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# Charybdotoxin Blocks Voltage-gated K<sup>+</sup> Channels in Human and Murine T Lymphocytes

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**ABSTRACT** A variety of scorpion venoms and purified toxins were tested for effects on ion channels in human T lymphocytes, a human T leukemia cell line (Jurkat), and murine thymocytes, using the whole-cell patch-clamp method. Nanomolar concentrations of charybdotoxin (CTX), a purified peptide component of *Leiurus quinquestriatus* venom known to block Ca<sup>2+</sup>-activated K<sup>+</sup> channels from muscle, blocked "type *n*" voltage-gated K<sup>+</sup> channels in human T lymphoid cells. The Na<sup>+</sup> channels occasionally expressed in these cells were unaffected by the toxin. From the time course of development and removal of K<sup>+</sup> channel block we determined the rates of CTX binding and unbinding. CTX blocks K<sup>+</sup> channels in Jurkat cells with a  $K_d$  value between 0.5 and 1.5 nM. Of the three types of voltage-gated K<sup>+</sup> channels present in murine thymocytes, types *n* and *n'* are blocked by CTX at nanomolar concentrations. The third variety of K<sup>+</sup> channels, "type *l*," is unaffected by CTX. Noxiustoxin (NTX), a purified toxin from *Centruroides noxius* known to block Ca<sup>2+</sup>-activated K<sup>+</sup> channels, also blocked type *n* K<sup>+</sup> channels with a high degree of potency ( $K_d = 0.2$  nM). In addition, several types of crude scorpion venoms from the genera *Androctonus*, *Buthus*, *Centruroides*, and *Pandinus* blocked type *n* channels. We conclude that CTX and NTX are not specific for Ca<sup>2+</sup>-activated K<sup>+</sup> channels and that purified scorpion toxins will provide useful probes of voltage-gated K<sup>+</sup> channels in T lymphocytes. The existence of high-affinity sites for scorpion toxin binding may help to classify structurally related K<sup>+</sup> channels and provide a useful tool for their biochemical purification.

## INTRODUCTION

Scorpion toxins have proved useful in probing the gating mechanisms and biochemistry of sodium channels in excitable cell membranes. Effects on either sodium channel inactivation or activation have been observed with various crude venoms and purified peptide toxins (Koppenhöffer and Schmidt, 1968; Cahalan, 1975; Wang and Strichartz, 1983; Simard et al., 1986; Gonoï and Hille, 1987). Biochemical studies using a purified toxin from *Leiurus quinquestriatus* venom have led to the identification of three distinct polypeptide components of mammalian sodium channels

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(reviewed in Catterall, 1986). Since little is known regarding the biochemical properties of the diverse family of potassium channels, there is considerable interest in finding toxins that bind tightly to  $K^+$  channels to provide a handle for their biochemical purification. Miller and colleagues purified a peptide constituent of *Leiurus quinquestriatus* scorpion venom, charybdotoxin (CTX), and demonstrated that it blocks the large conductance, calcium- and voltage-activated "maxi"-  $K^+$  channel from muscle transverse tubular membrane incorporated into planar lipid bilayers (Miller et al., 1985). CTX also blocks a lower conductance calcium-activated  $K^+$  channel present in molluscan cell bodies (Hermann and Erxleben, 1987), and has recently been shown to block rapidly inactivating *Shaker*  $K^+$  channels expressed in *Xenopus* oocytes after mRNA injection (MacKinnon et al., 1988). Some scorpion venoms reduce currents through voltage-activated potassium channels. Noxiustoxin (NTX), a peptide component of *Centruroides noxius* venom, reduces  $K^+$  current in squid axon (Carbone et al., 1982, 1987), and also blocks  $Ca^{2+}$ -activated  $K^+$  channels (Valdivia et al., 1988), although with lower affinity than CTX. A crude scorpion venom from *Pandinus imperator* has been shown to block and alter the gating of voltage-activated  $K^+$  channels in frog nerve and muscle membranes (Pappone and Cahalan, 1987; Pappone and Lucero, 1988).

Voltage-activated  $K^+$  channels are the major type of ion channel found in human T lymphocytes and related cell lines, and in murine T lymphocytes and thymocytes (reviewed in Lewis and Cahalan, 1988b). In murine lymphoid cells, three distinct types of  $K^+$  channels have been described, differing in their voltage dependence, inactivation and closing kinetics, and sensitivity to blockers such as tetraethylammonium (TEA). The most commonly found channel in either human or murine T cells is activated by depolarization to potentials above  $-40$  mV, accumulates inactivation during a series of depolarizing pulses repeated at 1-s intervals, is half-blocked by  $\sim 10$  mM TEA, and has a single-channel conductance in the range of 10–18 pS (Cahalan et al., 1985; Deutsch et al., 1986; DeCoursey et al., 1987; Lewis and Cahalan, 1988a). This channel has been named type *n*, for *normal*, because it is found in normal human T cells. A second type of  $K^+$  channel, called type *n'* for its similarity to type *n* channels, is found in a subset of murine thymocytes (Lewis and Cahalan, 1988a, b). Unlike type *n* channels, *n'* channels do not accumulate inactivation during repetitive depolarizations and are less sensitive to block by TEA. The third type of  $K^+$  channel, dubbed type *l* to mnemonically represent its *larger* single-channel conductance and its abundance in abnormally proliferating T cells from mice homozygous for the *lpr* mutation (Chandy et al., 1986), is activated at potentials above  $-10$  mV, accumulates little inactivation during repetitive depolarizations, is half-blocked by 50–100  $\mu$ M TEA, and has a single-channel conductance of 22–30 pS (DeCoursey et al., 1987; Lewis and Cahalan, 1988a).

Here we report that CTX selectively blocks both type *n* and *n'* voltage-gated  $K^+$  channels in human and murine T lymphocytes. As these channels are not activated by intracellular calcium ions, CTX cannot be regarded as specific for calcium-activated  $K^+$  channels. We also show that  $K^+$  channels are blocked by several types of crude scorpion venom and by noxiustoxin (NTX), isolated from *Centruroides sculpturatus*. High-affinity block of lymphocyte  $K^+$  channels by scorpion toxins suggests their use in both electrophysiological and structural studies of  $K^+$  channels. Some of these results have been reported in preliminary form (Sands et al., 1988).

## METHODS

*Patch-Clamp Recording*

Voltage-clamp experiments were performed on T lymphocytes and related cells from mice and men, using the whole-cell recording technique (Hamill et al., 1981; Cahalan et al., 1985). The cells under investigation were bathed in normal Ringer solution containing (in millimolar) 160 NaCl, 4.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4 with NaOH, and having an osmolarity of 290–320 mosm/liter. Electrodes were pulled from Accu-fill 90 Micropets (Becton, Dickinson & Co., Parsippany, NJ) in three stages, coated with Sylgard (Dow Corning Corp., Midland, MI) near their tips, and fire-polished. Pipettes had resistances of 2–7 MΩ when filled with the standard internal solution consisting of (in millimolar) 140 KF, 2 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, 11 K<sub>2</sub>EGTA, and 5 K-HEPES, pH 7.2. A List L/M-EPC 7 (Medical Systems, Great Neck, NY) or Axopatch 1 B (Axon Instruments, Inc., Burlingame, CA) patch-clamp amplifier was used in the voltage-clamp mode, and the output was filtered at 2 kHz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) before sampling unless otherwise noted. Series resistance compensation was not used when current amplitudes were <0.5 nA, since series resistance errors during maximal K<sup>+</sup> current activation were estimated to be <4 mV. For cells with larger current amplitudes, series resistance compensation of 70–80% was used. Capacity and leak currents were eliminated by subtracting a scaled version of the averaged current response to small depolarizing pulses below –70 mV. Command voltage was corrected for a liquid junction potential of –7 mV between the pipette solution and the bath. All experiments were conducted at room temperature (22–26°C).

*Cells*

A human T cell-derived cell line, Jurkat E6-1, was maintained in culture and used in most of the experiments. Human T lymphocytes were enriched by the sheep red cell rosetting technique from blood donated by healthy volunteers (Cahalan et al., 1985); the resulting T cell population was estimated to be >95% pure by fluorescence-activated cell sorter analysis (FACS IV sorter; Becton, Dickinson & Co., Mountain View, CA). Thymocytes from 4–8 wk-old BALB/c or C57BL/6 mice were isolated as previously described (Lewis and Cahalan, 1988a). For identification of cell subsets, thymocytes were stained with fluorescently labeled anti-CD4 and anti-CD8 monoclonal antibodies (Becton-Dickinson Immunocytometry Systems, Mountain View, CA) and placed in the recording chamber for visualization with a Zeiss IM-35 microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with epifluorescence optics as detailed elsewhere (Lewis and Cahalan, 1988a). Four distinct subsets could be identified: cells binding only anti-CD8 (mostly cytotoxic/suppressor T cells), cells binding only anti-CD4 (mostly helper T cells), cells stained with both antibodies (“double positives”), and cells that did not stain (“double negatives”). A few experiments were also performed on rat thymocytes, isolated by similar procedures from Wistar rats, 6 wk of age.

*Solutions and Toxins*

Solutions of CTX were prepared just before use by diluting frozen aliquots (5–80 μM) into Ringer solution. Bovine serum albumin (0.1 mg/ml) was added to all toxin solutions. For the equilibrium block experiments, the bath solution was changed during the recordings by perfusion of the entire recording chamber. In experiments on binding and unbinding rates, extracellular toxin concentration was altered rapidly by pressure ejection from a puffer pipette. The puffer pipette could be perfused with either TEA, as a test for the rapidity of solution exchange, or, in the same experiment, with CTX to determine the rate of CTX block.

The tip of the puffer pipette had an internal diameter of  $\sim 30 \mu\text{m}$  and was positioned within  $30 \mu\text{m}$  of the cell under study.

Dr. C. Miller (Brandeis University, Waltham, MA) provided samples of CTX isolated from *Leiurus quinquestriatus* venom and purified as previously described (Smith et al., 1986; Anderson et al., 1988). We used these samples (referred to below as CTX<sub>M</sub>) in most of the early experiments. A second sample of purified CTX (referred to as CTX<sub>G</sub>), previously characterized (Gimenez-Gallego et al., 1988), was provided by Dr. M. Garcia (Merck Institute, Rahway, NJ). The potencies of CTX<sub>M</sub> and CTX<sub>G</sub> for blocking K<sup>+</sup> channels in lymphocytes differed somewhat, possibly due to degradation of active toxin. Stock solutions of the toxins deteriorated slowly in the refrigerator; a sample of CTX<sub>G</sub> ( $77 \mu\text{M}$ ) lost 60% of its potency after 6 mo. A purified sample of NTX from *Centruroides* venom was provided by Dr. L. Possani (Baylor University, Houston, TX). Purified alpha scorpion toxin from *Leiurus quinquestriatus* was provided by Dr. W. Catterall (University of Washington, Seattle, WA). Apamin and venom samples from the scorpions *Androctonus australis*, *Buthus tamulus*, *Centruroides sculpturatus*, and *Tityus serrulatus*, were purchased from Sigma Chemical Co. (St. Louis, MO). A sample of *Pandinus imperator* venom was purchased from LaToxan (Rosans, France).

## RESULTS

### CTX Blocks K<sup>+</sup> Channels in Human T Cells

Human T cells and the cell line Jurkat express K<sup>+</sup> channels that open initially and then inactivate in response to maintained depolarization. Fig. 1 illustrates whole-cell

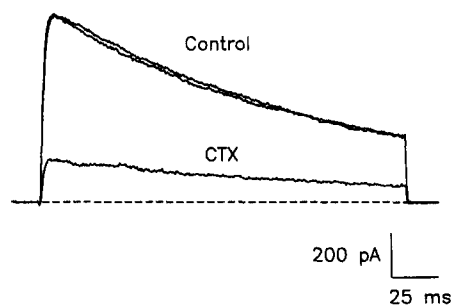


FIGURE 1. Reversible block of voltage-gated K<sup>+</sup> current by CTX. Type *n* K<sup>+</sup> currents were evoked by 200-ms depolarizing pulses to +30 mV before and after application of 2.6 nM CTX, and after washout of the toxin. Pulses were delivered every 30 s from a holding potential of -80 mV to minimize accumulation of inactivation due to repetitive depolarization.

recordings of K<sup>+</sup> current in a Jurkat cell before, during, and after application of CTX. The toxin reversibly blocked most (78% with 2.6 nM CTX) of the K<sup>+</sup> current. Equilibrium dose-response relations for block of K<sup>+</sup> channels were determined for two samples of CTX, obtained from Dr. C. Miller (CTX<sub>M</sub>) and from Dr. M. Garcia (CTX<sub>G</sub>). Fig. 2 shows the results of these experiments, with binding isotherms representing  $K_d$  values of 0.55 nM (CTX<sub>M</sub>) and 1.5 nM (CTX<sub>G</sub>) fitted to the data. K<sup>+</sup> channels in human T cells separated from human peripheral blood showed a similar sensitivity to CTX.

Although the inactivation of lymphocyte K<sup>+</sup> channels during sustained depolarizations makes it difficult to determine the possible voltage dependence of CTX binding under steady-state conditions, we explored the possibility that CTX might alter the apparent voltage dependence of the K<sup>+</sup> channels, either by producing voltage-dependent block or by interacting with the channel's gating mechanism. Fig. 3

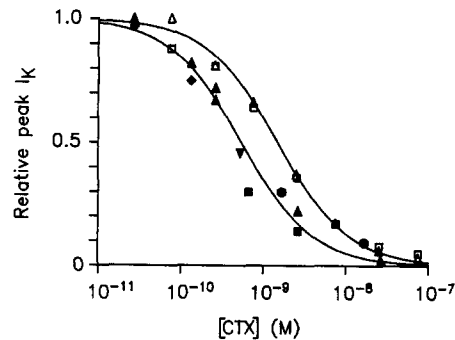


FIGURE 2. Equilibrium dose-response relations for K<sup>+</sup> channel block by CTX. Peak K<sup>+</sup> current was measured using the same protocol as in Fig. 1, in the presence of the indicated concentrations of CTX obtained from C. Miller (CTX<sub>M</sub>, five cells represented by filled symbols) or M. Garcia (CTX<sub>G</sub>, two cells, represented by open symbols). Single-site binding curves have been superimposed on the data points, and correspond to K<sub>d</sub> values of 0.55 nM (CTX<sub>M</sub>) or 1.5 nM (CTX<sub>G</sub>).

illustrates that CTX reduces the maximum K<sup>+</sup> conductance, but does not affect the voltage dependence of channel activation. K<sup>+</sup> currents were evoked before, during, and after application of a partially blocking dose of CTX<sub>M</sub>. All three families display similar half-maximal activation voltages, suggesting that CTX simply blocks the channels and does not shift the voltage dependence of activation of the unblocked channels.

We may represent the binding of CTX to a site S on the K<sup>+</sup> channel in terms of the following simple scheme:

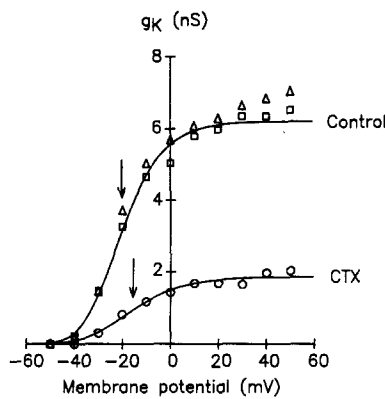
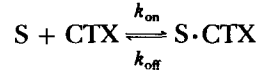


FIGURE 3. CTX does not alter the voltage dependence of K<sup>+</sup>-channel activation in Jurkat cells. K<sup>+</sup> currents were evoked by 200-ms pulses to the indicated potentials in the presence or absence of CTX. Chord K<sup>+</sup> conductance,  $g_K$ , was calculated from the peak K<sup>+</sup> current during each pulse, assuming a reversal potential of -80 mV. Squares, before CTX; circles, 1.7 nM CTX<sub>M</sub> in bath; triangles, after washout of toxin. Boltzmann curves of the form:

$$g_K = g_{K, \text{max}} / \{1 + \exp[(V_n - V)/k]\}^3$$

have been fitted to the data by a least-squares criterion, where  $g_K$  is the peak K<sup>+</sup> conductance at a given potential,  $g_{K, \text{max}}$  is the cell's limiting K<sup>+</sup> conductance,  $V_n$  is the voltage at which the "n-gate" is half-activated,  $V$  is membrane potential, and  $k$  expresses the voltage dependence of activation. Values for  $g_{K, \text{max}}$ ,  $V_n$ , and  $k$  are 6.21 nS, -34.3 mV, and 10.4 mV (control); and 1.88 nS, -33.4 mV, and 13.3 mV (CTX). The arrows indicate the membrane potential at which half the channels are activated: -20.3 mV for the control condition, and -15.5 mV in the presence of CTX.

To estimate  $k_{\text{on}}$  and  $k_{\text{off}}$  from the time constants of toxin wash-on and -off, we activated the  $\text{K}^+$  channels with brief depolarizations (to minimize inactivation) and applied the toxin by pressure ejection from a puffer pipette. The speed of solution exchange was assessed by measuring the rate of  $\text{K}^+$  channel block produced by rapid applications of TEA, a fast  $\text{K}^+$  channel blocker with a  $K_i$  of 10 mM (DeCoursey et al., 1984). Fig. 4 *A* shows the response of  $\text{K}^+$  current to the application of 10 mM TEA. Channel block is established along a roughly exponential time course, with a time constant,  $\tau$ , of 0.5 s. Fig. 4 *B* illustrates the reversal of bath-applied TEA block by puffing Ringer onto the cell; relief from block occurs with a time constant of 1.3 s. These estimates of the time constants for TEA block and unblock reflect the time course of local solution exchange, since TEA binding to and unbinding from these channels occurs on a submillisecond time scale (DeCoursey et al., 1987; Grissmer and Cahalan, 1989*a*). In contrast, rapid application of 2 nM CTX (Fig. 4 *C*) reduced the  $\text{K}^+$  current more slowly ( $\tau = 4.7$ s), with a time course representing that of toxin binding to its receptor on the  $\text{K}^+$  channel. These data correspond to an on-rate of  $5.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ . In another series of experiments, toxin-free Ringer solution was puffed onto cells bathed in 2 nM CTX to determine the toxin unbinding rate. The relief of block occurred with an estimated time constant of  $\sim 10$  s, representing an off-rate for CTX unbinding from the  $\text{K}^+$  channel of  $0.1 \text{ s}^{-1}$ . Based on the experimentally determined on- and off-rates, an equilibrium dissociation constant,  $K_d$ , of 1.8 nM can be calculated ( $K_d = k_{\text{off}}/k_{\text{on}}$ ). This value agrees well with the value of  $K_d$  determined from equilibrium block experiments using the same toxin sample (CTX<sub>C</sub>).

#### *Selectivity of CTX for Specific $\text{K}^+$ Channels*

In addition to  $\text{K}^+$  channels, Jurkat cells sometimes express voltage-activated  $\text{Na}^+$  channels. These  $\text{Na}^+$  channels are blocked by tetrodotoxin and have an inactivation time course that is dramatically slowed by a purified alpha scorpion toxin from *Leiurus quinquestriatus* venom (data not shown), which demonstrates their similarity to  $\text{Na}^+$  channels in electrically excitable cells. Fig. 5, *A* and *B* shows families of  $\text{Na}^+$  and  $\text{K}^+$  currents in the absence or presence of CTX, demonstrating selective block of the  $\text{K}^+$  channels. Fig. 5 *C* summarizes the results in a current-voltage plot of the peak inward current, demonstrating that CTX does not alter  $\text{Na}^+$  channel activation. This result illustrates the selectivity of CTX for  $\text{K}^+$  channels; despite their sensitivity to an alpha scorpion toxin,  $\text{Na}^+$  channels in Jurkat cells are not affected by CTX.

CTX is selective for specific types of voltage-activated  $\text{K}^+$  channels in lymphocytes. Most murine thymocytes express type *n*  $\text{K}^+$  channels similar in gating kinetics and pharmacological sensitivity to those of human T cells (Lewis and Cahalan, 1988*a, b*). Type *n* channels from murine thymocytes are also blocked by CTX, as shown in Fig. 6 *A*. A similar sensitivity to block by CTX was characteristic of type *n*  $\text{K}^+$  channels in double negative, double positive, and helper phenotype thymocytes from mice, as well as in rat thymocytes (data not shown). Two additional varieties of  $\text{K}^+$  channels, types *n'* and *l*, differing from type *n* channels in their kinetic properties and sensitivity to TEA, appear to be restricted to the cytotoxic/suppressor ( $\text{CD4}^- \text{CD8}^+$ ) subset of thymocytes (Lewis and Cahalan, 1988*a*). Fig. 6 *B* illustrates a cytotoxic/suppressor cell expressing mainly type *n'* channels, as judged by a lack of

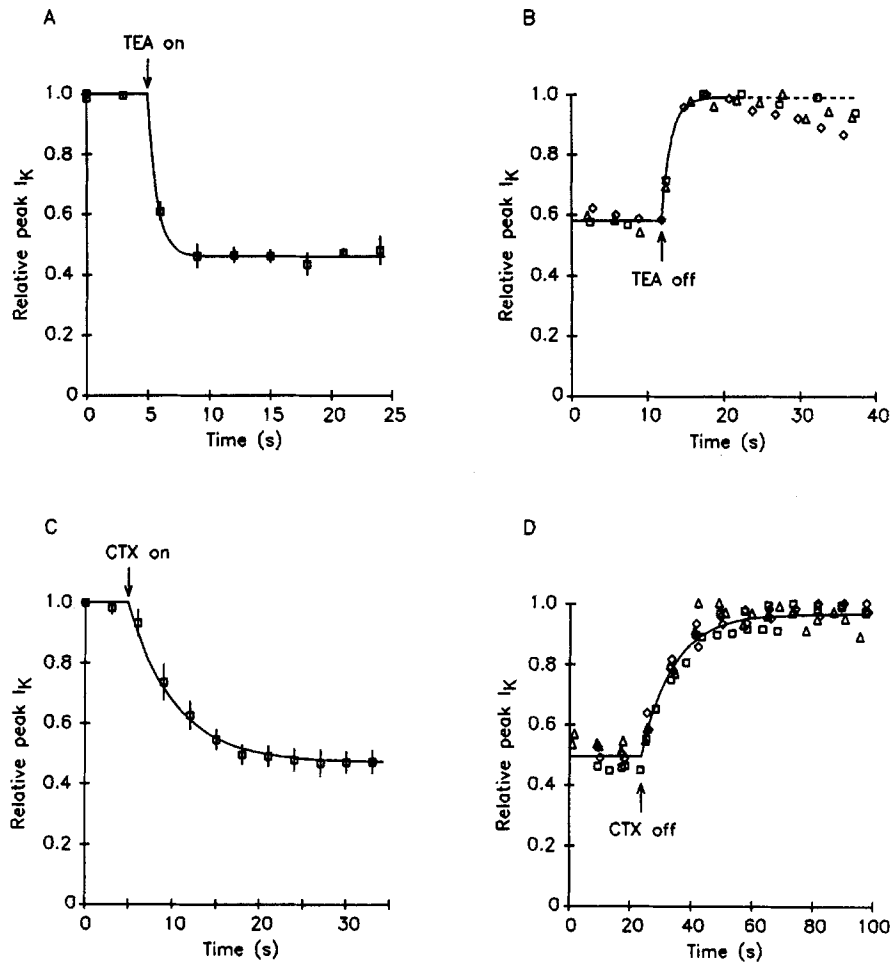


FIGURE 4. Measuring the on- and off-rates for block of K<sup>+</sup> channels by CTX. K<sup>+</sup> currents were evoked by 10-ms pulses from  $-80$  to  $+30$  mV delivered every 2–3 s, and their peak values were normalized to the maximum current amplitude of each experiment. In A and C, data were corrected for a measured amount of cumulative inactivation averaging  $\leq 0.5\%/s$ . An internally perfused puffer pipette placed within  $30\ \mu\text{m}$  of the cell was used to apply sequentially 10 mM TEA (A) and 2 nM CTX<sub>G</sub> (C) to cells bathed in Ringer, or to deliver Ringer solution to cells bathed in 10 mM TEA (B) or 2 nM CTX<sub>G</sub> (D). Results of two experiments on each of three cells are plotted in each panel. In A and C, symbols represent average  $\pm$ SD values of normalized peak K<sup>+</sup> currents, while in B and D, each type of symbol represents a separate experiment. In each case a single exponential curve was fitted by eye to estimate the time constant,  $\tau$ , for block or unblock. (A) K<sup>+</sup> current is blocked rapidly by 10 mM TEA, indicating rapid extracellular perfusion.  $\tau = 0.5$  s. (B) For cells in bath solution containing 10 mM TEA, Ringer applied from the puffer pipette promotes rapid recovery from TEA block.  $\tau = 1.3$  s. (C) 2 nM CTX<sub>G</sub> blocks K<sup>+</sup> current with  $\tau = 4.7$  s. (D) For cells bathed in 2 nM CTX<sub>G</sub>, Ringer applied from the puffer pipette relieves channel block with  $\tau = 10.4$  s.



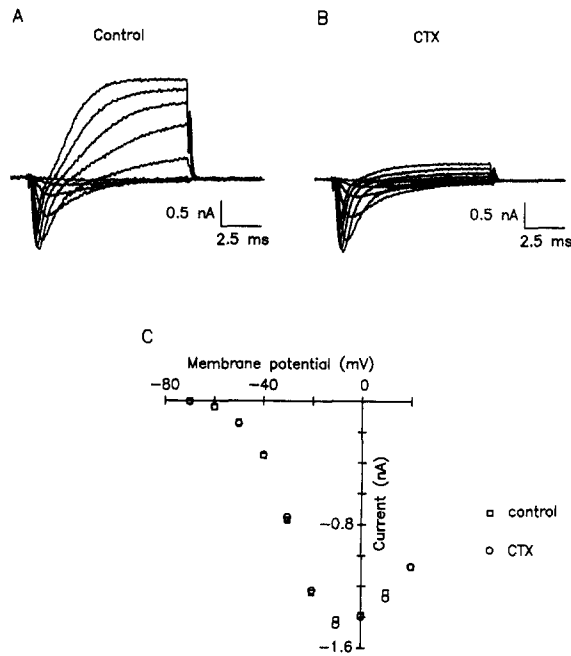


FIGURE 5. CTX does not block voltage-gated Na<sup>+</sup> channels in Jurkat cells. Data were filtered at 6 kHz. (A) Family of inward Na<sup>+</sup> and outward K<sup>+</sup> currents evoked by a series of pulses from -60 to +20 mV in 10-mV increments, delivered after a 100-ms prepulse to -100 mV (holding potential, -80 mV). (B) Family of currents evoked by the same series of pulses in the presence of 26 nM CTX<sub>c</sub>. The K<sup>+</sup> current is largely blocked, while Na<sup>+</sup> current is unaffected. (C) Na<sup>+</sup> current-voltage relation in the presence or absence of CTX, plotted using peak inward currents from A and B.

cumulative inactivation during repetitive depolarizations and a low sensitivity to block by TEA, with half-block occurring at 100 mM. As shown above for type *n* channels, application of CTX (8.5 nM) almost completely abolished type *n'* K<sup>+</sup> current. Some cytotoxic/suppressor cells express mainly type *l* channels, classified on the basis of little or no cumulative inactivation, rapid channel-closing kinetics, and

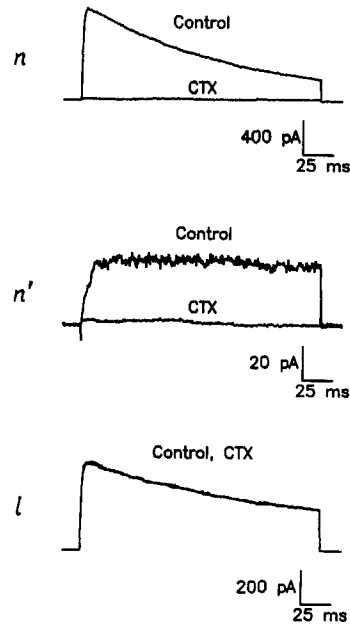


FIGURE 6. Selective block by CTX of K<sup>+</sup> channel subtypes in murine thymocytes. Each panel shows K<sup>+</sup> currents (types *n*, *n'*, or *l*) elicited by pulses from -80 to +30 mV in the absence or presence of 8.5 nM CTX<sub>M</sub> (*n* and *n'*) or 34 nM CTX<sub>m</sub> (*l*). CTX blocks type *n* (top) and *n'* (middle) currents, but has no effect on *l*-type channels (bottom). CTX-resistant current in the middle panel was due to a single type *l* channel in the cell. Channel subtypes were identified on the basis of use-dependent inactivation, tail-current kinetics, and sensitivity to block by TEA (Lewis and Cahalan, 1988a).

high sensitivity to TEA, with half-block occurring at  $\sim 100 \mu\text{M}$ . Unlike *n* and *n'* channels, type *l* channels are resistant to block by CTX. Fig. 6 C illustrates a cell that had been classified as type *l*, showing no effect of 34 nM CTX.

CTX provides a useful probe for the pharmacological separation of multiple channel types present in a given cell, as illustrated in Fig. 7. This cytotoxic/suppressor thymocyte had a bimodal conductance-voltage curve and both rapid and slow components to the tail current upon repolarization to  $-50 \text{ mV}$ , indicating the presence of multiple K<sup>+</sup> channel subtypes. Application of 8.5 nM CTX reduced the current during the depolarizing pulse by  $\sim 40\%$  by selectively blocking the slowly closing channels, as shown in Fig. 7 B. The remaining current represented K<sup>+</sup> flux through type *l* channels.

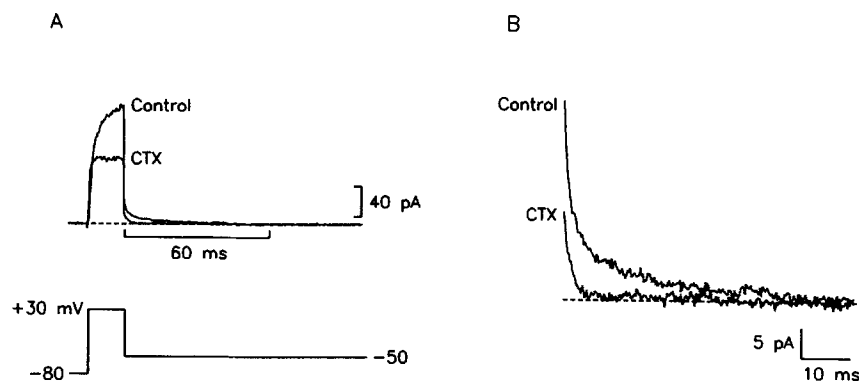


FIGURE 7. Separation of multiple K<sup>+</sup> current subtypes in a murine thymocyte using CTX. (A) Whole-cell K<sup>+</sup> current (*upper trace*) evoked by a brief depolarization to  $+30 \text{ mV}$  (*lower trace*) is partially blocked by 8.5 nM CTX. This cell displayed a combination of type *n'* and *l* K<sup>+</sup> channels, which produced two kinetic components in tail currents upon repolarization to  $-50 \text{ mV}$ ; a 60-ms segment of the data is shown at higher gain on an expanded time base in B. (B) CTX selectively blocks the type *n'* channels. The slowly decaying tail current component (type *n'*) is blocked by CTX, while the rapidly decaying component (from *l* channels) is unaffected.

#### *Other Scorpion Venoms and Toxins*

We examined other venoms and toxins for effects on K<sup>+</sup> currents in Jurkat cells. NTX, like CTX, is a potent blocker of type *n* K<sup>+</sup> current in lymphocytes. Fig. 8 illustrates families of K<sup>+</sup> and Na<sup>+</sup> currents before and after application of 4 nM NTX; K<sup>+</sup> currents are blocked reversibly by NTX, whereas Na<sup>+</sup> currents are unaffected. The NTX dose-response curve for K<sup>+</sup> channel block is shown in Fig. 8 C; the half-maximal blocking dose of 0.2 nM is somewhat lower than that for CTX. In further experiments we found that several crude scorpion venoms also blocked lymphocyte type *n* K<sup>+</sup> current, including venom from *Pandinus imperator* (10  $\mu\text{g}/\text{ml}$  blocked 80–90%), *Buthus tamulus* (1  $\mu\text{g}/\text{ml}$  blocked 51%), and *Androctonus australis* (10  $\mu\text{g}/\text{ml}$  blocked  $\sim 55\%$ ). Toxin from *Tityus serrulatus* had little effect on the K<sup>+</sup> current. Apamin, the venom from the honeybee (*Apis mellifera*), at 10  $\mu\text{g}/\text{ml}$  (4.9

$\mu\text{M}$ ) had no effect on the  $\text{K}^+$  current in Jurkat cells. These results illustrate that venoms from several species of scorpions contain toxins that block voltage-gated  $\text{K}^+$  channels.

#### DISCUSSION

We have demonstrated that purified samples of both CTX and NTX from *Leiurus* and *Centruroides* scorpions, respectively, block type *n* voltage-gated  $\text{K}^+$  channels in human and murine lymphoid cells. In addition, we have shown that CTX blocks a similar  $\text{K}^+$  channel (type *n'*) in mouse thymocytes, but does not affect either type *l*  $\text{K}^+$  channels or  $\text{Na}^+$  channels. Although type *n* and *n'* channels differ in their inacti-

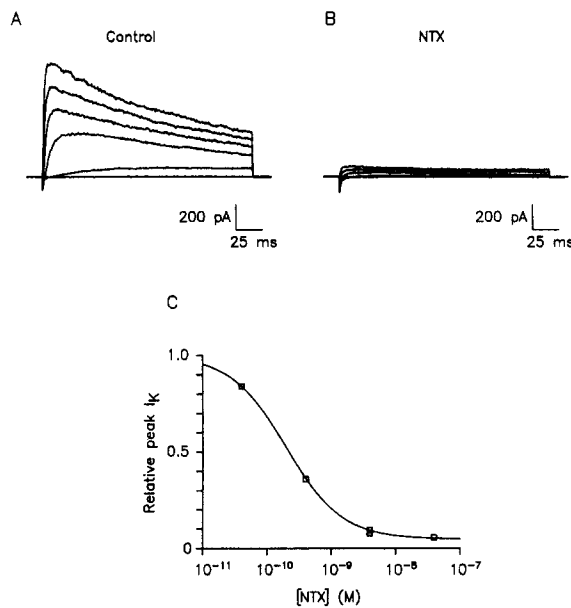


FIGURE 8. NTX blocks type *n*  $\text{K}^+$  channels but not  $\text{Na}^+$  channels in Jurkat cells. (A) Family of currents evoked by pulses from  $-50$  to  $+50$  mV in  $20$ -mV increments applied from a holding potential of  $-80$  mV. Depolarization elicits a rapid, inward  $\text{Na}^+$  current followed by outward  $\text{K}^+$  current. (B) Currents evoked by the same series of pulses in the presence of  $4$  nM NTX. The toxin substantially blocks the  $\text{K}^+$  channels but leaves  $\text{Na}^+$  channels unaffected. (C) Dose-response relation for block of type *n*  $\text{K}^+$  channels by NTX. Peak  $\text{K}^+$  current during pulses to  $+30$  mV is plotted against toxin concentration on a log scale. A single-site binding curve with  $K_d = 0.20$  nM has been fitted visually to the data; for the best fit at higher concentrations,  $5\%$  of the channels were assumed to be insensitive to the toxin.

vation properties and sensitivity to block by TEA (Lewis and Cahalan, 1988a), the fact that CTX blocks both with high affinity suggests that these two varieties of  $\text{K}^+$  channel share a common toxin receptor.

#### Mechanism of Block by CTX

Using a rapid local perfusion system we measured the kinetics of CTX block and recovery of macroscopic whole-cell  $\text{K}^+$  currents in Jurkat cells. Under the conditions

of our experiments (holding potential of  $-80$  mV, brief depolarizing test pulses to  $+30$  mV) the observed on- and off-rates of  $5.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  and  $0.1 \text{ s}^{-1}$ , respectively, probably represent the rates of binding to and unbinding from closed channels at  $-80$  mV. CTX binding to closed channels is indicated by the observation that channel block, assayed by the current evoked by a single test depolarization, can be fully established by exposure to the toxin at a constant holding potential of  $-80$  mV (data not shown). The equilibrium dose-response relation for the CTX<sub>C</sub> sample indicates a  $K_d$  value of  $1.5$  nM (Fig. 2), which is in good agreement with the calculated  $K_d$  value of  $1.8$  nM, computed from  $k_{\text{off}}/k_{\text{on}}$  measured with the same toxin sample. We conclude from these results that CTX is a potent and reversible K<sup>+</sup> channel blocker, binding with simple bimolecular kinetics to a site on the external surface of the channel.

Miller and colleagues studied CTX block of single Ca<sup>2+</sup>-activated "maxi"-K<sup>+</sup> channels and determined the influence of channel conformation (open vs. closed), membrane potential, external ionic strength, and internal [K<sup>+</sup>] on the toxin on- and off-rates (Anderson et al., 1988; MacKinnon and Miller, 1988). Binding of CTX to the lymphocyte K<sup>+</sup> channel is similar in some respects. In lymphocytes, the toxin can bind to either closed or open channels, since channels can be blocked at  $-80$  mV (see above), and there is no indication of recovery during a depolarizing pulse.  $K_d$  values are in the nanomolar range for both lymphocyte K<sup>+</sup> channels (Fig. 2) and Ca<sup>2+</sup>-activated K<sup>+</sup> channels (Anderson et al., 1988; Gimenez-Gallego et al., 1988). Toxin on- and off-rates are more rapid for the lymphocyte K<sup>+</sup> channel than for the Ca<sup>2+</sup>-activated K<sup>+</sup> channel. At comparable ionic strength, our measured on-rate (Fig. 4) is about 15-fold faster than that for closed, and twofold faster than that for open Ca<sup>2+</sup>-activated K<sup>+</sup> channels, while the off-rate for the lymphocyte K<sup>+</sup> channel is approximately 10-fold faster than the limiting off-rate for Ca<sup>2+</sup>-activated K<sup>+</sup> channels at negative potentials. As suggested for the Ca<sup>2+</sup>-activated K<sup>+</sup> channel in muscle (Anderson et al., 1988), scorpion toxin binding to lymphocyte K<sup>+</sup> channels appears to be influenced by a negative surface potential; raising external [Ca<sup>2+</sup>] reduced the apparent potency of *Pandinus* venom (data not shown). Although no voltage dependence of CTX block was detected in our experiments (Fig. 3), the depolarizing pulse durations used may have been too brief for significant unbinding to occur. Inactivation of type *n* K<sup>+</sup> channels during depolarizing pulses and slow recovery from inactivation complicate the kinetic analysis of CTX binding and unbinding, particularly at the level of single channels. Despite these uncertainties, the most likely conclusion is that CTX blocks lymphocyte K<sup>+</sup> channels by a simple plugging mechanism, as hypothesized for the Ca<sup>2+</sup>-activated K<sup>+</sup> channel (Anderson et al., 1988; MacKinnon and Miller, 1988).

#### *CTX as a Probe for a Family of K<sup>+</sup> Channels*

In addition to blocking Ca<sup>2+</sup>-activated K<sup>+</sup> channels from skeletal muscle in lipid bilayers (Miller et al., 1985), CTX blocks closely related Ca<sup>2+</sup>-activated K<sup>+</sup> channels in a variety of cells, including PC-12 cells (Hoshi and Aldrich, 1988), GH<sub>3</sub> cells (Gimenez-Gallego et al., 1988), cloned renal tubule cells (Guggino et al., 1987), and *Drosophila* muscle (Elkins et al., 1986). A smaller conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel from molluscan neurons is also blocked by CTX (Hermann and Erxleben,

1987). Despite their sensitivity to the toxin, type *n* K<sup>+</sup> channels from lymphocytes are not activated by intracellular Ca<sup>2+</sup> ions; instead, raising cytosolic [Ca<sup>2+</sup>] reduces the macroscopic K<sup>+</sup> current and accelerates the rate of channel inactivation (Bregestovski et al., 1986; Choquet et al., 1987; Grissmer and Cahalan, 1989b). Thus, block by CTX should not be considered as diagnostic for Ca<sup>2+</sup>-activated K<sup>+</sup> channels, as it applies to a subset of voltage-gated K<sup>+</sup> channels as well.

The *Shaker* gene in *Drosophila* encodes several functionally distinct K<sup>+</sup> channels differing in kinetic properties of inactivation (Kamb et al., 1988; Timpe et al., 1988). These channels have been termed "A" current channels, because their relatively fast inactivation kinetics are similar to those of the molluscan A currents described by Connor and Stevens (1971). In microelectrode recordings from *Drosophila* larval muscle, CTX was shown to block Ca<sup>2+</sup>-activated K<sup>+</sup> channels, but not to affect the A currents encoded by the *Shaker* gene (Elkins et al., 1986). The lack of effect on *Shaker*-related K<sup>+</sup> currents in *Drosophila* cells has been confirmed by Aldrich and colleagues, using patch-clamp techniques (Dr. R. Aldrich, personal communication). Recently, however, CTX was shown to block A currents expressed in *Xenopus* oocytes after *Shaker* mRNA injection (MacKinnon et al., 1988). The possible reasons for the difference in sensitivity of *Shaker* A currents in the fly as opposed to those expressed in *Xenopus* oocytes remain to be explored. Type *n* K<sup>+</sup> channels from lymphocytes inactivate about 10-fold more slowly than *Shaker* A currents during depolarizing pulses, but share the property of slow recovery from inactivation found in some variants of *Shaker* (Timpe et al., 1988). High affinity block by CTX of the Ca<sup>2+</sup>-activated K<sup>+</sup> channel, the *Shaker* K<sup>+</sup> channel, and type *n* and *n'* lymphocyte K<sup>+</sup> channels suggests an underlying structural similarity at the toxin-receptor site for these four members of a very large family of K<sup>+</sup> channels. Future studies using molecular genetic techniques may help to characterize the CTX binding site and further delineate this region of similarity.

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