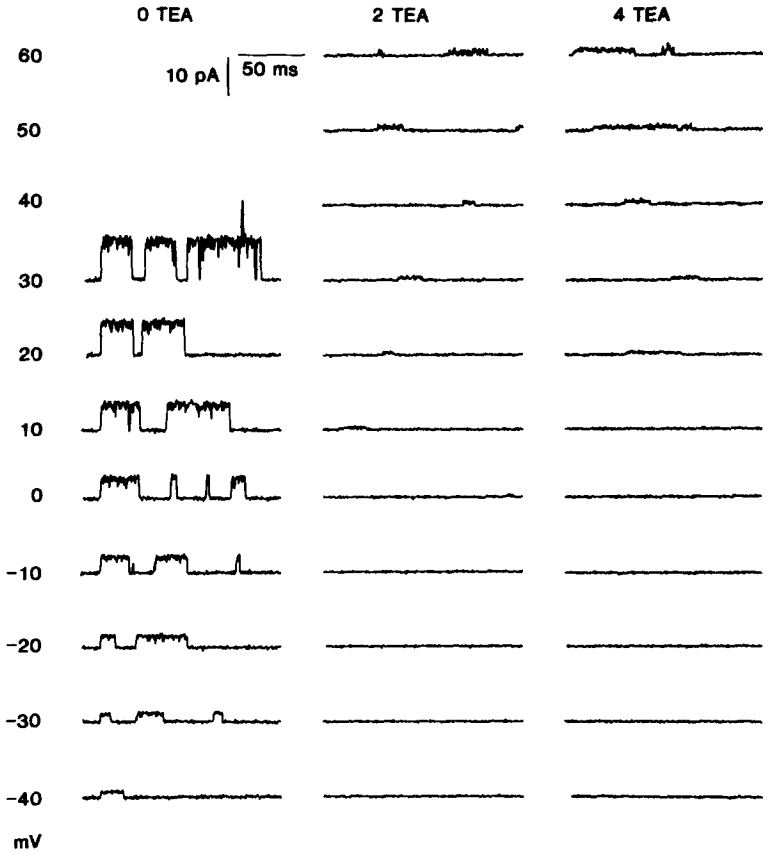

FIGURE 7. External TEA^+ is more potent in blocking the channel. Single-channel currents at various potentials from three inside-out patches with pipette solution containing 0, 2, or 4 mM TEA^+ (as indicated). Calibration bars valid for all traces.

potency between the actions of internal and external TEA^+ suggest the presence of separate receptors for their actions.

DISCUSSION

Selectivity and Blockade Property

The ion selectivity of the 150-pS Ca^{2+}-activated K^+ channel of the taenia myocyte resembles the selectivity pattern of the Ca^{2+}-activated K^+ channel (Blatz and

Magleby, 1984; Yellen, 1984; Benham et al., 1986) as well as that of the delayed rectifier channel (Chandler and Meves, 1965; Berman, 1970; Bezanilla and Armstrong, 1972; Hille, 1973) in many other types of cells. The channel is very selective to K^+, less permeable to NH_4^+ and Rb^+, and nearly impermeable to Na^+, Cs^+, Li^+, and TEA^+.

The value of 0.65 for P_{Rb}/P_K in an asymmetrical Rb^+ condition is consistent with those observed on Ca^{2+}-activated K^+ channel in other tissues: 0.67 in the cultured rat muscle cell (Blatz and Magleby, 1984) and 0.7 in the rabbit jejunal cell (Benham et al., 1986). These values are considerably larger than the conductance ratio, g_{Rb}/g_K, of 0.31. The discrepancy probably reflects some binding of Rb^+ to a saturable site in the channel to reduce the rate of its passage. Thus, the absolute channel conductance defined by currents is depressed by saturation, whereas the relative permeability defined by reversal potentials, which depends only on the ratio of ionic concentrations, is unaffected (Hille and Schwarz, 1978; Adams et al., 1981). We found some change in channel gating kinetics when Rb^+ is the charge carrier (Fig. 2 *C*), as was also reported by Blatz and Magleby (1984).

Our value of 0.50 for P_{NH_4}/P_K, determined by applying the independence principle to current amplitude, is significantly larger than what has generally been found with reversal potential measurements for the delayed rectifier channel (Bezanilla and Armstrong, 1972; Hille, 1973) and the Ca^{2+}-activated K^+ channel (Blatz and Magleby, 1984). The difference may indicate a failure caused by equating relative permeability with relative conductance, which implies that the independence principle does not apply to the Ca^{2+}-activated K^+ channel when NH_4^+ is the charge carrier. On the other hand, the absolute conductance in NH_4^+ based on the amplitude of current might actually be somewhat exaggerated, if NH_4^+ carried a larger current through the K^+ channel than theoretically predicted, as it did in the node of Ranvier (Hille, 1973) and in the endplate channel of the frog skeletal muscle (Adams et al., 1981).

It is known that internal Na^+ and Cs^+ interfere with outward K^+ current through the delayed rectifier channel, and that they can cause a region of negative slope conductance in the *I-V* curve (Chandler and Meves, 1965; Bezanilla and Armstrong, 1972; French and Shoukimas, 1985). With internal Na^+, regions of negative slope conductance in the single-channel *i-V* curves were readily seen (Fig. 5 *B*).

With internal Cs^+, no negative conductance range was observed with $[Cs^+]_i$ up to 40 mM. This observation differs from the results obtained on the delayed rectifier of the squid giant axon. Blockade by internal Cs^+ in the taenia myocyte channel is of low affinity. The $K_D(0)$ of 88 mM (at 20 mM of Cs_i^+) is close to that of 100 mM for chromaffin cells (Yellen, 1984). However, the blocking site in the taenia myocyte has an electrical distance of 0.61, which is significantly greater than Yellen's value of 0.25. Yellen's observation was obtained in a symmetrical K^+ condition. So this discrepancy is in line with the general trend that the value of d is lower with higher $[K^+]_o$ (French and Shoukimas, 1985).

Is the Channel a Single-Ion or Multi-Ion Pore?

In our experiments, all currents were studied in an asymmetrical K^+ concentration ($[K^+]_i$ 135/$[K^+]_o$ 5.4) within a physiological potential range. The current-voltage

relationship can be well described by the Goldman-Hodgkin-Katz constant-field relation (Fig. 2 *B* of Hu et al., 1989), which suggests that the Ca^{2+}-activated K^+ channel might obey the independence principle. Contradictions arise, however, from the following observations. First, the relative conductance of 0.31 for Rb^+ through the K^+ channel obviously deviates from the permeability ratio of 0.65 obtained from the shift of the reversal potential. Second, the internal Na^+ blocking site is calculated to be at an electrical distance of 1.2 at 20 mM Na^+. In a single-ion channel pore with the monovalent blocking ion Na^+ and the monovalent permeant ion K^+, the value of zd in Eq. 2 cannot exceed 1.0. (Hille and Schwarz, 1978; Yellen, 1984; Eisenman et al., 1986). Third, the dose-response curve of internally applied TEA^+ is steeper ($n = 1.4$) than can be explained by a theory with one occluding ion per channel (where $n = 1.0$). These discrepancies can be resolved by assuming that the Ca^{2+}-activated K^+ channel is a multi-ion pore with multi-occupied sites inside (Hille and Schwarz, 1978), like the delayed rectifier K^+ channel. It obeys the independence principle only in a physiological ionic environment and potential range, in the absence of less permeant or impermeant foreign ions.

TEA^+ and the Macroscopic Outward Current

There is a discrepancy between the effectiveness of TEA^+ in blocking the single Ca^{2+}-activated K^+-channel and its inability to block all outward current in the whole cell. Thus, on the freshly dispersed myocyte of the guinea pig urinary bladder (Klöckner and Isenberg, 1985), and in multicellular preparations of the guinea pig taenia coli (Inomata and Kao, 1979, 1985) $[TEA^+]_o$ of 150 and 135 mM markedly reduced but did not eliminate the outward current. Two possible reasons could be considered: there are different types of K^+ channels with different responsivenesses to TEA^+, and there are other ionic channels contributing to the macroscopic outward current in the intact cell. Three types of K^+ channels have been shown to occur in the taenia myocyte (Hu et al., 1989), but the evidence indicates that the macroscopic outward current during a 100-ms step depolarization can be largely accounted for by the 150-pS Ca^{2+}-activated K^+ channel. Moreover, the two other types of K^+ channel do not appear to respond fast enough to the 100-ms depolarizing step. In view of such considerations, the possibilty of other ionic channels (especially chloride channels) contributing to the macroscopic outward current assumes real significance, especially under conditions where the K^+ channel is blocked.

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