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Single-Channel K^+ Currents in *Drosophila* Muscle and Their Pharmacological Block

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Summary. Four types of nonvoltage-activated potassium channels in the body-wall muscles of *Drosophila* third instar larvae have been identified by the patch-clamp technique. Using the inside-out configuration, tetraethylammonium (TEA), Ba^{2+}, and quinidine were applied to the cytoplasmic face of muscle membranes during steady-state channel activation. The four channels could be readily distinguished on the basis of their pharmacological sensitivities and physiological properties. The K_{ST} channel was the only type that was activated by stretch. It had a high unitary conductance (100 pS in symmetrical 130/130 mM KCl solution), was blocked by TEA ($K_d \sim 35$ mM), and was the most sensitive to Ba^{2+} (complete block at 10^{-4} M). A Ca^{2+}-activated potassium channel, K_{CF}, 72 pS (130/130 mM KCl), was gated open at $>10^{-8}$ M Ca^{2+}, was the least sensitive to Ba^{2+} (K_d of ~3 mM) and TEA (K_d of ~100 mM), and was not affected by quinidine. K_2 was a small conductance channel of 11 pS (130/2 KCl, pipette/bath), and was very sensitive to quinidine, being substantially blocked at 0.1 mM. It also exhibited a half block at ~0.3 mM Ba^{2+} and ~25 mM TEA. A fourth channel type, K_3, was the most sensitive to TEA (half block < 1 mM). It displayed a partial block to Ba^{2+} at 10 mM, but no block by 0.1 mM quinidine. The blocking effects of TEA, Ba^{2+} and quinidine were reversible in all channels studied. The actions of TEA and Ba^{2+} appeared qualitatively different: in all four channels, TEA reduced the apparent unitary conductance, whereas Ba^{2+} decreased channel open probability.

Key Words barium · tetraethylammonium · quinidine · patch clamp · I_K · I_A · stretch-activated channel · Ca^{2+}-activated potassium channel

Introduction

The study of ion channels in excitable membranes has profited greatly in the last few years with the use of the fruit fly *Drosophila*. The repertoire of genetic and molecular techniques available in this organism provide powerful tools for solving problems concerning the molecular mechanisms and functional regulation of ion channels. The gene encoding the channel mediating the transient, rapidly inactivating potassium current, or I_A, has been cloned and sequenced, and its transcripts have been expressed in *Xenopus* oocytes (Kamb, Tseng-Crank & Tanouye, 1988; Timpe et al., 1989) and the fly (Gisselmann et al., 1989). Recently, site-directed mutagenesis of sequences coding for the I_A channel has produced alterations in inactivation kinetics (Hoshi, Zagotta & Aldrich, 1989; Papazian et al., 1989) and toxin binding (MacKinnon & Miller, 1989). The *Drosophila* sodium channel gene has also been cloned and sequenced (Loughney, Kreber & Ganetzky, 1989) and another gene, which underlies Ca^{2+}-activated potassium channel activity, is currently being cloned (Atkinson, Robertson & Ganetzky, 1989). It will be important to characterize the properties of normal ion channels in *Drosophila* to obtain baseline information for the proper interpretation of genetic and molecular data as they become available.

Voltage-clamp experiments on larval, pupal, and adult life stages have identified at least five ionic currents in muscle—four potassium currents and a Ca^{2+} current (Salkoff & Wyman, 1983; Wu & Haugland, 1985; Elkins, Ganetzky & Wu, 1986; Gho & Mallart, 1986; Wei & Salkoff, 1986; Singh & Wu, 1989; Wu et al., 1989; Haugland & Wu, 1990), and patch-clamp work has correlated a number of single-channel currents to their macroscopic counterparts (Solc, Zagotta & Aldrich, 1987; Zagotta, Brainard & Adrich, 1988; Komatsu et al., 1990). Single-channel studies have also identified a number of channels for which there are no known macroscopic counterparts (Solc & Aldrich, 1988; Zagotta et al., 1988; Komatsu et al., 1990). Although the individual macroscopic currents have been separated both genetically and pharmacologically, single-channel currents in *Drosophila* have not been subjected to the same rigorous pharmacological analysis. TEA, Ba^{2+}, and quinidine have been used to specifically block K^+ channel currents in a wide variety of organisms (Rudy, 1988; Castle, Haylett & Jenkinson, 1989). The patch-clamp and lipid bilayer techniques have allowed their use at the single-channel level where the kinetics and mechanisms of block have been more thoroughly examined (Miller, 1988; Davies et al., 1989).

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Information concerning the effects of channel blocking agents on single-channel currents in *Drosophila* would facilitate their identification process and could make their unknown macroscopic correlates more readily discernable. The present work describes four nonvoltage-activated single-channel potassium currents and presents the blocking characteristics of TEA and other K^+ channel blockers. The stretch-activated channel, K_{ST} (Zagotta et al., 1988), fast calcium-activated channel, K_{CF} (Komatsu et al., 1990), and two previously unreported channel types tentatively designated as K_2 and K_3 were studied using an enzyme-treated larval body-wall muscle preparation (K_0 and K_1 channel types have previously been described by Solc and Aldrich (1988) and Zagotta et al. (1988)). The relationship of these channels to macroscopic currents and to single-channel currents from different preparations is discussed.

Materials and Methods

The wild-type strain, Canton-S, of *Drosophila* was maintained at 20–23°C on a standard food medium. The body-wall muscles of third instar wandering larvae were used in all experiments. These muscles surround the body cavity of larvae and are responsible for locomotion (Crossley, 1978). Larval muscle membranes were prepared in two ways. The vesicle method resulted in severely disrupted muscle fibers with membrane vesicles or "blebs" attached to the remnant muscle surface whereas the *in situ* method led to an apparently more intact muscle membrane in a dissected larva. Single-channel properties were essentially the same regardless of the preparation. For both methods, third instar larvae were pinned down in a chamber flooded with normal saline (concentrations in mM; 2 KCl, 128 NaCl, 1.8 $CaCl_2$, 4 $MgCl_2$, 35.5 sucrose, 5 HEPES, buffered at pH 7.2), and cut open along the dorsal midline. The body wall was spread out, held with pins, and viscera and nervous system were removed. Further aspects of the vesicle preparation have been previously described (Komatsu et al., 1990). For the *in situ* muscle preparation, the dissected larva was incubated for ~5 min with 0.4 mg/ml protease solution (Sigma type XIV) in normal saline with no Ca^{2+} or Mg^{2+} added but buffered with 1 mM EGTA. The preparation was washed in normal saline for 30 min just before recording. Some of the more superficial muscle fibers contracted and detached one end from the cuticle as a result of this procedure. To prevent the muscles from completely detaching and rounding up, the dissected larva was kept in normal saline. It was then transferred to a recording chamber, and placed under an inverted microscope (Nikon Diaphot-TMD) with Nomarski optics where it was subsequently used for patch recording. The dissection and recording were conducted at room temperature 20–25°C.

Patch microelectrodes were fabricated from borosilicate glass, coated with Sylgard® (Dow Corning) along the shank, and the tip was fire polished. Electrode resistances prior to seal formation were typically 12–15 MΩ and most seals were in the range of 3–20 GΩ with a few as high as 100 GΩ. Formation of gigaohm seals on vesicles occurred >50% of the time, whereas seal formation on muscle fibers *in situ* had a much lower (<20%) success rate. However, on membrane patches excised from *in situ* preparations, the resultant seal would often remain stable for greater than 20 min even after many solution changes. The excised vesicle membrane seals generally lasted for <5 min and were often destroyed by solution changes. All of the data presented in the figures and tables were made from the *in situ* preparation.

The inside-out patch configuration was used for all experiments. The recording pipette solution was always normal saline with sodium completely replaced by potassium (130 mM KCl instead of 2 mM) to increase the inward driving force on potassium ions for inside-out patches. Inward currents from the pipette were represented as downward deflections. The perfusion system consisted of an array of drug-filled, adjacent tube outlets in the chamber to which the patch microelectrode tip could be positioned for rapid contact with new solution (modified after Yellen, 1982). Current-voltage relationships were determined in bath solutions modified from normal solution to achieve different potassium-sodium concentrations (in mM): 2 KCl/128 NaCl (normal saline), 65 KCl/65 NaCl, and 130 KCl/0 NaCl. Chloride salts of TEA and Ba^{2+} were used. TEA solutions were made by replacing sodium with TEA on an equimolar basis.

Current recordings were made using a List EPC-7 patch-clamp amplifier (Medical Systems), pulse coded with a PCM-1 modulator (Medical Systems) and taped on a VHS video cassette recorder (Hitachi, VT-94A). Except for Fig. 1 (*see* legend), chart recording traces were filtered at 250 Hz. For the stretch-activated channels in Fig. 2, signals analyzed for kinetics were filtered at 2000–3000 Hz (Krohn-Hite 3200R; 4th order variable low-pass filter) and digitized at 100–200 μsec per point. For other channels, filtering was done at 1000 Hz and sampling at 330–500 μsec per point. Kinetic analyses were conducted on an AST Premium 286 computer using P-Clamp software (version 5.01, Axon Instruments). Open- and closed-channel events were defined by a transition in current to >50% of the unitary current and events briefer than 0.1 msec were ignored. The single-channel data were made into an idealized record from which mean open and closed times and mean channel amplitudes were calculated. Frequency distribution histograms were plotted and fitted with 1–3 exponentials.

Results

The potassium concentration gradient at the beginning of each experiment was chosen primarily for technical reasons. The muscles required a low K^+ concentration (2 mM) in the bath to prevent detachment and rounding up. The pipette [K^+] of 130 mM was used because it imparted a considerable driving force for K^+ ions at 0 mV for channels in detached patches and thus enabled observations of spontaneous K^+ channel activity in the bath solution. This large gradient also allowed us to quantitatively analyze many more patches with seals in the low gigaohm range that would otherwise have been lost in the lower signal-to-noise ratio of 2/2 (pipette/bath) mM K^+. Patches were of an inside-out configuration. Attempts to form outside-out patches were unsuccessful. Open-channel activity was seen in the majority ($n > 250$) of patches using the *in situ* method.

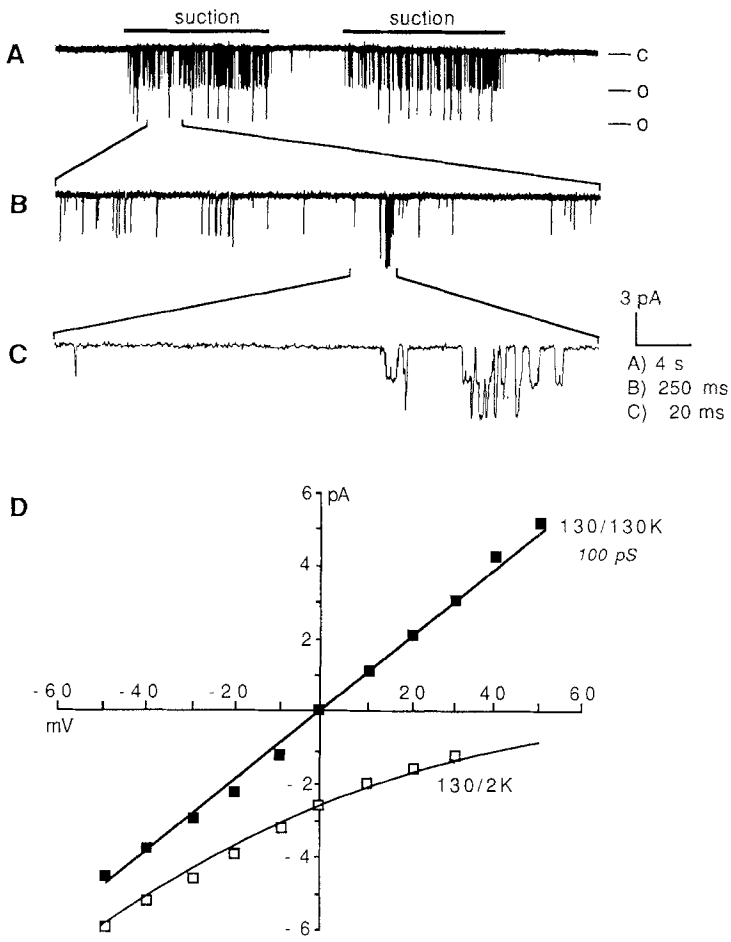

Fig. 1. (*A-C*) Single-channel currents observed upon application of negative pressure to an inside-out patch of muscle membrane at 0 mV are displayed at three different time scales. The pipette and bath solution contained 130 and 2 mM KCl, respectively (denoted 130/2 KCl, pipette/bath in the following figures). Downward deflections represent inward currents. Data were low-pass filtered at 2 kHz and sampled at 100 μsec/point. (*D*) Current-voltage plot for two concentrations of potassium in an inside-out patch. The 130/130 mM KCl data were fitted by linear regression and yielded a slope conductance of 100 pS. The constant field equation was used to determine the permeability coefficient for this curve (P_K = 2.0 $\times$ 10^{-13} cm^3/sec) which was then used to calculate the curve for the 130/2 KCl concentration

Typically, several channels were apparent just after seal formation but their activity subsided after only a minute or so. Infrequently, one or two channels would be active long enough to allow characterization. Many of the observed channels could not be included into one of the channel types described here and await eventual classification.

Four channel types were, however, readily distinguishable. All four channel types, K_{ST}, K_{CF}, K_2, and K_3, were spontaneously active at 0 mV and their steady-state characteristics are presented. In addition to these four channels, two types of voltage-gated channels were revealed by a voltage-pulse paradigm in patches which had no spontaneous activity. On the basis of their conductance, kinetics and pharmacology, they correspond to the A_1 (A-type muscle current) and the K_D (delayed rectifier type current) channels already described in detail in myocytes by Zagotta et al. (1988), in larval muscle vesicle by Komatsu et al. (1990) and in macroscopic voltage-clamp measurements by others in pupal and larval muscles (Salkoff & Wyman, 1983; Wu & Haugland, 1985; Gho & Mallart, 1986).

Stretch-Activated Channels

The single-channel events seen in Fig. 1*A-C* were evident upon application of negative pressure ($\geq$10 mm Hg) to the muscle membrane. K_{ST} channels (n = 10) were easily distinguished by a number of characteristics: pressure dependent activation, short mean open times (<2 msec), large unitary conductance and multiple channels in the same patch. Some stretch-activated channels showed low levels of activity, open probability (P_o) $\approx$ 0.01, without any applied negative pressure, which could have been due to distortion of the membrane by formation of the patch itself.

The current-voltage (*I-V*) plot in Fig. 1*D* shows the conductance for the channel at two different potassium concentrations. The linear regression of

the data at 130/130 mM KCl (recording pipette/bath), denoted by the straight line, yielded a slope conductance of 100 pS for both inward and outward currents from −50 to 50 mV, and a reversal potential (V_{rev}) of 0 mV. The other curve represents the predicted *I-V* relationship calculated from the Goldman-Hodgkin-Katz equation (Hodgkin & Katz, 1949) for a perfect K^+ electrode with a 130/2 K^+ gradient,

$$I_K = P_K(VF^2/RT)\{([K]_o - [K]_i e^{(VF/RT)})/(1 - e^{(VF/RT)})\} \quad (1)$$

where I is the single-channel current, P_K is the permeability coefficient, V is the membrane voltage, and F, R and T have their standard meanings. For the condition of symmetrical $[K^+]$, Eq. (1) is simplified (Eq. (2)) and the permeability of the channel can be determined.

$$P_K = \{RT/F^2[K^+]\}(\Delta I_K/\Delta V). \quad (2)$$

The slope conductance of 100 pS at 130/130KCl in Fig. 1*D* thus gives the permeability of the open channel: $P_K = 2.0 \times 10^{-13}$ cm³/sec. Using this value for the P_K in Eq. (1), the curve was generated for the 130/2 (pipette/bath) K^+ concentration giving a slope conductance of 59 pS at 0 mV and a calculated reversal potential of about 105 mV. The close fit of the data to the curve (Fig. 1*D*) suggests a substantial potassium-to-sodium permeability ratio. In one patch where 65 mM NaCl was substituted for 65 mM KCl, the shift in reversal potential indicated a K : Na permeability ratio of ~7 : 1.

Drosophila K_{ST} channels displayed a burst-like activity with short open times interrupted by very brief closed times in response to stretch. The bursts were separated by long closures with only occasional activity (Fig. 1*A-C*). Voltages from −50 to 50 mV did not activate the channel or substantially affect its P_o during stretch-induced activity. K_{ST} channels were always accompanied by at least one other K_{ST} channel or some dissimilar channel type(s). However, the low open probability (P_o) of K_{ST} channels in the absence of strong suction resulted in virtually no simultaneous open events such that a kinetic analysis of open times could be made (assuming that K_{ST} channels in the same patch had similar kinetic properties). Examination of open-state durations made on three patches yielded open-time distributions fit by single exponentials with time constants (τ_0) between 1.5 and 1.8 msec, (Fig. 2*A* and *B*). No open-time durations longer than 13 msec (>1800 events) were seen.

In contrast to open-event durations, analysis of closed durations in patches containing multiple channels could be seriously distorted even when P_o is low. The opening of a second channel would truncate the true duration of the first channel's long closures. However, a second channel would not be expected to produce interruptions of very brief closed transitions at low P_o. Analysis of closed-time histograms demonstrated a short closed state with τ_c values of about 0.5 msec, which did not vary in patches with different P_o. A long closed state was also indicated with τ_c ranging from 71 to 187 msec in patches of P_o = 0.05 to 0.02, but these values, as stated above, could be influenced by a second channel opening. Inspection of the traces in Fig. 1 also leads to the conclusion that there is both a short closed state as well as one much longer.

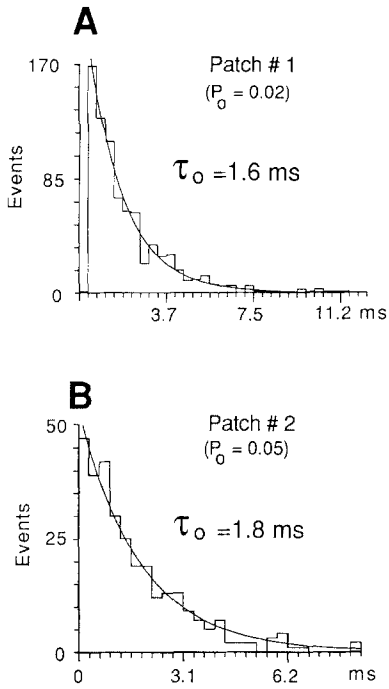

Fig. 2. Frequency histograms for open events in two representative K_{ST} channels at 0 mV (130/2 KCl, pipette/bath). (*A* and *B*) Distribution of open channel times is fit by a single exponential function with a time constant of 1.6 (bin size = 375 μsec) and 1.8 msec (bin size = 310 μsec), respectively. Data were filtered at 2 kHz and sampled at 200 μsec/point for *A*. For *B*, data were filtered at 3 kHz and sampled at 100 μsec/point

TEA applied to the cytoplasmic membrane face was found to reversibly block the stretch-activated single-channel currents in a dose-dependent fashion. The current amplitude decreased to 70, 40, and 32% of normal (Fig. 3*A*) for both channels in the patch at 10, 40, and 100 mM TEA, respectively. No obvious change in the channel kinetics was observed at these concentrations. The slope conductance was determined at 10 mM TEA and found to be 44 pS, a reduction to 68% of the control value of 64 pS in 130/2 KCl (Fig 3*B*). The remaining fractional single-channel current (current amplitude in TEA divided

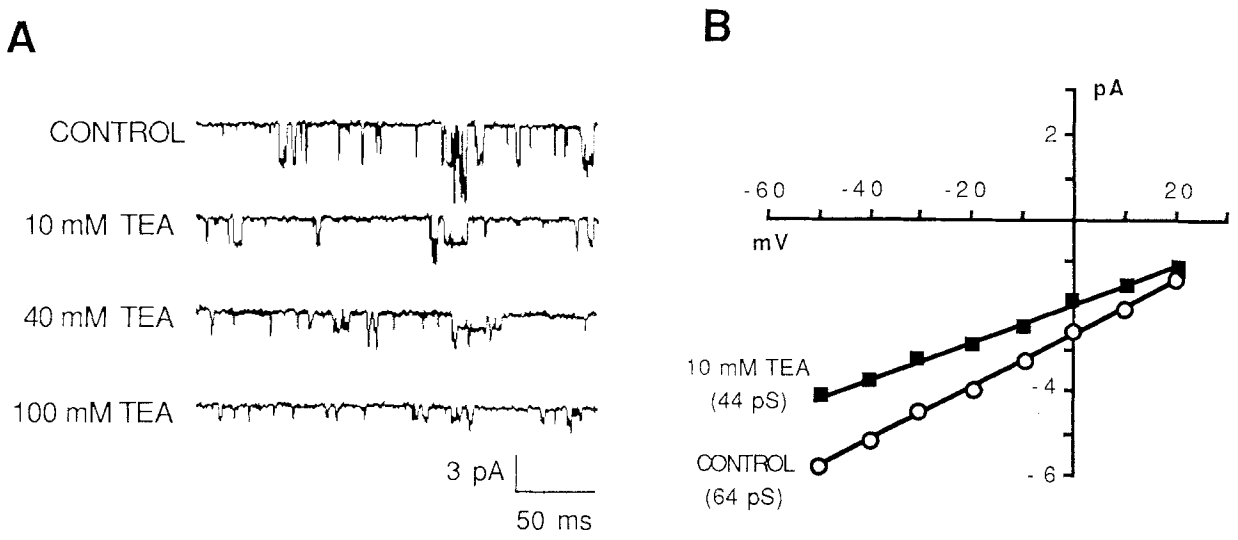

Fig. 3. The effect of TEA on K_{ST} single-channel current amplitude. (*A*) At least two active channels are present in this inside-out patch held at 0 mV (130/2 KCl, pipette/bath). Data were filtered at 2 kHz, and sampled at 100 μsec/point. (*B*) Slope conductance (linear regression) in the same patch demonstrates a substantial reduction with 10 mM TEA

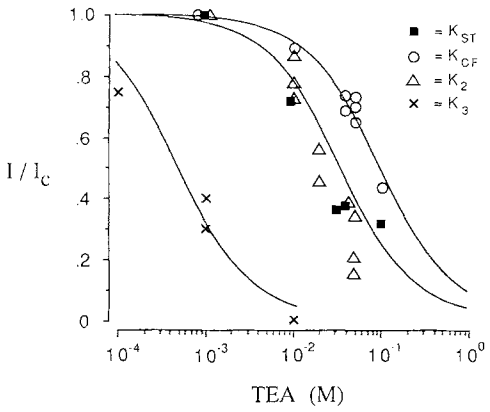

Fig. 4. Dose-response curve of TEA effect on single-channel current for four types of potassium channels. Data from individual patches (n = 2–5) for each channel type are represented as follows. K_{ST} = squares, K_{CF} = circles, K_2 = triangles, and K_3 = crosses. The solid lines are calculated from Eq. (3) (*see* text) and fit by eye to data for comparative purposes

by control current amplitude, I/I_c) was plotted against the concentration of TEA in log scale (Fig. 4, squares). The continuous curves in Fig. 4 were drawn according to the equation

$$I/I_c = 1/(1 + [\text{TEA}]/K_d). \quad (3)$$

This equation describes a simple bimolecular binding reaction with one molecule of TEA binding and blocking the channel with a dissociation constant of K_d. The K_d for the K_{ST} channel was estimated to be ~35 mM, from the concentration exhibiting 50% block.

Ba^{2+} was applied to a stretch-activated channel in one patch, and the lowest concentration used, 100 μM, suppressed both spontaneous and pressure-induced openings. The effects of Ba^{2+} were reversible. K_{ST} channels were not apparently regulated by internal Ca^{2+} because no obvious differences in open probability or current amplitude were observed during stretch-initiated activity when the normal saline (with 1.8×10^{-3} M Ca^{2+}) was replaced with a low Ca^{2+} solution (10^{-8} M buffered with EGTA) at the inner membrane surface (*data not shown*).

K_{CF} Channels

The calcium-dependent potassium channel (Komatsu et al., 1990) was readily discernable when the Ca^{2+} concentration was reduced to ~10^{-8} M (Fig. 5*A*). Between 1.8×10^{-3} and 10^{-6} M Ca^{2+}, K_{CF} channels generally exhibited an open probability greater than 0.5. On average, their open probability began to decrease near 10^{-7} M Ca^{2+} though this value varied from 5×10^{-7} to 5×10^{-8} M Ca^{2+} for different patches. In most patches there was a complete block at 10^{-8} M Ca^{2+}. The mean conductance at symmetrical 130 KCl for K_{CF} channels in patches excised from *in situ* preparations was 74 pS (Fig. 5*B*). The slope conductances at 0 mV in 130/65 and 130/2 KCl were 57 and 30 pS, respectively. From the reversal potential shift introduced by ion replacement, the potassium-to-sodium permeability

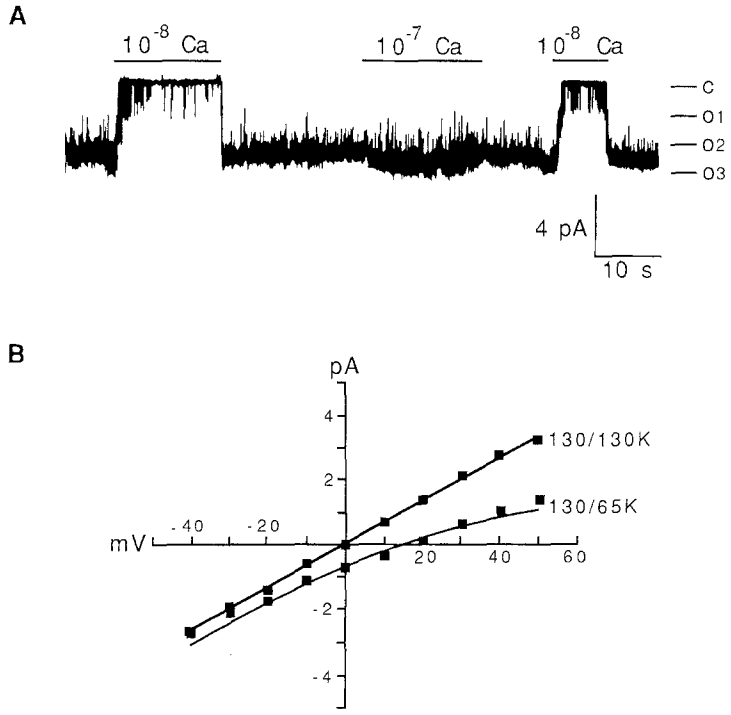

Fig. 5. (*A*) Effect of EGTA-buffered Ca^{2+} concentrations on an inside-out patch with at least three active I_{CF} channels recorded at 0 mV. Bath Ca^{2+} concentration was 1.8 mM. Low Ca^{2+} severely reduces the open probability of the channels in this patch. Data were filtered at 250 Hz. (*B*) *I-V* relationship of a K_{CF} channel at two different potassium concentrations. The slope conductance was taken from the linear regression of the data at 130/130 KCl to calculate the K^+ permeability of the open channel; $P_K = 1.3 \times 10^{-13}$ cm³/sec. Using this value, the solid curve was generated from Eq. (1) for the 130/65 KCl data. The potassium: sodium selectivity ratio was calculated from the reversal potential (V_{rev}) shift introduced by the bath solution change: $[K]_i$ from 130 to 65 mM; $[Na]_i$ from 0 to 65 mM. Estimated on the basis of the following equation: $V_{rev} = (RT/F) \ln [(P_{Na}[Na]_o + P_K[K]_o)/(P_{Na}[Na]_i + P_K[K]_i)]$ where V_{rev} is the reversal potential and P the permeability, the $P_K : P_{Na}$ ratio was approximately 50 : 1

ratio was calculated to be ~50 : 1 (*see* Fig. 5 legend). Two or more K_{CF} channels were frequently present in the same patch on 6 out of 13 occasions.

Internal TEA reduced the single-channel current amplitude of K_{CF} (Fig. 6*A*) but to a lesser extent than the other three channels. Half block was estimated at about 100 mM (Fig. 4, circles). Neither 1 mM 4-AP nor 100 μM quinidine applied internally lowered the open probability or single-channel current amplitude of K_{CF}, consistent with previous studies of macroscopic I_{CF} (Gho & Mallart, 1986; Singh & Wu, 1989).

Gho and Mallart (1986) have reported that Ba^{2+} reduces I_{CF} current. The present study, showed that the effect of internally applied Ba^{2+} on single-channel currents was to lower the P_o without altering the unit conductance (Fig. 6). The relationship of Ba^{2+} concentration to open probability can be seen in Fig. 7 (circles). The data points are fit to Eq. (4) with a K_d of about 320 μM:

$$P_o/P_{oc} = 1/(1 + [Ba^{2+}]/K_d) \quad (4)$$

where P_{oc} is the open probability when no Ba^{2+} was applied. The K_d for Ba^{2+} block was approximately 3 mM, more than an order of magnitude higher than for the other channels described herein. Analysis of open- and closed-time distributions yielded at least one open state and two closed states. The Ba^{2+} block manifested itself primarily on decreasing the open-time constant. Values for τ_o dropped from roughly 1000 to 10 msec at 10 mM Ba^{2+}. The closed times did not increase much (τ_{c1} = 2, 6 and 8 msec and τ_{c2} = 53, 87 and 80 msec for 0.1, 1.0 and 10 mM Ba^{2+}, respectively, *data not shown*) over this Ba^{2+} range (τ_{c1} and τ_{c2} for 0 mM Ba^{2+} were not determined due to the low number of channel closing events).

K_2 Channels

The K_2 channel is a small conductance (11 pS) channel (Fig. 8*A*), with a relatively high open probability that exhibits two open and two closed states (Table 1). There was no substantial voltage dependency on P_o in the ($n > 8$) channels observed. The K_2 channel was blocked by TEA (Fig. 8*B*). The effect of TEA on the K_2 channel was a reversible decrease in apparent open-channel conductance in a dose-dependent fashion (Figs. 4 and 8*B*). The half block for TEA was about the same as for the K_{ST} channel. Quinidine at 100 μM also reduced the current amplitude considerably (Fig. 8*C*) but did so to such an extent that determining the change in the P_o was very difficult.

Application of Ba^{2+} to the cytoplasmic surface of the membrane had no appreciable effect on current amplitude but caused a reversible decrease in P_o that was concentration dependent (Figs. 7 and 9). To discern what channel states were being affected by Ba^{2+}, a kinetic analysis was carried out. The K_2

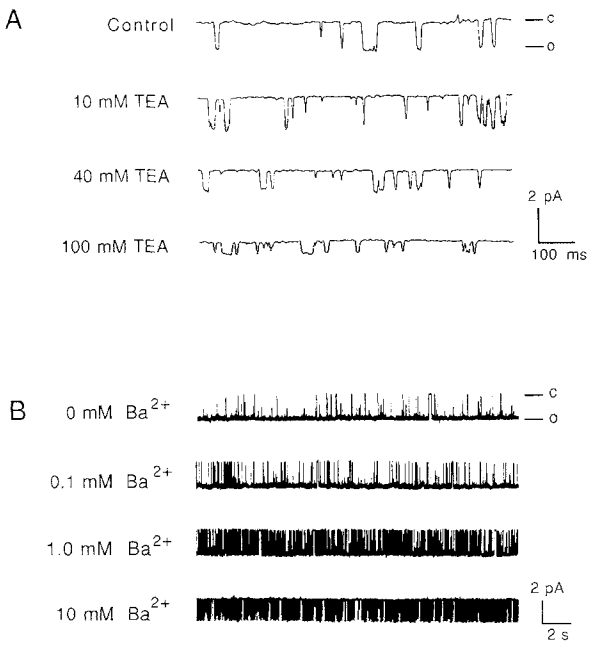

Fig. 6. Block of a single K_{CF} channel. (*A*) TEA reduces the single-channel current amplitude in a dose-dependent fashion. (*B*) A different patch exposed to three Ba^{2+} concentrations. Both recordings were at 0 mV and an internal Ca^{2+} concentration of 1.8 mM (130/2 KCl, pipette/bath). Data were filtered at 250 Hz

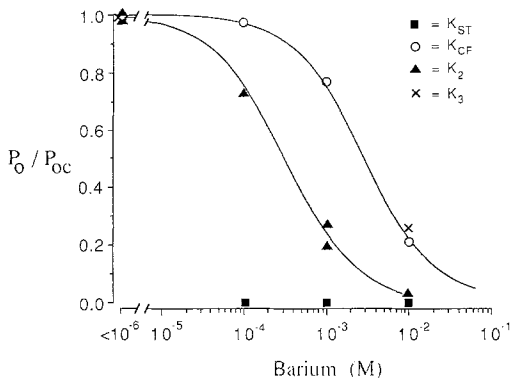

Fig. 7. Dose-response curves for Ba^{2+} from three channel types. K_{CF} = circles, K_2 = triangles; K_3 = cross (observed at only one concentration). All data collected at 130/2 KCl, 0 mV. Solid lines are calculated from Eq. (4) and are fit by eye for comparative purposes

channel, when active, typically had a high P_o (0.63 for the channel in Fig. 9), and was interrupted by brief closures. Table 1 summarizes values for the two open-time constants and the three closed-time constants for the frequency histograms (*not shown*) collected under various Ba^{2+} concentrations. Two patches, each containing only one K_2 channel, were analyzed in detail and produced very similar time constants. The open-time constants (τ_{o1} and τ_{o2}) decreased somewhat at the higher Ba^{2+} levels. The first and second closed-time constants remained stable over the concentration range used. A third time constant was needed to describe the closed-time distribution when Ba^{2+} was added and it increased substantially at higher concentrations. The large increase in its value may be misleading in that the lower concentrations of Ba^{2+} resulted in too few long closed states to provide an accurate fit for the third closed state. Apparently, the main effect of Ba^{2+} was to induce this long nonconducting state.

K_3 Channels

The K_3 channel was the rarest channel studied—only three examples were observed. Its identity as a potassium channel was based on the fact that potassium was the only ion with an inward driving force at 0 mV and that submillimolar TEA effected a block. The channel was easily distinguished by its high sensitivity to TEA (Fig. 10*A* and *B*); half block occurred at less than 1 mM (Fig. 4), a concentration well below the K_d's for the other channels, and no current was detectable at 10 mM. At 0 mV (130/2 KCl) its amplitude was 1.2 pA, larger than K_2 and smaller than K_{ST} and K_{CF}. The slope conductance was not determined. The open and closed histograms (*data not shown*) suggest two open and two closed states. As with the other channel types, the open probability of K_3 decreased with Ba^{2+} (Fig. 10*C*). The degree of block appeared to be in the same range as for the K_{CF} channels (Fig. 7). Both the TEA and Ba^{2+} effects were reversible. Quinidine at 100 μM had no apparent effect on the channel P_o or its conductance (*data not shown*).

Discussion

Data presented in this study of *Drosophila* were mostly taken from enzyme-treated, third instar larval muscles *in situ*. This preparation was chosen to approximate a normal in vivo situation and to take advantage of the preparation for which much macroscopic voltage-clamp data exist (Salkoff & Wyman, 1983; Singh & Wu, 1989). The K_{CF} channel described here, and the two voltage-dependent channels, A_1 and K_D, mentioned at the beginning of Results have macroscopic counterparts in larval muscles. In contrast, the K_{ST}, K_2 and K_3 channel types exhibit no clear correlation to any macroscopic current so far identified. Studies by Zagotta et al. (1988) and Komatsu et al. (1990) have characterized A_1 and K_D type single channels in some detail. In addition,

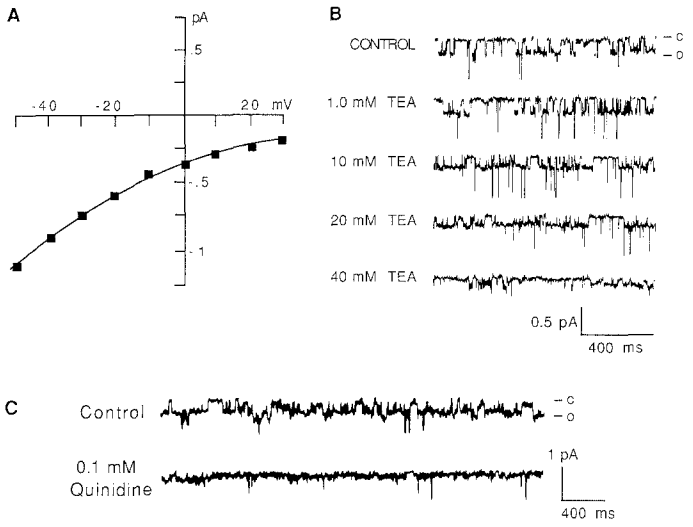

Fig. 8. Current-voltage relation and effect of fast blockers on K_2 channel. (*A*) The slope conductance (calculated between −40 and 20 mV, least squares fit) for this inside-out patch at 130/2 KCl (pipette/bath) was 11 pS. (*B*) Open-channel current amplitude at 0 mV was reduced by increasing concentrations of TEA. Two larger and different channels were intermittently active in these traces and at least one was reduced at the higher TEA concentrations. (*C*) Quinidine also reduced the single-channel current through the same patch as above. Data were filtered at 250 Hz

Table 1. Open probability and time constants of barium block for the K_1 channel

$[Ba^{2+}]$ (mM)	P_o	Open (msec)		Closed (msec)		
		τ_{o1}	τ_{o2}	τ_{c1}	τ_{c2}	τ_{c3}
0.0	0.63	1.6	11	1.0	6.8	n.o.
		(1.7)	(14)	(1.5)	(14)	(n.o.)
0.1	0.46	1.7	9.9	0.8	4.2	30
1.0	0.17	1.2	8.7	0.9	7.1	70
		(0.5)	(7.2)	(1.1)	(6.1)	(51)
10.0	0.02	0.9	3.3	0.9	5.5	623

A second channel from a different patch was analyzed at 0 and 1.0 mM Ba^{2+} (values in parentheses). Recordings were filtered at 1 kHz and sampled at 500 μsec/point.
n.o.—not observed.

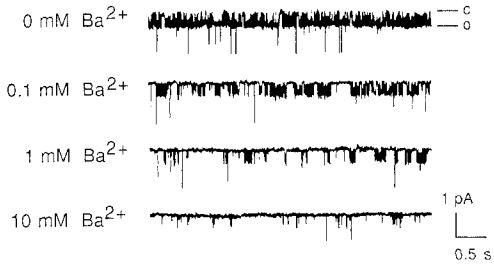

Fig. 9. The effect of Ba^{2+} on a K_2 channel at 0 mV. Data are from the same channel as in Fig. 8. Data were filtered at 250 Hz

some physiological details of K_{CF} (Komatsu et al., 1990) and K_{ST} (Zagotta et al., 1988) channels in muscle membrane have been described. Although examples of different channel types can be found in the various preparations, similarities among the better identified channel types described suggest that enzyme treatment, cell culture conditions, and developmental stage may not substantially affect most properties of these channels. It is interesting to note that K_{ST} and K_{CF} channels, examined in the present study with a nonstandard K^+ distribution across the membrane, still showed properties very similar (*see below*) to those in the other studies (Zagotta et al., 1988; Komatsu et al., 1990).

Pharmacological Block of *Drosophila* K^+ Channels

Our primary intent of the blocker studies was to enable a pharmacological separation of single-channel currents in a preparation amenable to genetic analysis. As more K^+ channels are discovered which have less distinct or overlapping kinetic properties, a pharmacological analysis will become a more important approach to channel identification (Wei et al., 1990). However, some general features of the blocking action of TEA and Ba^{2+} have emerged from the experiments on the four types of *Drosophila* K^+ channels. Ba^{2+} always acted as a slow channel blocker by reducing P_o, and TEA behaved as a very fast channel blocker causing a reduction in the apparent channel conductance without affecting P_o appreciably (*see* summary in Table 2).

Depending on the cell type, TEA can block K^+ channels from inside, outside, or from both sides of the membrane (Armstrong & Binstock, 1965; *see also* review by Stanfield, 1983). In this preparation, TEA was applied only to the cytoplasmic surface of the inside-out patch membrane. The effect of TEA in many previous reports has been to lower the ap-

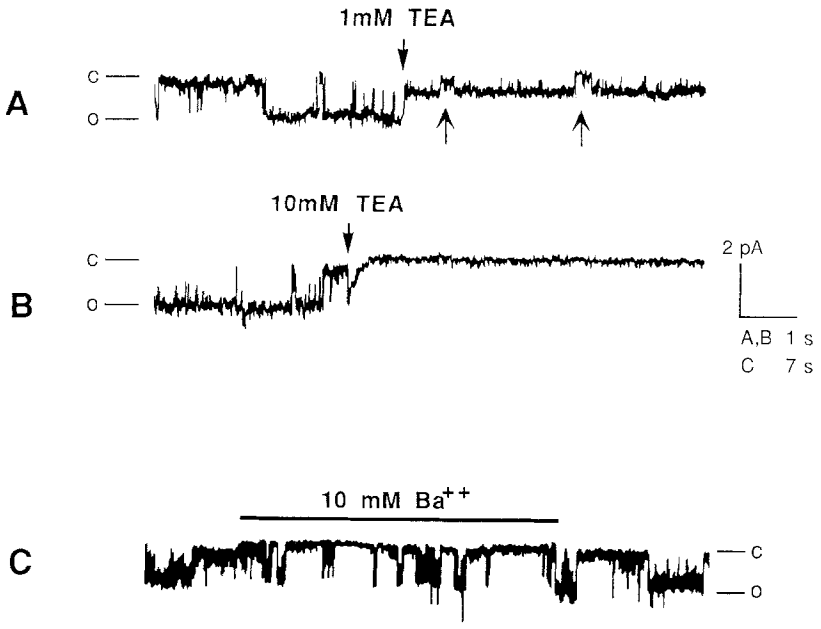

Fig. 10. Effect of TEA and Ba^{2+} on a K_3 channel at 0 mV. (*A* and *B*) The upper trace shows normal channel open events decreasing in amplitude upon application of 1 mM TEA (arrows at brief closings). At 10 mM TEA, the channel appears completely blocked. Curvilinear part of trace at onset of 10 mM TEA demonstrates the lowered single-channel current amplitude as the TEA concentration increases during the 500-msec period. (*C*) Ba^{2+} lowers the open probability of the K_3 channel. A slight dc shift occurred during Ba^{2+} application

Table 2. The effect of blocking agents on the four potassium channel classes

Channel type	TEA		Ba^{2+}		Quinidine	
	Action	K_d (mM)	Action	K_d (mM)	Action	K_d (mM)
K_{ST}	$\downarrow g$	~35	$\downarrow P_o$	<0.01	ND	ND
K_{CF}	$\downarrow g$	~100	$\downarrow P_o$	~3.0	None	—
K_2	$\downarrow g$	~25	$\downarrow P_o$	~0.3	$\downarrow g$	<0.1
K_3	$\downarrow g$	<1	$\downarrow P_o$	ND	None	—

g: single-channel conductance; P_o: open probability; ND: not determined.

parent single-channel conductance by virtue of blocking and unblocking very rapidly, suggesting that TEA rapidly bound and unbound to its binding site in the channel's pore (Wong & Adler, 1986; Shuster & Siegelbaum, 1987). In most cases, there is only a weak voltage dependence to this block (Inoue et al., 1986; Shuster & Siegelbaum, 1987; but *see also* Blatz & Magleby, 1984), indicating a binding site that is not very deep within the pore. The reduced current amplitude in the presence of TEA prevented detailed kinetic analyses of the blocking behavior in this study. However, results from all four channel types are consistent with the above view.

Ba^{2+} is a very potent K^+ single-channel blocker often operating in the micromolar range (*see* reviews by Rudy, 1988; Castle et al., 1989). It is considered to be a slow channel blocker (Hille, 1984) because its effect is usually limited to reducing the open probability; conductance values change little or not at all (Brown, Loo & Wright, 1988; Sheppard, Giraldez & Sepulveda, 1988). Unlike TEA, Ba^{2+} applied to the cytoplasmic surface typically induces a long-lived nonconducting state in Ca^{2+}-activated channels that can last for hundreds of milliseconds to seconds. It is thought that these closed intervals represent the binding of a single Ba^{2+} molecule to the open channel which it blocks. Because of a strong voltage dependency to the block, the binding site is thought to lie well within the pore (Vergara & Latorre, 1983; Miller, Latorre & Reisin, 1987). Results of applying Ba^{2+} to the cytoplasmic surface of larval muscle membrane are consistent with the above. Ba^{2+} substantially reduced the open-state probability but not the single-channel amplitude. In both the K_2 and K_{CF} channels, the open-time constants decreased, although to very different degrees. Their decrease is consistent with the Ba^{2+} ion entering the pore while it is open and blocking it, thereby reducing the time spent conducting. The K_2 channel showed a new

Ba^{2+}-induced closed state, whereas the K_{CF} channel had somewhat longer closed times. The apparent lack of a new closed state in K_{CF} channels could be explained by the possibility that the blocked state had a similar time constant to one of the existing closed states and could not be readily distinguished.

Quinidine applied to the internal surface blocked K_2 channels but not K_{CF} or K_3 channels. It may act as a fast channel blocker since its major effect was to reduce single-channel current amplitude.

K_{ST} Channels

Our results indicated that *Drosophila* K_{ST} channels were not voltage gated and that Ca^{2+} at the inner membrane surface was not necessary for opening. It is unlikely that second messengers are necessary for channel opening (though they may modulate it) because no cytoplasmic constituents were available to the inside-out membrane patches used in this study. These factors strongly imply that gating is a direct consequence of stretching the membrane.

Zagotta and colleagues (1988) reported that 10 mM TEA applied to either the internal or external membrane surface of *Drosophila* cultured myocytes caused a reduction in single-channel currents to about 50% of normal. In the present study with larval channels, internally applied 10 mM TEA led to a 30% reduction in slope conductance; the K_d was about 35 mM (*see* Fig. 4). Ba^{2+} was very effective at micromolar concentrations in reducing the open probability of this channel. The K_{ST} channels in myocytes appear to be very similar to those reported here on the basis of general kinetics, lack of voltage dependence, large slope conductance, and high selectivity for K^+ over Na^+. Zargotta et al. (1988) recorded from larval muscle fibers in the cell-attached mode and stated that K_{ST} channels were indistinguishable from their myocyte counterparts. Together, these data indicate that the mode of patch clamping and the different saline compositions do not alter the basic properties of the K_{ST} channel. The role of K_{ST} channels in the larval body-wall muscle of *Drosophila* and of stretch-activated channels in general is still open to question (*see* review by Morris, 1990).

K_{CF} Channels

Two types of macroscopic Ca^{2+}-activated K^+ channels have been described in the body-wall muscles of larvae and flight muscles (Elkins et al., 1986; Gho & Mallart, 1986; Wei & Salkoff, 1986; Singh & Wu, 1989). The transient, rapidly inactivating current has been termed I_{Acd}, I_{Ac}, and I_{CF}, whereas the slowly activating channel has been termed I_C, I_{Kc}, and I_{CS}. We have chosen to use I_{CF} and I_{CS} for the fast and slow Ca^{2+}-activated potassium currents, respectively (*see* Singh & Wu, 1989) and K_{CF} and K_{CS} for their single-channel counterparts. Single-channel currents corresponding to I_{CF} have been characterized in the larval muscle vesicle preparation (Komatsu et al., 1990). This type of Ca^{2+}-activated potassium channel is absent in inside-out patches of muscle vesicles in the *slowpoke* mutant (Komatsu et al., 1990). The *slowpoke* mutation deletes only the macroscopic I_{CF} current, not I_{CS} in adult flight (Elkins et al., 1986) and larval (Singh & Wu, 1989) muscles.

The Ca^{2+}-activated potassium channels described in this paper were recorded exclusively from inside-out patches and showed conductance values, selectivity of K^+ over Na^+, and a range of activation consistent with those previously described for the channel mediating I_{CF} (Komatsu et al., 1990). The K_{CF} channel was blocked by internally applied TEA ($K_d \approx 100$ mM) and Ba^{2+} ($K_d \approx 3.0$ mM). Previously these agents (>2.7 mM Ba^{2+} and 20 mM TEA) have blocked I_{CF} when applied extracellularly in voltage-clamp studies (Gho & Mallart, 1986). The K_{CF} channel of *Drosophila* demonstrates an unusual pharmacology in that most Ca^{2+}-activated channels in other species are blocked by micromolar internal Ba^{2+} and low millimolar internal TEA (Rudy, 1988; Castle et al., 1989). However, K_{CF} is blocked by charybdotoxin (Elkins et al., 1986) in common with other Ca^{2+}-activated channels.

K_2 Channels

The K_2 channel possessed a similar conductance and kinetics with the K_D channel described in embryonic cultured myocytes by Zagotta et al. (1988), in larval muscle vesicles by Komatsu et al. (1990), and in larval neurons (Solc & Aldrich, 1988). The decrease in conductance upon application of internally applied TEA was also similar to the cultured neuronal K_D channel described by Yamamoto and Suzuki (1989). Its sensitivity to millimolar TEA and micromolar quinidine were consistent with the K_D channel as seen in larval muscle voltage-clamp studies (Wu & Haugland, 1985; Gho & Mallart, 1986; Singh & Wu, 1989). However, the K_2 channel departed from this similarity in that it was seen at a steady holding potential of 0 mV and could be active for tens of seconds to minutes. It was also active at −50 mV, far below the activation threshold of K_D channels (Wu & Haugland, 1985). Whether K_2 is a K_D channel somehow modified by enzyme treatment or some other manipulation, or whether it is a separate entity is unknown.

Conclusion

Using TEA and Ba^{2+}, the four channels described in this report can be readily separated at the single-channel level (*cf.* Figs. 4 and 8). The K_3 channel was uniquely sensitive to low TEA concentrations and the K_{ST} channel was two to three orders of magnitude more sensitive to Ba^{2+} than the other channels (Fig. 7) and was the only channel sensitive to stretch. The K_{CF} channel was the least affected by TEA but could be closed by reducing the Ca^{2+} concentration to $\sim 10^{-8}$ M. The K_2 channel was similar to the K_{ST} channel with regard to TEA half block but was sensitive to Ba^{2+} at a concentration intermediate to the other three channels.

Mutations which specifically affect only one channel type (e.g., *Shaker* and *slowpoke*) present an excellent opportunity to identify single-channel currents and their macroscopic counterparts (Solc et al., 1987; Komatsu et al., 1990). However, the drawback of using a single criterion such as mutational or drug effect is that the analysis is usually statistical, and based upon many repeated observations—a slow process with the *Drosophila* preparations to date. The use of pharmacology allied with mutations could substantially improve the speed and reliability with which channels are identified. The contribution of a channel type to the function of the excitable membrane into which it is embedded can then be more fruitfully explored.

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