Influence of permeating ions on potassium channel block by external tetraethylammonium

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1. Block by external tetraethylammonium (TEA) was examined on currents carried by potassium (K^+) and sodium (Na^+) through the cloned delayed rectifier K^+ channel Kv2.1.

2. In the presence of high intracellular K^+ and high extracellular Na^+, currents through the Kv2.1 channel were carried almost exclusively by K^+. In the absence of both intracellular and extracellular K^+, large currents were carried by Na^+ through the Kv2.1 channel.

3. External application of 30 mM TEA blocked K^+ currents through Kv2.1 by 87%. The same external concentration of TEA had no effect on Na^+ currents through this channel.

4. As the K^+ concentration was increased between 0 and 140 mM, the percentage of current blocked by TEA progressively increased from 0 to 87%.

5. These data indicate that block of K^+ channels by external TEA depends on the permeating ion. Furthermore, these data indicate that in the presence of Na^+ and absence of K^+, TEA does not bind to the channel, and that addition of low concentrations of K^+ facilitates TEA binding.

Extracellular application of tetraethylammonium (TEA) blocks delayed rectifier K^+ channels by binding to a site in the pore region of the channel (Armstrong & Hille, 1972; Yellen, Jurman, Abramson & MacKinnon, 1991; Taglialatela, Vandongen, Drewe, Joho, Brown & Kirsch, 1991). Different K^+ channels have different sensitivities to external TEA, with the K_D (dissociation constant) for block varying from less than 0·4 mM to greater than 100 mM (Stuhmer *et al.* 1989; Taglialatela *et al.* 1991; Kavanaugh, Hurst, Yakel, Varnum, Adelman & North, 1992). K^+ channels are composed of four independent subunits that combine to form a single channel (Jan & Jan, 1989; MacKinnon, 1991). TEA appears to interact symmetrically with all four subunits (Heginbotham & MacKinnon, 1992; Kavanaugh *et al.* 1992). One amino acid in particular, at position 449 in the *Shaker* K^+ channel, appears to be a critical component of binding of extracellular TEA to the channel. The presence of tyrosine or phenylalanine at this position on all four subunits confers high affinity TEA binding (Heginbotham & MacKinnon, 1992). Replacement of tyrosine or phenylalanine at this position in from one to four subunits successively reduces the potency of TEA for block of K^+ currents. Taken together, these data have led to a model whereby external TEA plugs the pore by 'co-ordinate' interaction of TEA with all four subunits.

The delayed rectifier K^+ channels in rat superior cervical ganglion (SCG) neurons and chick dorsal root ganglion (DRG) neurons display a unique permeation mechanism (Zhu & Ikeda, 1993; Callahan & Korn, 1994). Under normal physiological conditions, these channels are highly selective for K^+ over Na^+. Unlike most voltage-gated K^+ channels, however, these channels are highly permeant to Na^+ when both external and internal K^+ are removed. When carried by K^+, delayed rectifier currents in DRGs are inhibited approximately 70% by 10 mM external TEA (Callahan & Korn, 1994). However, Na^+ currents through the same channel are completely insensitive to TEA at 10 mM. This suggested that K^+ channel sensitivity to external TEA depended on the ion that was permeating the pore. However, neurons contain many different K^+ channels, which precluded the unequivocal determination of whether Na^+ was conducting through a TEA-insensitive K^+ channel or a TEA-sensitive channel that lost its TEA sensitivity when Na^+ was the permeant ion.

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A K^+ channel clone from human brain cortex, Kv2.1, also utilizes the permeation mechanism that allows Na^+ to conduct in the absence of intracellular or extracellular K^+ (Korn & Ikeda, 1995). This channel has similar activation and inactivation gating properties as the SCG and DRG delayed rectifier. The data presented below demonstrate unequivocally that the sensitivity of this K^+ channel to external TEA is dependent on the ion that permeates the channel. Preliminary accounts of these data have been presented in abstract form (Korn, Zhu, Lewis & Ikeda, 1995).

METHODS

Potassium channel expression

Kv2.1 potassium channels (Genbank L02840), cloned from human brain cortex, were expressed in a mouse fibroblast cell line (L-cells) that contained no relevant ion channels. L-Cells were cultured in minimal essential medium (MEM) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, 1% glutamine and non-essential amino acids. Cells were injected with cRNA to Kv2.1, prepared as described previously (Ikeda, Solar, Zuhike, Joho & Lewis, 1992; Korn & Ikeda, 1995). cRNA was diluted from a stock solution of 1–2 $\mu g\ \mu l^{-1}$ to a final concentration of 0·2–1 $\mu g\ \mu l^{-1}$ with a solution of 0·1% fluorescein dextran (10 000 MW, Molecular Probes, Eugene OR, USA) in H_2O. This solution was centrifuged at 16 000 *g* for 10 min to remove particulates. Approximately 1 μl of the supernatant was then removed and loaded into a micropipette for injection. Injections were made with a pressure of 10–18 kPa for a duration of 0·3 s. After incubation (37 °C, 5% CO_2 in air) in culture media for 14–24 h, injected cells were identified with an inverted microscope equipped with an epifluorescence unit.

Electrophysiology

Whole-cell currents were recorded with a patch-clamp amplifier (Axopatch 200A, Axon Instruments) at room temperature (21–24 °C) using the standard whole-cell patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Recording pipettes were pulled from Corning 7052 glass (Garner Glass Co., Claremont, CA, USA) and had resistances of 1–6 MΩ when filled with pipette solutions. Cell membrane capacitance and resistance were electronically compensated, typically by 80%. Traces were digitized at 2–5 kHz and filtered at 1–2 kHz using the 4-pole Bessel filter built into the amplifier. Solutions used are listed in the figure legends. External solutions were changed by gravity flow from a large bore pipette. Current traces were digitally corrected for linear leakage as determined from hyperpolarizing pulses. Currents during the depolarizing voltage step were measured at the end of the voltage step. Tail current amplitudes were measured isochronally 5 ms following repolarization.

RESULTS

With normal physiological solutions (high K^+ inside, high Na^+ outside), depolarization elicits outward delayed rectifier K^+ currents through Kv2.1 channels (Fig. 1*A* and *B*). Upon removal of both intracellular and extracellular K^+, Kv2.1 channels become permeant to Na^+ (Korn & Ikeda, 1995). Consequently, in the presence of high external Na^+ and no internal or external K^+ (with *N*-methylglucamine (NMG^+) to replace internal K^+), depolarization evokes inward Na^+ currents (Fig. 1*C* and *D*). Outward currents were not observed at voltages as high as +80 mV, which indicates that NMG^+ did not pass through the channel to any measureable extent.

Previous reports demonstrated that outward K^+ currents through Kv2.1 channels were inhibited by external TEA with a K_D of approximately 5 mM (Taglialatela *et al.* 1991). We obtained similar results; 3 mM TEA inhibited outward K^+ currents by 53·8 ± 1·8% (mean ± S.E.M., $n = 4$; Fig. 2*A*). To compare directly TEA-induced block of currents carried by K^+ and Na^+, we examined the effects of 30 mM TEA on inward currents carried exclusively by K^+ (Fig. 2*B*) or Na^+ (Fig. 2*C*). Channels were activated by depolarization of the membrane to various voltages, and the effect of TEA was examined on K^+ or Na^+ tail currents that followed repolarization to −80 mV. At a concentration of 30 mM, TEA inhibited K^+ currents by 88·0 ± 1·5% ($n = 3$; Fig. 2*B*). Currents measured during the depolarizing voltage step were similarly affected (Fig. 2*B*). In contrast, Na^+ currents were unaffected by the same concentration of TEA ($n = 6$; Fig. 2*C*).

Low concentrations of K^+ inhibit Na^+ current through Kv2.1 channels by binding to a site within the channel pore and competitively inhibiting Na^+ from conducting through the channel (Korn & Ikeda, 1995). K^+ inhibits Na^+ currents with an IC_{50} of < 3 mM, which indicates that K^+ binds to one or more sites in the channel pore at concentrations between 1 and 10 mM. Furthermore, at K^+ concentrations between 1 and 10 mM, currents through Kv2.1 channels are carried by both Na^+ and K^+ (Korn & Ikeda, 1995). The data in Fig. 2 demonstrate that TEA blocked currents carried exclusively by K^+ but did not block currents carried exclusively by Na^+. The experiments described in Figs 3 and 4 distinguished whether TEA binding was dependent on the presence of K^+ or whether TEA only blocked currents carried by K^+.

As previously shown (Fig. 2*C*), application of 30 mM TEA had no effect on currents carried by Na^+ in the absence of K^+ (Fig. 3*A*, top panel, and *B*). Application of 3 mM K^+ in the absence of TEA inhibited Na^+ currents by 75·3 ± 3·4% (Fig. 3*A*, bottom panel, and *B*). Subsequent addition of 30 mM TEA in the presence of 3 mM K^+ blocked the remaining Na^+ current by 56·8 ± 4·3% (Fig. 3*A*, bottom panel, and *B*).

The dependence of TEA block on $[K^+]$ is shown in Fig. 4. In Fig. 4*A*, the control currents were evoked in the presence

of external Na^+ plus increasing concentrations of external K^+ (currents in the presence of 140 mM K^+ were recorded in the absence of Na^+). Currents were normalized to match the magnitude of the current during the step to +20 mV. Superimposed on these traces are currents recorded after addition of 30 mM TEA. The percentage block by TEA increased with increasing concentrations of K^+ (Fig. 4*B*). These data indicate that the addition of even very low concentrations of K^+ facilitated block by TEA.

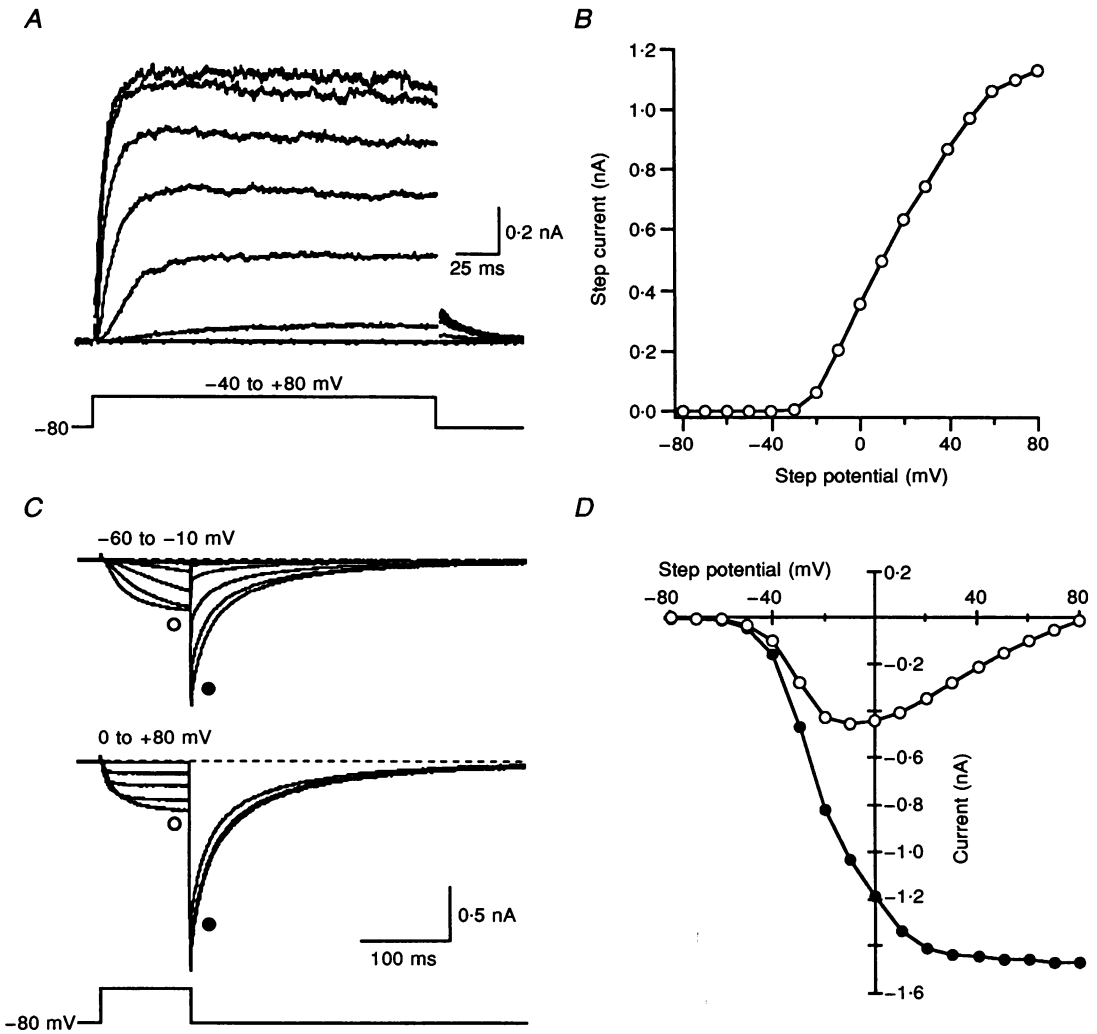

Figure 1. Removal of K^+ permits Na^+ to conduct through Kv2.1 channels

A, K^+ currents evoked by depolarizations from a holding potential of −80 mV to incremental voltages between −40 and +80 mV. Repolarization potential was −40 mV. Dashed line marks 0 current level. External solution (mM): 140 NaCl, 5·4 KCl, 2 $CaCl_2$, 0·8 $MgCl_2$, 10 Hepes, 15 glucose; pH 7·4 (NaOH); osmolality 295 mosmol kg^{-1}. Internal solution (mM): 125 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 Hepes, 4 MgATP, 0·1 NaGTP; pH 7·2 (KOH;, osmolality 270 mosmol kg^{-1}. *B*, *I–V* relationship measured from cell in Fig. 1*A*, measured isochronally 190 ms into the 200 ms depolarization. *C*, currents carried by Na^+ following removal of both intracellular and extracellular K^+. Depolarizations ranged from −60 to −10 mV (top family of traces) and from 0 to +80 mV (bottom family). Holding and repolarization potentials were −80 mV. External solution (mM): 135 NaCl, 2 $CaCl_2$, 10 Hepes, 45 sucrose; pH 7·4 (NaOH); osmolality 340 mosmol kg^{-1}. Internal solution (mM): 155 NMG, 1 $CaCl_2$, 11 EGTA, 10 Hepes, 20 HCl, 14 Tris creatine phosphate, 4 Mg-ATP, 0·3 Na-GTP; pH 7·2 (methanesulphonic acid); osmolality 315 mosmol kg^{-1}. *D*, current–voltage relationships measured at the end of the depolarizing step (○) or 5 ms following repolarization (●).

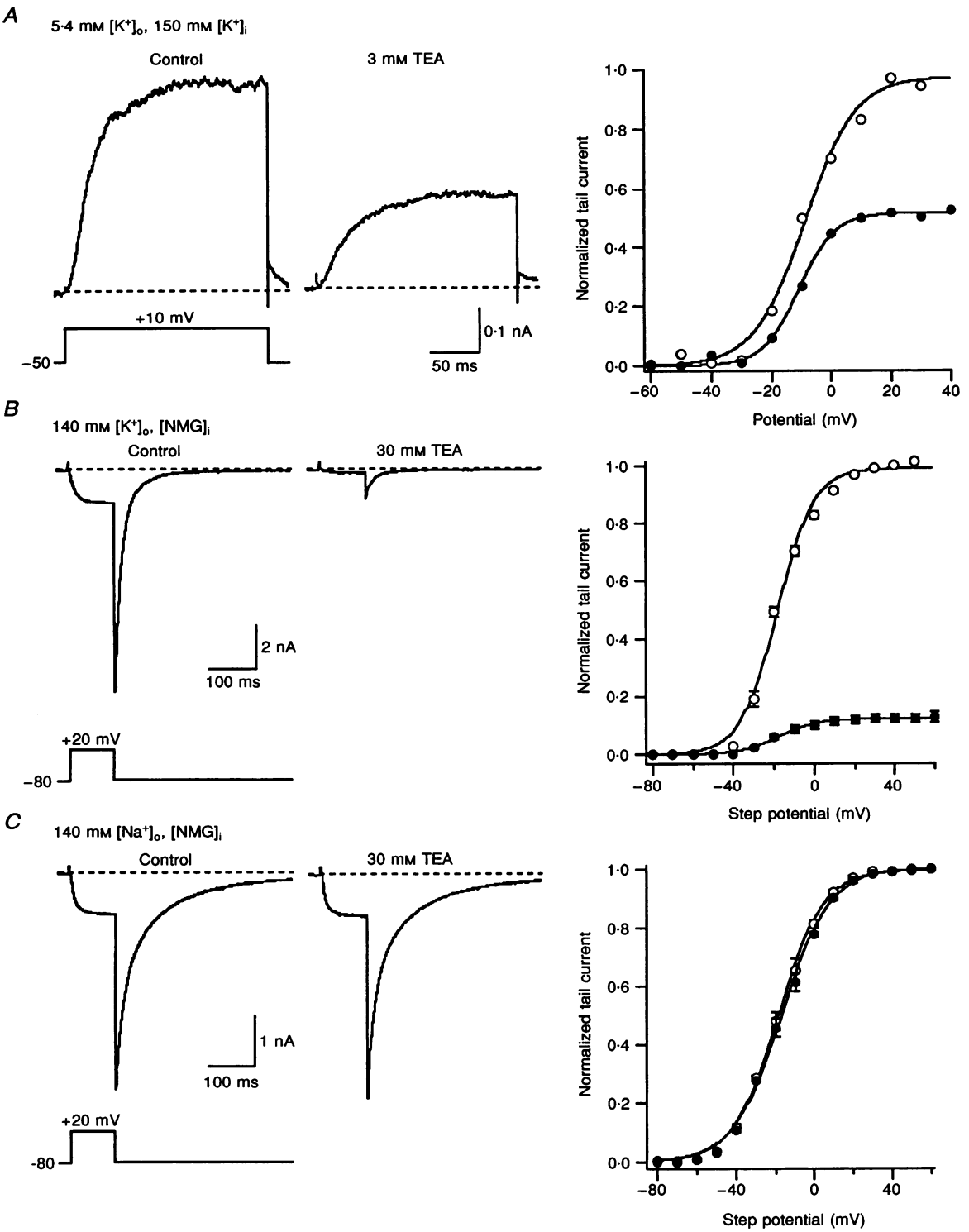

Figure 2. Inhibition of K^+ currents but not Na^+ currents by external TEA

A, left panel, outward K^+ currents evoked by depolarization to +10 mV from a holding potential of −50 mV in the absence (control) and presence of 3 mM TEA. *A*, right panel, activation curves, averaged from 2 cells, in the absence (○) and presence (●) of 3 mM TEA. Internal and external solutions as in Fig. 1*A*. *B*, left panel, inward K^+ currents in the absence (control) and presence of 30 mM TEA. *B*, right panel, activation curves, averaged from 3 cells. External solution as in Fig. 1*C* except with K^+ salts substituted for Na^+ salts (± 30 mM TEA-Cl). Internal solution as in Fig. 1*C*. *C*, left panel, inward Na^+ currents in the absence (control) and presence of 30 mM TEA. *C*, right panel, activation curves, averaged from 6 cells. Internal and external solutions as in Fig. 1*C*. Activation curves were fitted to a modified Boltzmann function (cf. Korn & Ikeda, 1995), normalized to the maximum control current.

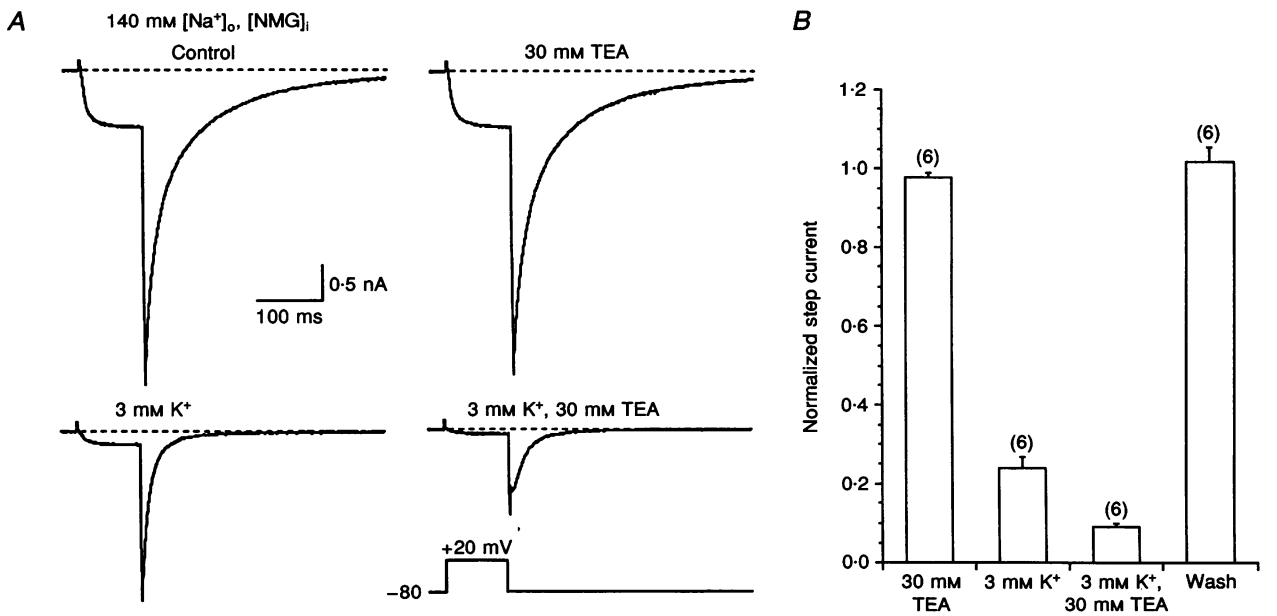

Figure 3. TEA blocks Na^+ current in the presence but not absence of K^+

A, Na^+ currents evoked by depolarization to +20 mV from a holding potential of −80 mV in the absence of K^+ or TEA (control), in the presence of 30 mM TEA (top right), in the presence of 3 mM K^+ (bottom left), and in the presence of 3 mM K^+ plus 30 mM TEA (bottom right). *B*, summary of the results from 6 cells. Wash demonstrates that currents returned to the control value upon removal of both K^+ and TEA.

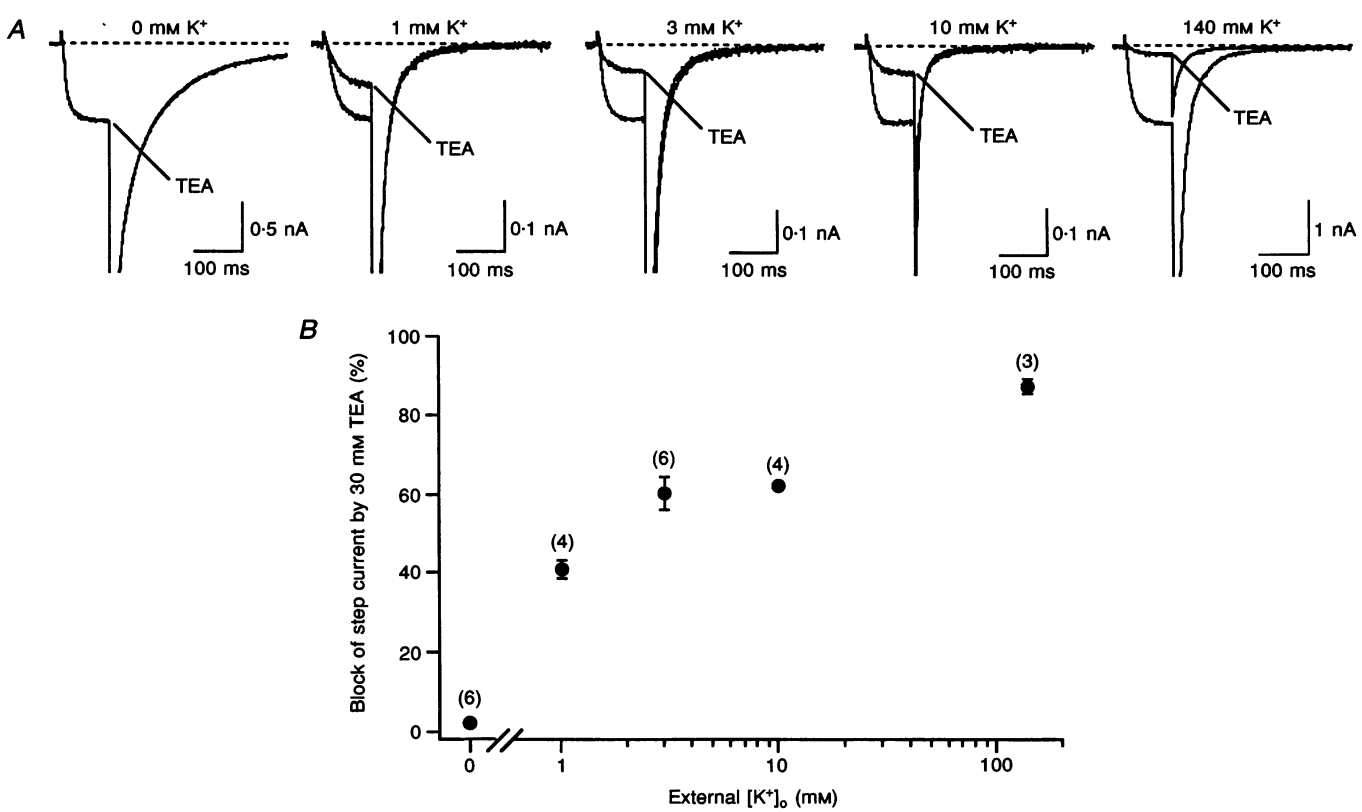

Figure 4. Dependence of TEA block on K^+ concentration

A, control traces (unmarked) illustrate inward currents in the presence of 140 mM Na^+ and increasing external $[K^+]$ (except 140 mM K^+ trace, which was evoked in the absence of Na^+). Currents were normalized to match the magnitude of the control step current. The traces marked TEA were evoked after application of 30 mM TEA in the presence of the $[K^+]$ noted above the traces. Currents are from different cells. *B*, percentage block of step current (current at +20 mV in the presence of K^+) by 30 mM TEA, as a function of $[K^+]$.

DISCUSSION

The observation that TEA inhibited K^+ currents but not Na^+ currents through the channel can be explained by one of two hypotheses. First, TEA may have bound to the channel but not prevented Na^+ from conducting. Second, TEA may have bound to the channel in the presence of K^+ but not in the presence of Na^+ and the absence of K^+. Although we have not conclusively ruled out the first possibility, two observations argue against it. First, delayed rectifier K^+ channels are composed of four subunits that are believed to form a central pore region (Jan & Jan, 1989; MacKinnon, 1991). TEA is believed to bind to all four subunits and block the central axis of the conduction pathway (Heginbotham & MacKinnon, 1992; Kavanaugh *et al.* 1992). Although it is possible that this binding could take place and permit Na^+ to pass by unimpeded, the geometry that would underly a situation in which TEA bound to all four subunits and left space for Na^+ but not K^+ to pass is not obvious. Perhaps more importantly, addition of just 1 mM K^+ allowed TEA to significantly inhibit Na^+ current through the channel. This observation would require that Na^+ could pass by TEA unimpeded with no K^+ present but not with some K^+ present.

The alternative possibility, that TEA bound to the channel in the presence of K^+ but not Na^+, is supported by the observation that TEA did not inhibit Na^+ currents at all in the total absence of K^+ but significantly inhibited Na^+ currents upon addition of low concentrations of K^+. Furthermore, the K^+ concentration range over which TEA block was facilitated is virtually identical to the range over which K^+ blocks Na^+ conductance through the channel (Korn & Ikeda, 1995). These data suggest that binding of K^+ but not Na^+ within the channel allows the formation of the TEA binding site.

Block by K^+ is quite voltage dependent, which indicates that the binding site(s) for K^+-induced inhibition of Na^+ current is well within the membrane field (in the chick DRG delayed rectifier channel, K^+ was calculated to bind to a site ~50% of the distance into the membrane field; Callahan & Korn, 1994). In contrast, TEA apparently binds to a sight very near the external mouth of the channel, as it is not very sensitive to membrane voltage (Armstrong & Hille, 1972; Taglialatela *et al.* 1991; Heginbotham & MacKinnon, 1992). Taken together, these data suggest that binding of K^+ to one or more sites relatively remote from the TEA binding site alters the structure of the pore and promotes the formation of the TEA binding site.

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