# Conduction Properties of the M-Channel in Rat Sympathetic Neurons

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ABSTRACT We have investigated the conduction properties of the M-channel in rat superior cervical ganglion neurons. Reversal potentials measured under bi-ionic conditions yielded a permeation sequence of Tl > K > Rb > Cs > $NH_4$ > Na. Slope conductances gave a conductance sequence of K > Tl > $NH_4$ > Rb > Cs. M-current was shown to exhibit a number of features atypical of potassium channels. First, the conduction of monovalent cations relative to K was very low. Second, the nature of the permeant ion did not affect the deactivation kinetics. Third, M-current did not exhibit anomalous mole-fraction behavior, a property suggestive of a multi-ion pore. Finally, external Ba, which is a blocker of M-current, showed a preferential block of outward current and had much less effect on inward current. The permeability sequence of the M-channel is very similar to other K-selective channels, implying a high degree of conservation in the selectivity filter. However, other conduction properties suggest that the pore structure outside of the selectivity filter is very different from previously cloned potassium channels.

## INTRODUCTION

The M-current is a voltage-dependent potassium current that possesses the unique characteristic of sustained activation. It was described first in bullfrog sympathetic ganglion cells (Brown and Adams, 1980) and has since been found in many areas of the central and peripheral nervous system (for review, see Brown, 1988). It is activated in the subthreshold range for action potential initiation and contributes most of the sustained membrane current in this range. Suppression of M-current by neurotransmitters such as acetylcholine results in membrane depolarization and increased input resistance, making the cell more likely to fire action potentials. Thus, modulation of the M-current is a potent pathway for regulating neuronal excitability (Adams et al., 1982).

In addition to sustained activation, the M-current in rat sympathetic neurons has several features that distinguish it from other potassium currents. The activation range ($V_{1/2}$ = −45 mV) (Constanti and Brown, 1981) is hyperpolarized with respect to the delayed rectifier ($V_{1/2}$ = −6 mV) (Belluzzi et al., 1985b) and the fast-inactivating A-current ($V_{1/2}$ = −30 mV) (Belluzzi et al., 1985a). The activation/deactivation kinetics are substantially slower than other voltage-activated potassium currents. Deactivation rate constants are ~10 times slower at equivalent potentials (Owen et al., 1990). Finally, the pharmacology of the M-current seems unique. It is not sensitive to the classical potassium channel blockers tetraethylammonium or 4-aminopyridine (Robbins et al., 1992), but it is highly sensitive to external barium (Ba) ions ($K_i$ = 300 μM) (Stansfeld et al., 1993). Thus, although the M-current is classified as a voltage-gated potassium current similar to the delayed rectifier and rapidly inactivating A-current, it has properties that allow it to be distinguished from these currents.

Although the kinetics and pharmacology of the M-current have been well described, the conduction properties of the M-channel have been examined only recently in bullfrog sympathetic neurons (Block and Jones, 1995). In this paper we describe the permeation sequence and other conduction properties of the M-channel in rat superior cervical ganglion cells and examine whether the M-channel is a multi-ion pore. We have found that although the selectivity of the M-channel for monovalent cations is similar to other potassium channels, other pore properties, in particular the lack of anomalous mole-fraction behavior and the effect of external Ba ions, are quite unusual.

## MATERIALS AND METHODS

### Tissue culture

Superior cervical ganglion neurons were cultured from 18- to 21-day-old Sprague-Dawley rats as described elsewhere (Beech et al., 1991). Cells were used for recording after 4–7 days in culture. Dishes were treated with 5-fluoro-2′-deoxyuridine (1 mM) and uridine (2.5 mM) for 24 h after 4 days to inhibit glial proliferation.

### Electrophysiological recording

Isolated neurons were voltage-clamped in the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA). Fire-polished electrodes (2–4 MΩ), pulled from borosilicate glass KG-33 (Friedrich and Dimmock, Millville, NJ) and coated in "Sticky Wax" (Kerr, Romulus, MI), contained (in mM): KAcetate (130), KCl (20), $MgCl_2$ (1.5), HEPES (10), EGTA (0.2), $CaCl_2$ (0.02; calculated free $[Ca^{2+}]$ = 80 nM), $Na_2ATP$ (1.5), pH 6.7. An acidic intracellular pH reduces rundown of M-current (Brown et al., 1989). For anomalous mole-fraction experiments, the nystatin perforated-patch technique was used (Horn

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and Marty, 1988). Nystatin was dissolved in dimethyl sulfoxide (75 mg/ml), sonicated for 30 s, and diluted 1:250 in intracellular solution. No difference in M-current kinetics or pharmacology was observed when recorded using the whole-cell or nystatin perforated-patch configuration. Recording began when the series resistance remaining uncompensated reached <12 MΩ. Uncompensated series resistance under whole-cell recording conditions was 2–5 MΩ. Series resistance compensation was typically 80–90%. Cells were superfused by gravity (12 ml/min) with a HEPES-buffered external solution at room temperature.

To determine the permeation sequence, the external solution contained (in mM): *N*-methyl-D-glucamine (NMDG) (135), aspartic acid (135), X-Acetate (15), MgAcetate (5), CaAcetate (0.1), CdAcetate (0.2), D-glucose (30), HEPES (10), pH 7.4 (adjusted using acetic acid), where X is the test cation. Monovalent cations tested were potassium (K), rubidium (Rb), thallium (Tl), ammonium ($NH_4$), and cesium (Cs). To determine whether sodium (Na) and NMDG were permeant through the M-channel, we used 150 mM NaAcetate or NMDG. Activity coefficients relative to K were Tl (0.94), Rb (1.0), $NH_4$ (0.93), Na (0.99), Cs (1.0) (Robinson and Stokes, 1959). Acetate salts were used to avoid the problem of TlCl precipitation. As a reference bath ground, we used a saturated KAcetate agar bridge. Junction potentials associated with solution changes varied by <2 mV and were not adjusted for.

To resolve whether the M-current exhibited anomalous mole-fraction behavior, the external solution contained a total of 150 mM K, Rb, or Tl or a mixture of two cations. Otherwise, the solution was the same as above. Deactivation currents were measured after a voltage step from −40 to −70 mV and plotted as conductance. Reversal potentials (Erevs) were determined from instantaneous currents (measured upon voltage steps from −40 mV), which were sensitive to oxotremorine M (10 μM), a muscarinic receptor agonist (Bebbington et al., 1966).

The relative conductance of monovalent cations to K was determined by measurement of the slope of instantaneous inward currents in the presence of 15 mM external cation. M-current was activated by a depolarizing step from −70 to −30 mV (1 s), and the voltage was steppedhyperpolarized to different membrane potentials. The instantaneous current was determined by extrapolation to $t = 0$ of an exponential fit to the current decay (10–150 ms). Leak current was corrected by subtracting current responses to negative voltage pulses without the preceding depolarization.

It was important that M-current be recorded in isolation from other voltage-dependent currents, in particular the delayed rectifier and fast-inactivating A-current. The delayed rectifier was blocked with 10 mM tetraethylammonium (Cl salt), except when Tl was the external cation ($K_i$ = 1 mM; $n$ = 8). 4-aminopyridine (10 mM) was included in all experiments to reduce A-current (Belluzzi et al., 1985a) and delayed rectifier ($K_i$ = 0.3 mM; $n$ = 3). In addition, we used a deactivating protocol to visualize M-current. The tail current recorded in these conditions was sensitive to oxotremorine M, with the oxotremorine M-sensitive component displaying the same deactivation kinetics as the nonsubtracted tail current (control: 165 ± 26 ms; oxotremorine M: 178 ± 30 ms; mean ± SEM, $n$ = 3). All measurements were made in the absence of oxotremorine M unless stated otherwise.

For kinetic analysis, M-current relaxations were fit with a single exponential from 100 to 1000 ms after the voltage step. A single exponential best described the time course of deactivation under these conditions.

All values are expressed as mean ± SEM. The significance of the results was compared using a paired Student's $t$-test and considered to be statistically significant if $p < 0.1$.

## Chemicals

Standard laboratory chemicals, including nystatin, were purchased from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO). Oxotremorine M was purchased from Research Biochemicals International (Natick, MA).

# RESULTS

## Permeability of M-channels to monovalent cations

To determine the permeability of the M-channel to monovalent cations, shifts in Erev were measured under bi-ionic conditions when extracellular K (15 mM) was replaced with a test cation. The cell membrane potential was held at −30 mV and steppedhyperpolarized for 1 s to reveal the slow inward relaxation as the M-current deactivated. Fig. 1 compares the M-current recorded in K and several test cations. There was a large negative shift in Erev when K was replaced with $NH_4$. Smaller changes in Erev were observed with Tl and Rb; this is more clearly illustrated in the current-voltage relationships plotted in Fig. 2. The instantaneous current, taken at the beginning of the jump, and the steady-state current, taken after deactivation of the M-current, are shown. Erev is the intersection of the two lines. Mean Erev of five ions tested under these conditions was K = −46 ± 1.1 mV ($n$ = 9); Tl = −36 ± 0.8 mV ($n$ = 6); Rb = −52 ± 1.7 mV ($n$ = 6); Cs = −87 ± 2.0 mV ($n$ = 4); and $NH_4$ = −96 ± 1.9 mV ($n$ = 5).

Tl, K, Rb, $NH_4$, and Cs were thus all permeant through the M-channel. We also examined whether Na or NMDG could permeate the M-channel. Cells were bathed in 150 mM external cation, and the M-current was deactivated by membrane hyperpolarization from −30 mV (Fig. 3). In high Na solution, M-current deactivation reversed negative to −100 mV. Thus, Na can permeate the M-channel, albeit very poorly. Mean Erev in these high ionic conditions was K = +10 mV ($n$ = 5); Na = −115 ± 4.1 mV ($n$ = 5); and Cs = −44 ± 2.4 mV ($n$ = 5). Only outward currents were observed in NMDG; no inward current was measured, even at membrane potentials negative to −150 mV ($n$ = 3).

The selectivity sequence of the M-channel for monovalent cations is summarized in Table 1. The permeability

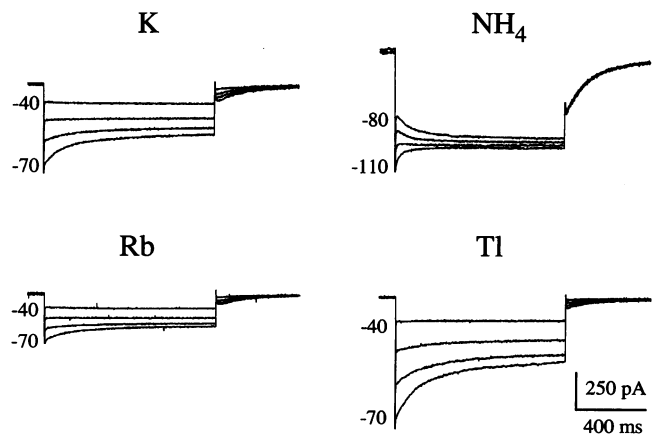


FIGURE 1 M-current recorded in one cell with different external cations. The cell was whole-cell voltage-clamped at −30 mV. M-current was revealed by hyperpolarizing voltage steps (1 s) in 10-mV increments. External permeant cation concentration was 15 mM. The current recorded in Tl was unusually large in this cell. In most cells, Tl conductance was lower than K conductance (see text).

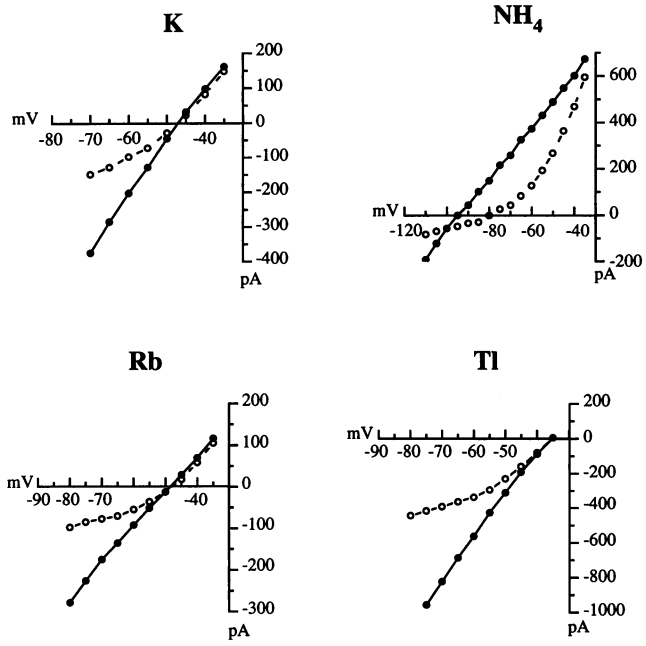


FIGURE 2 Current-voltage relationships for the cell illustrated in Fig. 1 with each permeating ion. Instantaneous current (●) was measured at the beginning of the voltage step (at time = 100 ms) and steady-state current (○) was measured at the end of the voltage step. Erev is the intersection of the two curves. A second intersection was approached at the holding potential and represents a crossover between deactivation and activation of M-current. Erev in this cell was K = −47 mV; $NH_4$ = −100 mV; Rb = −50 mV; and Tl = −35 mV.

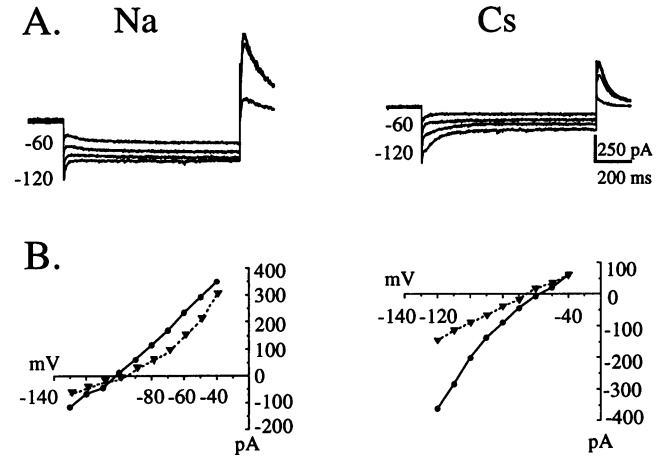


FIGURE 3 M-current deactivation relaxation recorded in 150 mM external Na and Cs. (*A*) The neuron was whole-cell voltage-clamped at −30 mV, and M-current was deactivated by 10-mV incremental hyperpolarizing voltage steps (1 s duration). Upon repolarization, the transient A-current was activated because of removal of inactivation by the negative voltage step. M-current relaxations in 150 mM Na consisted of deactivation of sustained outward current, with current reversing at −107 mV. Replacement of Na with Cs caused a shift of the Erev to ~−40 mV. Hyperpolarizing voltage steps revealed M-current deactivation of inward current carried by Cs. (*B*) Current-voltage relationship for the cell illustrated in *A*. Instantaneous current, measured at time = 100 ms (*solid line*), and steady-state current, measured at the end of the jump (*dashed line*), are plotted.

relative to K ($P_X/P_K$) of an external monovalent cation X is defined by the relationship obtained from the Nernst-Planck electrodiffusion equation

$$P_x/P_K = ([K]_o/[X]_o)e^{-(F\Delta Erev/RT)}$$

where $\Delta Erev$ is the shift in Erev, and $F$, $R$, and $T$ have their usual thermodynamic meanings (Goldman, 1943; Hodgkin and Katz, 1949). Relative ionic permeability in 15 mM external cation was Tl (1.5) > K (1.0) > Rb (0.8) > Cs (0.2) > $NH_4$ (0.1). The M-channel showed a slight permeability to Na and was essentially impermeable to NMDG. In 150 mM external cation, the relative permeability was K (1.0) > Cs (0.13) > Na (0.008) > NMDG.

Relative conductance is another measure of ion selectivity which reflects the ability of an ion to traverse the pore. Slope conductance of the M-channel was estimated by measurements of instantaneous currents evoked by hyperpolarizing voltage steps from −30 mV, thus reflecting the conductance through channels active at this potential (Fig. 4). The selectivity sequence based on relative conductance is different from that based on shifts in Erev (Table 1). Mean conductances in 15 mM external cation were K = 10.8 ± 1.4 nS ($n$ = 7); Tl = 2.9 ± 0.7 nS ($n$ = 5); $NH_4$ = 1.9 ± 0.2 nS ($n$ = 4); Rb = 1.2 ± 0.2 nS ($n$ = 5); and Cs = 0.8 nS ($n$ = 2).

## Is the M-channel a multi-ion pore?

Calcium and potassium channels display interactions between permeant ions such as channel block and anomalous mole-fraction behavior that suggest that they are multi-ion pores (Hille and Schwarz, 1978). Block of macroscopic current by addition of a low concentration of another permeant ion implies that a channel can be occupied by more than one ion at the same time (Almers and McCleskey, 1984; Hess and Tsien, 1984). In three cells, addition of 10 mM Rb inhibited M-current (recorded in 150 mM K) by 48 ± 4.2%. M-current was recorded in K, Rb, and varying mixtures of the two cations, maintaining a total external cation concentration of 150 mM, to examine whether the M-channel can display anoma-

**TABLE 1. Permeability and conductance series of macroscopic M-current**

| Ion (X) | $P_X/P_K$ | $g_X/g_K$ |
|---|---|---|
| Tl | 1.5 | 0.28 |
| K | 1.0 | 1.00 |
| Rb | 0.8 | 0.11 |
| $NH_4$ | 0.10 | 0.23 |
| Cs | 0.20 | 0.10 |

Reversal potentials were measured under bi-ionic conditions, with 15 mM and 150 mM monovalent cation in the external and internal solutions, respectively. The permeability of each ion X relative to K was calculated according to the equation described in the text. Relative conductance was determined from the slope of the instantaneous current-voltage relationship for M-current (see Materials and Methods).

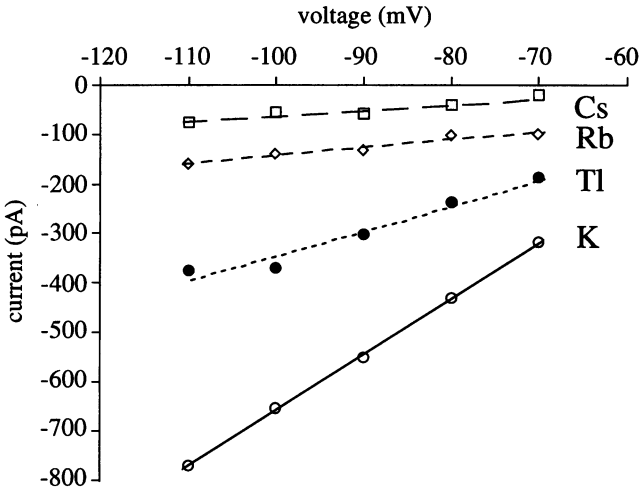


FIGURE 4 Instantaneous current-voltage relationship for M-current in one cell in different external cations. The cell was whole-cell voltage-clamped at −70 mV. M-current was activated by a depolarizing voltage step to −30 mV for 1 s. From this potential the membrane voltage was stepped negative, and the instantaneous current was plotted against voltage. Leak current was corrected by subtracting current responses to the same voltage pulses from −70 mV.

lous mole-fraction behavior. Conductance of potassium channels saturates at high ionic concentrations (>300 mM) (Eisenman et al., 1986; Heginbotham and MacKinnon, 1993; Lu and MacKinnon, 1994). The probability of observing an anomalous mole-fraction effect should be increased by using a permeant ion concentration of 150 mM, which would increase the likelihood of two ions residing simultaneously in the pore.

Deactivation tail currents in different mole-fractions of Rb are shown in Fig. 5 *A*. The membrane potential was held at −30 mV and stepped to −70 mV to visualize M-current relaxations. The deactivation tail amplitude decreased with increasing concentrations of Rb. The relationship between the conductance and mole fraction is shown in Fig. 5 *B*. Titration of K with Rb caused the relative conductance to decrease, but it did not go through a conductance minimum as expected for anomalous mole-fraction behavior. The change in Erev observed upon replacement of K with Rb also did not exhibit a local minimum. In two cells, M-current was recorded using different mole-fractions of K and Tl (150 mM), and in five cells, M-current was recorded with Tl and Rb (15 mM). No anomalous mole-fraction effect was observed in these conditions. In addition, the oxotremorine M-sensitive component of current, measured in either Rb or Tl, did not exhibit anomalous mole-fraction behavior (data not shown).

## Deactivation kinetics

Both native and cloned potassium currents exhibit a slowing of deactivation when Rb is used instead of K as the permeant ion (Swenson and Armstrong, 1981; Silver et al., 1994; Zagotta et al., 1994). The time course of M-current

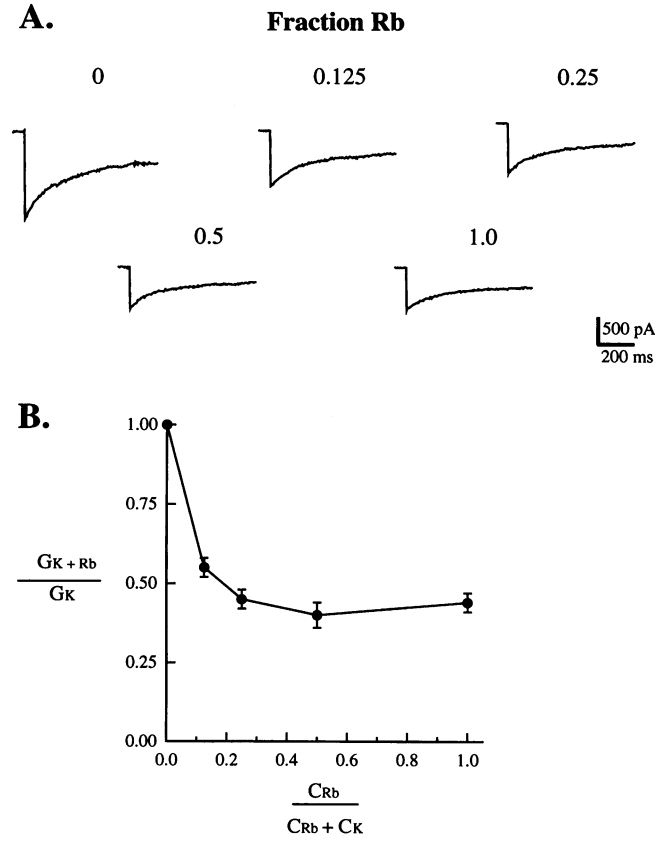


FIGURE 5 M-current deactivation recorded in different mole-fractions of K and Rb. (*A*) Deactivation tail currents evoked by a 1-s jump from −40 to −70 mV in different mixtures of K and Rb (150 mM total). As Rb was titrated for K, the M-current deactivation relaxation decreased progressively in amplitude. (*B*) Mean relative conductance for six cells plotted as a function of mole-fraction of Rb. There was no significant difference between the conductance in 0.5 Rb and in 1.0 Rb ($p > 0.1$).

deactivation induced by hyperpolarization was unaffected by different external cations (15 mM) (Table 2). There was also no significant difference in the deactivation time constant (stepping from −40 to −70 mV for 1 s) recorded in the presence of 150 mM K (168 ± 33 ms; $n = 7$) or Rb (152

**TABLE 2. Effect of monovalent cations on M-current deactivation kinetics**

| | τ (ms) | | |
|---|---|---|---|
| Ion (X) | −50 mV | −80 mV | *n* |
| K | 225 ± 18 | 156 ± 18 | 5 |
| Rb | | 147 ± 6 | 3 |
| Tl | 214 ± 34 | 171 ± 15 | 4, 3 |
| $NH_4$ | 187 ± 18 | 127 ± 17 | 4 |
| Na | 213 ± 6 | 125 ± 7 | 4 |
| Cs | 216 ± 23 | | 3 |

Deactivation time constants (τ) for M-current relaxations in different external cations (15 mM). Currents were evoked by stepping hyperpolarized for 1 s from a holding potential of −30 mV to either −50 or −80 mV. M-current relaxations were too small to be fit in Rb at −50 mV and Cs at −80 mV because of the proximity to the reversal potential. M-current relaxations were best fit by a single exponential (see Materials and Methods).

± 25 ms; $n = 7$; $p > 0.1$). The voltage-dependence of the deactivation time constant was very similar when recorded in K, Tl, Rb, and $NH_4$ (15 mM). The time constant decreased *e*-fold for a 60–80 mV hyperpolarization, as described previously for M-channels in rat superior cervical ganglion (Owen et al., 1990). When Na was the only external permeant cation, the voltage-dependence was increased to *e*-fold in 40 mV. Thus, with the exception of Na, the permeant ion seems to have little effect on the gating properties of the M-channel.

### Effect of external Ba on M-current

M-current is sensitive to block by low concentrations of external Ba (Constanti et al., 1981). However, M-current relaxations recorded in 15 mM external cation were differentially sensitive to external Ba, with more block being observed for outward current than for inward current (Fig. 6). Ba (1 mM) inhibited inward current by ~15%, whereas outward current was inhibited by 50%. In contrast, oxotremorine M (10 μM) inhibited both inward and outward M-current by the same amount (~50%). A similar effect was observed in the presence of 150 mM external K (data not shown). Ba block did not appear to be voltage-sensitive within the range studied. Ba did not significantly alter the kinetics of channel gating. For example, the mean time constant of deactivation at −80 mV (evoked by hyperpolarization from −30 mV) was 106 ± 13 ms in control and 84 ± 8 ms in 1 mM Ba ($p > 0.1$; $n = 5$). The time constant of activation (evoked by depolarization from −50 to −20 mV) was 129 ± 12 ms in control conditions and 136 ± 19 in 1 mM Ba ($p > 0.1$; $n = 4$).

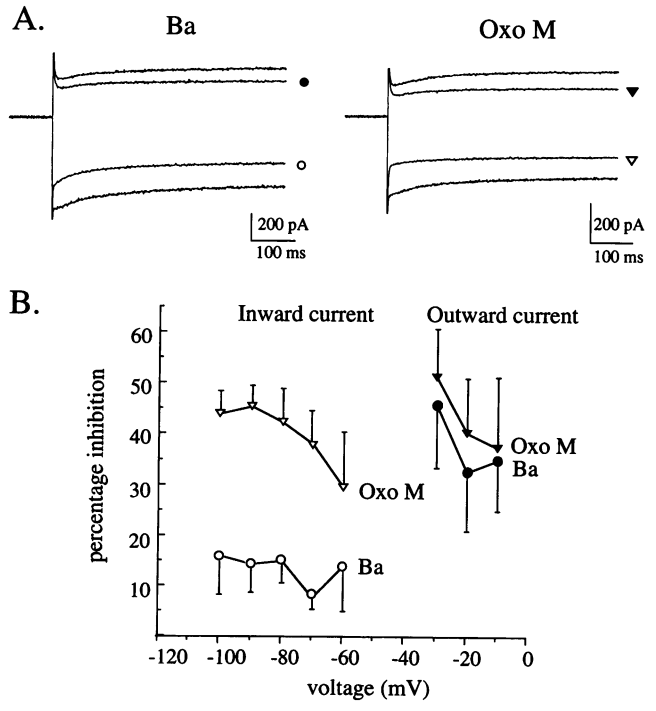


FIGURE 6 Suppression of M-current by Ba and oxotremorine M. (*A*) M-current deactivation was evoked by a 1-s voltage step to −70 from −30 mV. Activation was elicited by a 30-mV depolarization from −50 mV. Application of 1 mM external Ba (*left*, ○ inward current, ● outward current) or 10 μM oxotremorine M (*right*, ▽ inward current, ▼ outward current) illustrate that Ba suppressed more outward than inward current, whereas oxotremorine M had equal effects on both activation and deactivation relaxations. Ba principally reduced the instantaneous current with little effect on the time-dependent relaxations evoked by a negative voltage step. Holding currents have been zeroed for clarity. (*B*) Effect of external Ba and oxotremorine M on M-current relaxations evoked by the protocol shown in *A*; $n = 4$ or 5 cells for each point.

## DISCUSSION

### Selectivity of the M-channel for monovalent cations

The M-channel exhibits a high selectivity for K over other cations. The relative conductance of ions through the channel was K > Tl > $NH_4$ > Rb > Cs. This sequence was different from the permeability sequence based on shifts in Erev (Tl > K > Rb > Cs > $NH_4$ > Na). This phenomenon has been observed for other potassium channels (Heginbotham and MacKinnon, 1993; Park, 1994). It is interesting to note, however, that all permeant ions were much less conductive than K through the M-channel (see Table 1). For example, Tl, which was more permeant than K (based on shifts in Erev), had a relative conductance of only 0.28. Permeability can be considered a measure of how easily an ion enters the channel pore and conductance an indication of how rapidly the pore is traversed (Hille, 1975). We speculate that the M-channel pore possesses a large and nonselective outer vestibule like other K channels. Only K is able to permeate quickly, however, whereas other ions are retarded.

Potassium channels share a similarity in their selectivity for monovalent cations (Tl > K > Rb > $NH_4$); however, some differences do exist. For example, Cs and Na are not permeant through large Ca-activated K channels in skeletal muscle (Blatz and Magleby, 1984), delayed rectifier in myelinated nerve (Hille, 1973) and lymphocytes (Shapiro and DeCoursey, 1991), and inward rectifier in starfish egg (Hagiwara and Takahashi, 1974). In contrast, Cs but not Na is permeant through small Ca-activated K channels in chromaffin cells (Park, 1994). The M-channel was permeable to both Na and Cs; in addition, it showed similar permeability of $NH_4$ and Cs.

The molecular determinants of ionic selectivity have been established using cloned potassium channels. The pore region of all cloned potassium channels is highly conserved, with the sequence (T-X-G-Y-G) proposed to be the K selectivity filter (Heginbotham et al., 1994). The *Shaker* potassium channel (Heginbotham and MacKinnon, 1993) and the renal potassium channel ROMK2 (Chepilko et al., 1995) belong to distinct molecular families with very different primary structure, gating mechanisms, and function. These two channels show high homology in the pore selectivity filter, however, and have identical permeation se-

quences (K > Rb > $NH_4$ > Cs). Given the similarity in the permeation sequence of the M-channel with other potassium channels, it seems likely that it will also share this molecular motif in the pore region.

### Anomalous mole-fraction effect

An anomalous mole-fraction effect was not observed for the M-current. This was unexpected because all potassium channels in which it has been examined exhibit anomalous mole-fraction behavior (Hagiwara and Takahashi, 1974; Eisenman et al., 1986; Wagoner and Oxford, 1987; Heginbotham and MacKinnon, 1993; Park, 1994; Korn and Ikeda, 1995). In accord with our results, Block and Jones (1995) did not observe anomalous mole-fraction behavior for M-current in bullfrog sympathetic ganglion cells. It is possible that the expected increase in conductance upon complete replacement of one ion by another was not observed because ions other than K exhibit very low conduction. Therefore, the channel may not be able to conduct a measurable increase in ion movement.

It remains uncertain whether the M-channel is a multi- or a single-ion pore. The inhibition of M-current by low concentrations of Rb is consistent with multi-ion occupancy of the pore (Hess and Tsien, 1984) but by itself is not sufficient proof against single-ion occupancy.

### Kinetic implications

Different external cations did not affect the deactivation kinetics of the M-current. This is in contrast to delayed rectifier channels in squid giant axon (Swenson and Armstrong, 1981; Matteson and Swenson, 1986) and cloned *Shaker* potassium channels (Zagotta et al., 1994). The deactivation kinetics of these channels were slowed markedly when Rb was the permeating ion, and it is proposed that this occurs because the channel cannot close when the pore contains an ion (Matteson and Swenson, 1986). Rb exhibits a longer residency within the pore than K does and thus decreases the apparent channel closing rate. The lack of effect of the permeant ion on M-current deactivation kinetics suggests that although other ions exhibit a longer pore residency time than K does, they apparently do not impede channel closing.

### Block of M-current by Ba

M-current is blocked by low concentrations of Ba (Constanti et al., 1981; Stansfeld et al., 1993) acting from the outside but not from the inside (Robbins et al., 1992); however, the mechanism of action of Ba on M-current is not known. In this study, outward M-current was blocked more effectively by Ba than inward current was, with no voltage-dependence being apparent. It is possible that Ba binds within the pore of the M-channel, such that ions permeating from one side are differentially affected compared to those entering from the other side. Alternatively, Ba may act allosterically to inhibit M-current. Further experiments at the single-channel level will be necessary to elucidate the mechanism by which Ba interacts with the M-channel. Clearly the inhibition of M-current by Ba is very different from Ba block of other potassium currents. For example, many voltage- and Ca-activated potassium channels are blocked by low concentrations of internal Ba and high concentrations of external Ba acting at a single site in the pore that is accessible from both sides of the membrane (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980; Armstrong et al., 1982; Vergara and Latorre, 1983; Benham et al., 1985; Miller et al., 1987). Inward rectifier channels are sensitive to external Ba block in the same concentration range as M-channels ($K_i$ = 0.65 mM) (Standen and Stanfield, 1978), but the block is voltage-dependent and does not preferentially affect current in one direction.

## CONCLUSION

The cloning of a number of potassium channels has allowed tremendous advances in the understanding of the molecular mechanisms of permeation and selectivity. The permeation properties of the M-channel can be compared with other potassium channels, particularly those that have been cloned. The permeation sequence of the M-channel to monovalent cations was the same as other potassium channels, suggesting that the five amino acid selectivity filter sequence will be conserved. Other properties of M-channel permeation were quite different, including the inability to demonstrate anomalous mole-fraction behavior, the low conductance of ions through the channel, the lack of effect of permeant ions on macroscopic deactivation kinetics, and the inhibition by Ba of outward but not inward current. These unusual aspects of conduction suggest that regions of the pore will have a molecular structure very different from other potassium channels.

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