

Charybdotoxin block of Ca^{2+} -activated K^+ channels in colonic muscle depends on membrane potential dynamics

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Frey, Bret W., Andreas Carl, and Nelson G. Publicover. Charybdotoxin block of Ca^{2+} -activated K^+ channels in colonic muscle depends on membrane potential dynamics. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C673–C680, 1998.—Charybdotoxin (ChTX) is a specific blocker of Ca^{2+} -activated K^+ channels. The voltage- and time-dependent dynamics of ChTX block were investigated using canine colonic myocytes and the whole cell patch-clamp technique with step and ramp depolarization protocols. During prolonged step depolarizations, K^+ current slowly increased in the continued presence of ChTX (100 nM). The rate of increase depended on membrane potential with an e -fold change for every 60 mV. During ramp depolarizations, the effectiveness of ChTX block depended significantly on the rate of the ramp (50% at 0.01 V/s to 80% at 0.5 V/s). Results are consistent with a mechanism in which ChTX slowly “unbonds” in a voltage-dependent manner. A simple kinetic model was developed in which ChTX binds to both open and closed states. Slow unbinding is consistent with ChTX having little effect on electrical slow waves recorded from circular muscle while causing depolarization and contraction of longitudinal muscle, which displays more rapid “spikes.” Resting membrane potential and membrane potential dynamics are important determinants of ChTX action.

voltage dependence; calcium-activated potassium channels; colonic motility; model; maxi-potassium channels

CALCIUM-ACTIVATED POTASSIUM (BK or maxi- K^+) channels are abundantly expressed in a wide variety of smooth muscles, but their physiological role in these tissues continues to be controversial. The development of a number of specific blockers of BK channels such as charybdotoxin (ChTX; Ref. 22) and iberiotoxin (IbTX; Ref. 8) has allowed a more rigorous examination of the role of these channels in intact tissues. For example, Suarez-Kurtz et al. (28) have tested the effects of ChTX on smooth muscles from several sources. They report that depolarization and contraction are stimulated by ChTX in guinea pig bladder, taenia coli, and aorta but not in portal vein, uterus, or trachea. Because all of these tissues generously express BK channels, reasons for these differences in ChTX sensitivity remain unclear.

In a previous study (3), we investigated the effects of *Leiurus quinquestriatus* scorpion venom ChTX on electromechanical activity in intact muscle strips of isolated longitudinal and circular layers of canine colon to evaluate the physiological roles of these channels. The effects of ChTX in tissues were compared with effects on whole cell outward currents in enzymatically isolated myocytes from both muscle layers. In the circular layer, ChTX does not affect in vitro basal electrical activity

but alters responses to excitatory agonists. The lack of an effect on basal activity has been used to argue that BK channels do not play a role in the repolarization phase of the slow wave (15). In contrast, in longitudinal muscle strips, ChTX depolarizes membrane potential and increases burst duration as well as spiking frequency, resulting in increases in the force of spontaneous contractions. Despite these observations, no differences have been found in current density (pA/pF) as well as in ChTX, voltage, or Ca^{2+} sensitivity of BK channels in myocytes isolated separately from the two muscle layers (3).

In the present study, we investigated the voltage- and time-dependent kinetics of ChTX block of BK channels in colonic myocytes to determine whether the apparent difference in ChTX sensitivity in longitudinal and circular muscle strips may be due to the type of electrical activity displayed by these cells [spiking vs. nonspiking and resting membrane potential (RMP)]. The aim was to compare the ChTX sensitivity of Ca^{2+} -activated K^+ currents [$I_{\text{K}(\text{Ca})}$] elicited by different voltage protocols and to develop a quantitative model of ChTX binding to BK channels that describes these kinetics.

METHODS

Preparation of cells. Dogs of either sex were killed with pentobarbital sodium (100 mg/kg), and the colon was removed. Circular and longitudinal muscle strips were dissected from the proximal colon, viewed under a dissecting microscope. Muscle strips were separated into small pieces and placed in a Ca^{2+} -free Hanks' solution containing (in mM) 125 NaCl, 5.36 KCl, 15.5 NaHCO_3 , 0.336 Na_2HPO_4 , 0.44 KH_2PO_4 , 10 glucose, 2.9 sucrose, and 5 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 0.11 mg/ml ATP, 1 mg/ml collagenase type III (Worthington), 2 mg/ml trypsin inhibitor (Sigma), 0.1 mg/ml protease type XIV (Sigma), and 1 mg/ml bovine serum albumin (Sigma) at pH 7.4 (adjusted with KOH at 37°C). After a 20- to 30-min incubation period, strips were rinsed in Ca^{2+} -free solution and briefly agitated using a vortex mixer to disperse cells. Isolated cells were collected and added to minimum essential medium Eagle (Sigma) supplemented with (in mM) 0.5 CaCl_2 , 5 MgCl_2 , 4.17 NaHCO_3 , and 10 HEPES. Cells were stored at 4°C and used within 1–8 h. Fully relaxed myocytes with smooth-appearing membranes adhered to the glass bottom of a recording chamber (volume of ~300 μl) mounted on the stage of a Nikon TMS inverted microscope.

Whole cell patch-clamp recordings. Currents were recorded using the cell-attached or whole cell mode of the patch-clamp technique (10). High-resistance seals (>5 G Ω) were formed using borosilicate electrodes (1.5–3 M Ω). An Axopatch 1D amplifier (Axon Instruments) was used for current recordings. Data were digitized at sampling rates of up to 10 kHz

and filtered at 500 Hz. Capacitance was compensated for, and residual capacitance current was digitally removed. Current from hyperpolarizing pulses (represented by x , in mV) was multiplied by (test potential – holding potential)/ x and subtracted from currents recorded during test pulses. Series resistance (between 4 and 8 M Ω) was not compensated for. Current amplitudes were below 1,500 pA, resulting in a voltage error of <12 mV. Data are corrected for a –10-mV liquid junction potential.

Solutions. The bath solution initially contained (in mM) 140 NaCl, 5 KCl, 2 CaCl_2 , 1.2 MgCl_2 , 10 dextrose, 10 HEPES, and 5 tris(hydroxymethyl)aminomethane at pH 7.4. In most experiments, CaCl_2 was replaced by equimolar MnCl_2 to remove Ca^{2+} inward currents. The standard pipette solution contained (in mM) 20 KCl, 110 potassium gluconate, 5 MgCl_2 , 2.5 K_2ATP , 0.1 Na_2GTP , 2.5 Na_2 creatine phosphate, 5 HEPES, and 1 1,2-bis(2-aminophenoxy)ethane- N,N,N,N' -tetraacetic acid at pH 7.0. All patch-clamp experiments were carried out at room temperature (24°C).

ChTX was obtained from Peptides International (Louisville, KY). During all procedures involving ChTX, the drug (100 nM, unless otherwise noted) was added at least 2 min before voltage-clamp protocols. During this time, cells were held at –50 mV.

Modeling and data analysis. The model illustrated in Fig. 4 can be represented as a series of four simultaneous, coupled differential equations. To solve these equations, numerical integration was performed with the Gear method (12). The fraction of channels in the open state (O in Fig. 4) was converted to a current using the Goldman-Hodgkin-Katz equation (13) to account for the effects of driving force, with intracellular K^+ concentration = 140 mM and extracellular K^+ concentration = 5 mM.

For acquisition and analysis of patch-clamp data, pCLAMP software (Axon Instruments, version 5.5.1) was used. Data are expressed as means \pm SE. Levels of significance were calculated using the Student's t -test where appropriate.

RESULTS

Block of BK channels by ChTX: voltage ramps vs. steps. To determine the effectiveness of ChTX block under conditions comparable to components of electrical activity in vivo, ChTX block of large-conductance BK channels was assessed using both step and ramp depolarizations. Myocytes were isolated from the circular layer of canine proximal colon. Inward Ca^{2+} currents were suppressed by replacement of bath Ca^{2+} with 2 mM Mn^{2+} . Cells were either held at –50 mV and then step depolarized for 3 s to test potentials of 0 to +100 mV in 10-mV increments (Fig. 1, *A* and *B*, *top*) or held at –50 mV and then ramped from –100 to +100 mV within 4 s [ramp rate (dV/dt) = 0.05 V/s; Fig. 1, *A* and *B*, *bottom*]. Because ChTX blocks BK channels with slow kinetics (11), one might expect that ChTX might be more potent in blocking current elicited by step depolarizations than current elicited by ramp depolarizations. However, our experiments showed that ChTX was significantly more potent when 0.05 V/s ramp depolarizations were used (Fig. 1).

The outward currents shown in Fig. 1 (*A* and *B*, *top*) are composed of delayed rectifier K^+ currents (I_{dK}) as well as $I_{\text{K(Ca)}}$. The activation curve of BK channels is shifted toward positive potentials, allowing current attributable to I_{dK} to be partially distinguished from

$I_{\text{K(Ca)}}$. To reduce the amount of I_{dK} through inactivation (3), cells were held at 0 mV for at least 2 min before step depolarization to test potentials of 0 to +100 mV. In Fig. 1 (*A* and *B*, *middle*), reduced I_{dK} and total current are demonstrated, especially at lower test potentials. By comparing currents elicited from the –50-mV holding potential with those from the 0-mV holding potential, it can be seen that ChTX continued to suppress the noisy $I_{\text{K(Ca)}}$, particularly at more positive potentials. The continued presence of I_{dK} shown in Fig. 1 (*B*, *top*) suggests that ChTX does not significantly affect I_{dK} .

In Fig. 1 (*A* and *B*, *bottom*), the degree of ChTX block depends on the specific protocols used to measure binding. At +80 mV, ChTX (100 nM) blocked $61 \pm 1\%$ ($n = 5$ cells) of current elicited by 3-s step depolarizations. At the same test potential, following a ramp depolarization at a rate (dV/dt) of 0.05 V/s, ChTX blocked $73 \pm 2\%$ of current elicited by ramps ($n = 6$ cells). At this ramp rate, the degree of ChTX block differed compared with step depolarizations ($P < 0.03$). However, the effectiveness of ChTX suppression of currents elicited by voltage ramps was found to depend significantly on the rate of ramp depolarization. Figure 2 shows some examples of the degree of block of whole cell current at different ramp rates. ChTX (100 nM) was less effective in suppressing current during slow ramps (Fig. 2*A*) than current during faster ramps (Fig. 2, *B* and *C*). The degree of block was proportional to the concentration of ChTX, suggesting that availability of drug was not a limiting factor (3). These data suggest that ChTX may be less effective during slow ramps due to voltage-dependent “unbinding.”

To determine if the dependence of ChTX block on ramp rate might be due to such a mechanism, currents were measured during prolonged (30–60 s) step depolarizations. Figure 3 shows an example of current traces recorded from a colonic myocyte during 60-s depolarizations to +80 mV. Under control conditions (i.e., in the absence of ChTX), current was sustained at a relatively constant level throughout the 60-s period. In the presence of 100 nM ChTX, current was initially suppressed by >80% compared with control. The current amplitude relaxed to 60% at 250 ms following depolarization and then declined to a steady-state level with little apparent block remaining in this experiment (Fig. 3). In four experiments, current suppression averaged $68 \pm 7\%$ when measured at 250 ms compared with $22 \pm 10\%$ once steady state was achieved ($P < 0.01$). This current relaxation appeared to be due to a slow unbinding of ChTX during the sustained depolarization. The unbinding resulted in a current relaxation that was well fit by a single exponential function. The average time constant of relaxation in seven myocytes isolated from the circular muscle layer at +80 mV was 8.7 ± 2.1 s. With the use of the same protocol, a similar time constant for unbinding was observed in cells isolated from the longitudinal layer (9 s; $n = 2$).

Model of ChTX block of BK channels. These findings prompted the development a kinetic model of ChTX block to test if results were consistent with a simple unbinding mechanism and to quantitatively describe

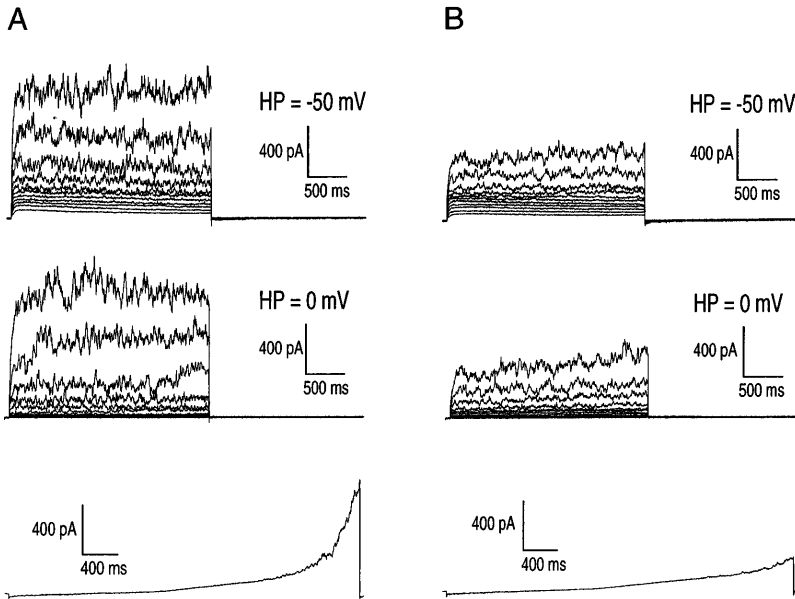


Fig. 1. Block of Ca^{2+} -activated K^+ current [$I_{\text{K}(\text{Ca})}$] by charybdotoxin (ChTX). Outward currents were recorded from myocytes isolated from the circular layer of canine colon. With the use of a step depolarization protocol from a holding potential (HP) of -50 mV to test potentials of 0 to $+100$ mV in 10 -mV increments, both delayed rectifier K^+ current (I_{dK}) and $I_{\text{K}(\text{Ca})}$ were apparent (A and B, top traces). Over the same range of test potentials, I_{dK} largely inactivated when cells were held at 0 mV for >2 min, leaving primarily $I_{\text{K}(\text{Ca})}$ (A and B, middle traces). Voltage ramps (-100 to $+100$ mV) were performed with a ramp rate (dV/dt) = 0.05 V/s (A and B, bottom traces). Compared with control (A), $I_{\text{K}(\text{Ca})}$ in the presence of ChTX (100 nM, B) was reduced by $\sim 75\%$ during voltage ramps. Current reduction was less ($\sim 65\%$) when elicited by 3 -s step depolarizations.

the dependence of ChTX block on ramp speed. The model also allows the efficacy of ChTX block to be predicted under any membrane potential conditions, including *in vivo* electrical activity. The gating of BK channels in skeletal muscle cells (myotubes) has been investigated in great detail by Magleby and co-workers (Refs. 17–20). They found that distributions of open and closed-time intervals were best described by the sum of three to four and six to eight exponential components, respectively, suggesting the existence of at least three open and six closed states (19). The kinetics of open-open and closed-closed transitions are rapid compared with ChTX binding. Therefore, for the present study, we have used a simplified model (Fig. 4), which considers only one open-close transition and assumes that ChTX blocks to closed and open states with the same affinity.

As shown in Fig. 5, the activation time constant of $I_{\text{K}(\text{Ca})}$ in the range of $+50$ to $+90$ mV was largely independent of voltage. However, channel activity (open channel probability as a function of potential) follows a Boltzmann relationship (5). Therefore, k_1 was set to the average rate at all voltages (30.9 ms^{-1}) and k_{-1} was assigned a voltage dependence according to $k_{-1} = c[1 + e^{(V_{1/2} - V)/K}]$, where the voltage at half-maximal activation ($V_{1/2}$) is 110 mV and the slope factor (K) is 17 mV (5). The constant, c , related to the number of channels per cell, is a simple scale factor that controls the magnitude of the response and the potential where detectable current can be observed. To match experimental results, c was set to 1.0 s^{-1} .

The on-rate (k_2) for ChTX binding was determined using a “recovery from unbinding” protocol illustrated in Fig. 6A. In the presence of 100 nM ChTX, cells were initially stepped from a holding potential of 0 to $+80$ mV for 30 s to assess the total range of block and to achieve a steady-state “unbound” condition. This was followed by a recovery period (Δt) at 0 mV ranging from 3 to 120 s. Cells were then returned by step depolariza-

tion to $+80$ mV when the degree of ChTX block was once again determined. The difference in current at the beginning of the second depolarization compared with steady-state current was normalized to the total range of block in current measured during the initial depolarization. The profile of recovery from unbinding followed an exponential decline and is illustrated in Fig. 6B. From these data, k_2 was determined to be 18.1 s^{-1} .

Rates of unbinding were measured using 30 -s depolarizations over a range of potentials up to $+120$ mV. The degree of block was difficult to curve fit at potentials below $+60$ mV. However, between $+60$ and $+120$ mV (illustrated in Fig. 7, see also Fig. 3), results of ChTX unbinding of currents were well fit by single exponential functions. Rates were found to be voltage dependent, ranging from an average of 13 s at $+60$ mV to 5 s at $+120$ mV. The voltage dependence of off-rates could be described by a function of the form $k_{-2} = 0.027e^{V/60 \text{ mV}} \text{ s}^{-1}$. In other words, an e -fold change in off-rate occurred for every 60 mV.

Simulation of ChTX block during step and ramp depolarizations. With the use of the parameters described above, simulations were performed over a range of membrane potentials and voltage protocols. An example of a response to a voltage step from a holding potential of -50 mV to a test potential of $+80$ mV is shown in Fig. 8. During the 30 -s step depolarization, the model demonstrates a current relaxation, in good agreement with empirical data shown in Fig. 3. The current relaxation is due to a decline in the fraction of channels in the blocked (either closed-blocked or open-blocked) states, and the magnitude of initial block depends on initial conditions (holding potential, corresponding in tissues to RMP).

The model was also used to compute the effects of ChTX on current elicited by voltage ramps (Fig. 9). We found that (in agreement with experimental data) ChTX block was significantly dependent on dV/dt . With the use of slower ramps (Fig. 9A), ChTX was less

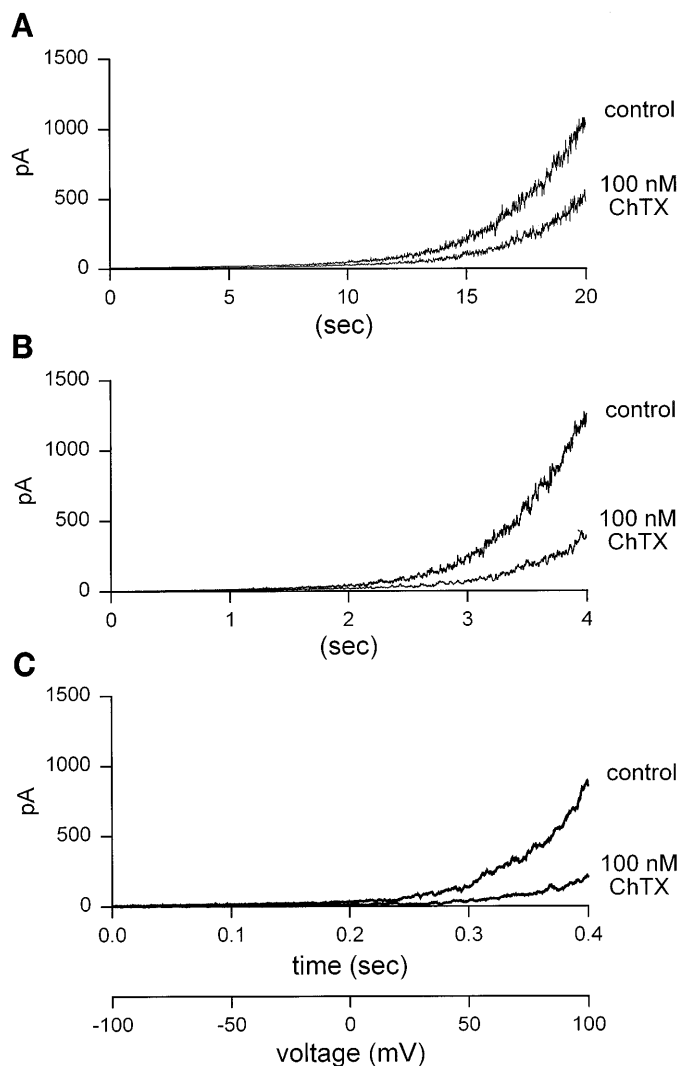


Fig. 2. ChTX block depends on ramp rate. Reduction of $I_{\text{K}(\text{Ca})}$ by ChTX was weaker during slow ramps compared with faster ramps (0 mV holding potentials). At a ramp rate (dV/dt) of 0.01 V/s (A), current was reduced by 50% in the presence of ChTX (100 nM). At a rate of 0.05 V/s (B), current was reduced by 68%, and at 0.5 V/s (C) current was reduced by 78%. All current traces were recorded from the same myocyte isolated from the circular layer.

effective than during faster ramps (Fig. 9, B and C). The decreased effectiveness of ChTX during slow ramps was primarily due to a significant decline in the fraction of channels in the closed-bound (C_b) state during the voltage ramp (Fig. 10A). On the basis of simulations, there was also a progressive decline in the fraction of channels in the closed state and even a decline in the fraction of channels in the open-bound (O_b) during the later part of the slowest voltage ramp (see Fig. 10A). This is due to the flux of channels in transition from the C_b state through the O_b to the open state. During more rapid voltage ramps, more channels remain in bound states (Fig. 10, B and C).

DISCUSSION

The electrical activities of circular and longitudinal muscle layers of the canine proximal colon differ signifi-

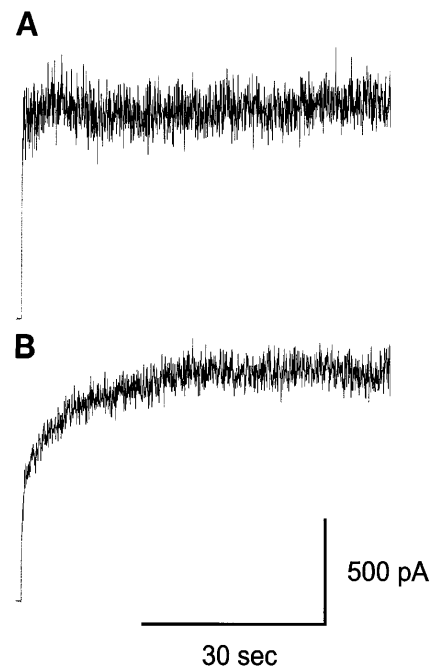


Fig. 3. ChTX block weakens during prolonged depolarization. ChTX binding was determined during sustained step depolarizations from a holding potential of 0 to +80 mV. Compared with control (A), relaxation of block was observed in the presence of 100 nM ChTX (B). When curve fit with a single exponential function, the time constant of this current relaxation was 8.7 s.

cantly (5). The RMP of cells through the thickness of the circular layer varies as a function of distance from the submucosal border (RMP of about -80 mV) to the myenteric layer (RMP of about -40 mV; Ref. 26). Cells near the submucosal border of the circular layer generate slow waves consisting of an upstroke to about -25 to -35 mV (rate of rise <1 V/s), a partial repolarization to about -30 to -40 mV, and a sustained plateau potential that persists for several seconds before repolarization to the RMP (26, 27). Cells in the longitudinal muscle layer of the canine colon have a more positive resting potential (about -60 mV) and exhibit low-

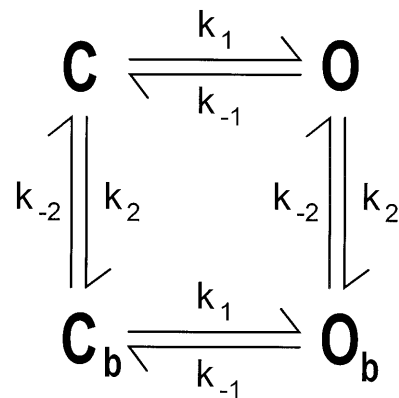


Fig. 4. Model of ChTX block of Ca^{2+} -activated K^+ (BK) channels. C and O represent the closed (nonconducting) and open states, respectively. C_b and O_b similarly correspond to closed and open bound nonconducting states, where ChTX binds to both open and closed states with the same affinity. Rate constants k_1 , k_{-1} , k_2 , and k_{-2} were determined as described in the text.

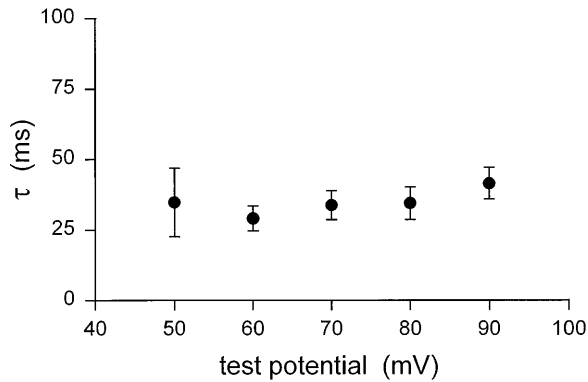


Fig. 5. Time constant of BK channel activation is independent of test potential. Rates of activation during 2-s step depolarizations were measured from a holding potential of 0 mV to a range of test potentials (+50 to +90 mV in 10-mV increments) in 11 colonic myocytes. Time constants (τ) were determined from curve fits of a single exponential function using pCLAMP software. SE bars represent at least 5 measurements. No significant voltage dependence of τ was found. Average time constant of activation (all cells at all potentials) was 30.9 ± 2.2 ms ($n = 41$ measurements).

amplitude oscillations in membrane potential (myenteric potential oscillations), with individual Ca^{2+} action potentials superimposed (7, 14, 27). These spikelike action potentials (SLAPs; Ref. 16) have a much more rapid rate of rise (1–4 V/s), reach a peak at about -10 to $+10$ mV, and repolarize rapidly with a total duration <50 ms (29).

Although both tissues express BK channels with identical properties (i.e., voltage sensitivity, Ca^{2+} sensitivity, ChTX sensitivity) and similar current density (pA/pF), the effect of ChTX on these tissues is strikingly different (3). Bath-applied ChTX (100 nM) has no effect on basal (i.e., unstimulated by agonist) electrical slow waves in the circular muscle layer but increases both spiking and contraction generated by the longitudinal muscle layer (3). Data in the present study suggest that part of this difference may be due to the type of electrical activity (spiking vs. nonspiking) as well as RMPs in the two muscle layers.

To measure binding/unbinding rates, it was necessary during patch-clamp studies to use a positive range of membrane potentials compared with physiological conditions. During patch-clamp protocols, there may be additional differences compared with recordings from tissues, including intracellular Ca^{2+} concentrations and the effects of temperature. Despite these differences, it is evident that unbinding may occur, in which simulations using the model presented in Fig. 4 suggest that there is $>50\%$ unbinding during the time course of a colonic slow-wave event (average duration of 6.3 s) compared with $<1\%$ unbinding during SLAPs with durations of <50 ms (see Figs. 3 and 8). These results suggest that experiments using “slow blockers” (11) such as ChTX or IbTX during spontaneous electrical activity in intact tissues need to be interpreted with caution.

In circular muscle, BK channels likely contribute to the process of slow-wave repolarization (4). However, the relative contribution of BK channels compared with

other voltage-dependent K^+ channels and inactivation of Ca^{2+} currents remains controversial (for review, see Ref. 23). Part of the reason for this controversy is the lack of effect of BK blockers such as ChTX and IbTX on basal slow-wave activity. The lack of effect argues in favor of little or no contribution to the repolarization process by BK channels (e.g., Ref. 15). However, as shown in the present study, substantial unbinding may occur during a slow wave, since repolarization follows a prolonged depolarization.

Previous studies (3) have also shown that, although ChTX does not alter basal slow-wave activity in circular muscle strips, it can alter responses to excitatory agonists. In the presence of 10^{-6} M acetylcholine (ACh), ChTX (100 nM) increased slow-wave duration by 0.6 s and slow-wave amplitude by 4.4 mV, resulting in a doubling of the force of phasic contractions. ACh alone causes both membrane depolarization (2) and a sustained elevation in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) (25). Both factors might contribute to the apparent increase in ChTX effectiveness. The Ca^{2+} -channel opener BAY K 8644 caused an increase in $[\text{Ca}^{2+}]_i$ as well as an RMP of 8.5 mV over control. The further addition of ChTX (100 nM) caused further depolarization (2.5 mV). Therefore, agonist-induced

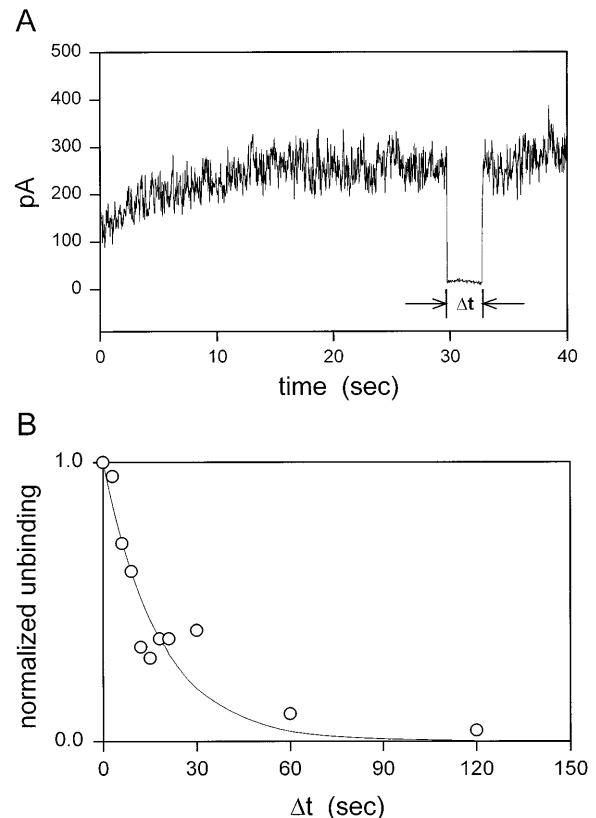


Fig. 6. On-rate of ChTX block at 0 mV. *A*: on-rate of ChTX block was estimated using initial step depolarization (30 s) to +80 mV that was followed by recovery to 0 mV for varying durations [recovery period (Δt) = 3–120 s] and a second step depolarization to +80 mV. *B*: degree of ChTX block (i.e., difference current) at the beginning of the second step depolarization was normalized to difference in current during the initial 30-s step depolarization. When fitted by a single exponential, time constant for recovery at 0 mV was 18.1 s.

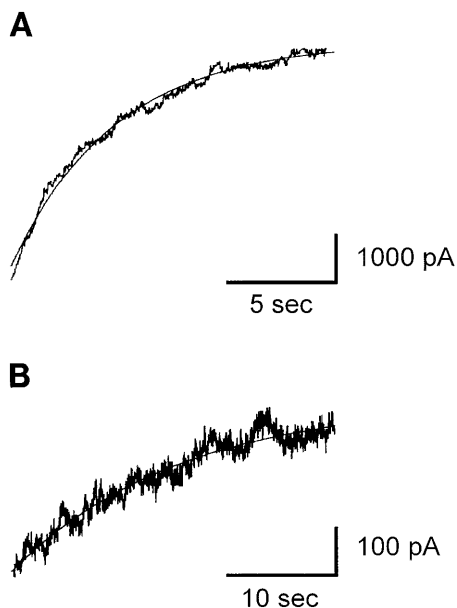


Fig. 7. Voltage dependence of unbinding. Colonic myocytes were stepped from a holding potential of 0 mV to test potentials of +120 mV (A) and +60 mV (B) in the presence of 100 nM ChTX. Rates of unbinding were found to be voltage dependent. Curve fits (smooth traces) represent rates of 4.9 s at +120 mV and 15.5 s at +60 mV.

membrane depolarization as well as changes in $[\text{Ca}^{2+}]_i$ may alter the dynamics of ChTX block of BK channels.

Differences in the ability to buffer intracellular Ca^{2+} might result in dramatically different magnitudes and time courses of submembrane Ca^{2+} transients and subsequent activation of BK channels. Under conditions of low-intracellular Ca^{2+} buffering (whole cell voltage-clamp conditions utilizing 0.1 mM ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid in the pipette solution), smooth muscle cells from the longitudinal layer of canine colon often display spontaneous transient outward currents (STOCs), whereas cells from the circular muscle layer do not

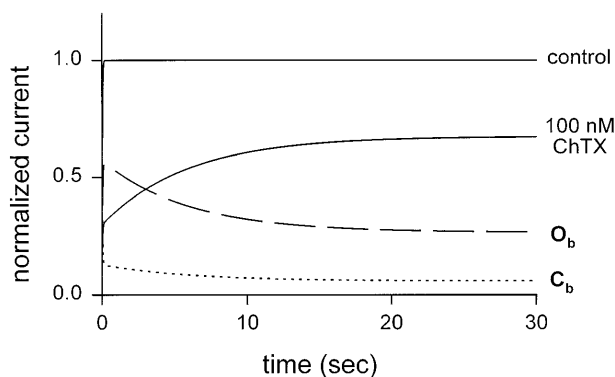


Fig. 8. Simulation of responses to a voltage-clamp step. A simulation of responses to a voltage step from -50 to $+80$ mV was performed using numerical techniques. Fraction of channels in the open state was converted to current using the Goldman-Hodgkin-Katz equation. Simulation of a 30-s step depolarization mimics the relaxation of ChTX block observed in myocytes (as illustrated in Fig. 3). This relaxation is due to a decline in the fraction of channels in the blocked states and depends on initial conditions (i.e., fraction of channels starting off in C_b and O_b states).

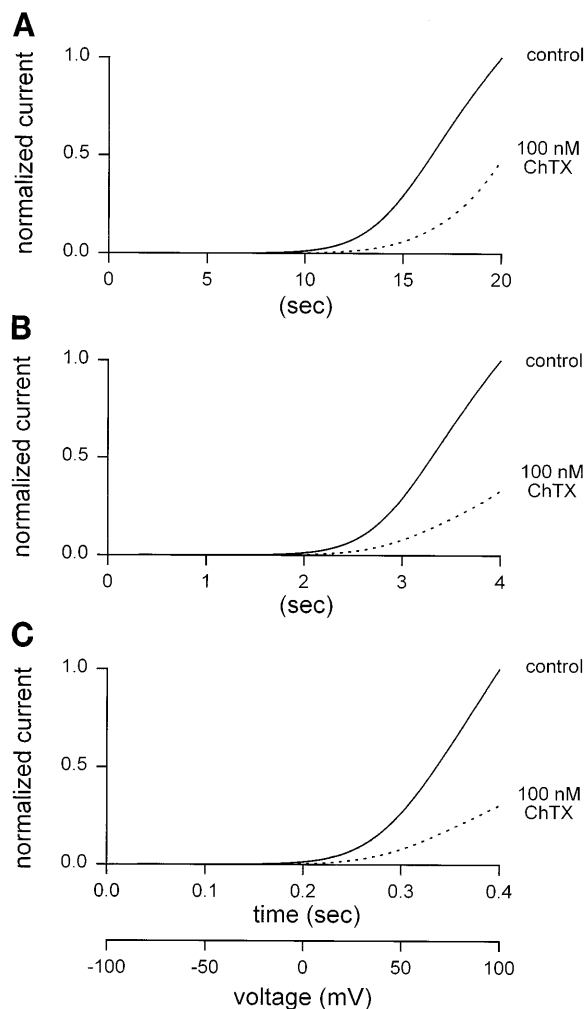


Fig. 9. Simulation of currents in response to voltage ramps. Simulations of responses to voltage ramps from -100 to $+100$ mV over a range of rates from 0.01 to 0.5 V/s were performed using numerical techniques. Normalized currents in absence (control) and presence of ChTX were computed using the Goldman-Hodgkin-Katz equation. In agreement with experimental observations (Fig. 2), ChTX was less effective in suppressing current elicited by slow ramps (A, 0.01 V/s) compared with current elicited by faster ramps (B and C, 0.05 and 0.5 V/s, respectively).

display STOCs (Carl, unpublished observations). It is possible that longitudinal muscle cells reach higher transient submembrane Ca^{2+} levels than circular cells. This would result in a larger degree of activation of BK channels in the longitudinal layer that would result in a larger ChTX sensitivity of this muscle layer, even if all other channel properties were the same.

Suarez-Kurtz and colleagues (28) have pointed out that it is difficult to relate the effects of ChTX and IbTX from one tissue or species to another. However, some of the variability they reported may be attributed to the electrical activities in each tissue. Spontaneous contractions in tissues that were not affected by ChTX (portal vein and uterus) were prolonged (up to 1 min) compared with tissues stimulated by ChTX (bladder, taenia coli, and aorta). Prolonged depolarizations (associated with contractions) may allow time for relaxation of ChTX block. Guinea pig trachea showed no significant

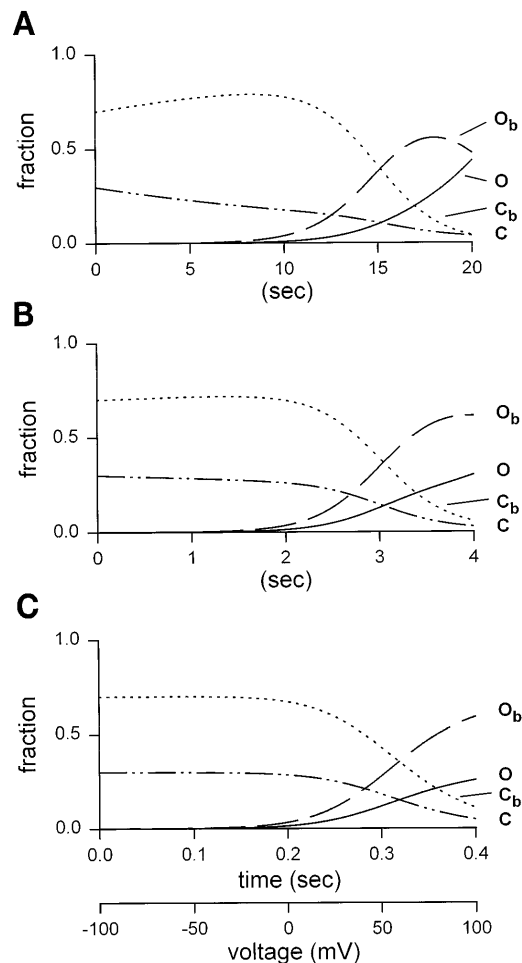


Fig. 10. Simulation of channel activity in response to voltage ramps. Fractions of channels occupying each state (see model in Fig. 4) in the presence of 100 nM ChTX under the same ramp protocols as Fig. 9 are shown. During slow ramps (A, 0.01 V/s), there is a leveling off and even a decline in the fraction of channels in the O_b state at positive potentials. During faster ramps (B and C, 0.05 and 0.5 V/s, respectively), more channels remain in blocked states.

response to ChTX; however, this tissue showed no spontaneous activity (in the presence of indomethacin) and may possess an excess of K^+ channels to remain in a hyperpolarized state.

Voltage ramps are widely used as an experimental protocol to rapidly assess current-voltage relationships in a variety of cell types (e.g., Ref. 6). Ramp rates (dV/dt) are selected to be slow compared with channel activation kinetics but rapid compared with inactivation to directly observe current-voltage relationships. When characterizing BK channels that do not inactivate (at room temperature), these criteria are easy to meet. On the other hand, these criteria are difficult or impossible to meet when studying, for example, I_{dK} that rapidly inactivate. When applying voltage ramps in the presence of an agonist or antagonist, in addition to considering activation and inactivation rates, rates of binding and unbinding must also be considered (as illustrated in Fig. 2). Because ChTX binding/unbinding is relatively slow and voltage dependent, responses

vary depending on ramp rate, even when well below the rates of channel opening.

The model presented in Fig. 4 is a simplified view of BK channel kinetics and ChTX binding. McManus and Magleby (19) have reported that there are at least three open states and five to six closed states that contribute to current dynamics. Similar results have been obtained from channels purified from aortic smooth muscle and reconstituted in planar lipid bilayers (9). However, the open-to-open and closed-to-closed transitions occur rapidly and are not apparent over the time frame used to observe ChTX binding/unbinding kinetics. In our model, ChTX binds with equal affinity to both the open and closed state of BK channels where BK channels continue to cycle through closed-blocked and open-blocked states. Alternatively, the voltage dependence of ChTX block may be due to different affinities of ChTX to closed and open channel states. For example, in BK channels incorporated into planar lipid bilayers, Anderson and colleagues (1) found an off-rate of ChTX block independent of channel state but a sevenfold faster on-rate for the open state compared with the closed state. Our results are not contradictory to these findings but rather indicate that the macroscopic behavior of $I_{K(Ca)}$ is well described by a simplified model using identical rate constants. The mechanism of ChTX block involves a simple 1:1 binding near the external mouth of the channel, thereby occluding ion flux through the channel. Although direct evidence for closed-blocked to open-blocked transitions was not given by Anderson et al. (1), these authors speculate that the channel indeed may cycle through the blocked states on the basis of identical off-rates. This is analogous with Ba^{2+} block of BK channels, and earlier work had clearly shown such interconversions (21). The voltage dependence of the off-rate (k_{-2}) with an e -fold change every 60 mV is less than that reported by Anderson and colleagues (1), in which they report an e -fold change in rate every 28 mV. This, in part, may be due to the positive range of potentials used in our studies in which clear unbinding could be measured. The simple model (Fig. 4) can be used to generate simulated responses to a variety of protocols that are in excellent agreement with experimental responses, as well as to provide a basis to analyze and interpret experimental results.

In summary, the dependence of ChTX block on the dynamics of membrane potential changes might be the basis for explaining a wide variety of apparently contradictory results in the literature: 1) The ability of ChTX to alter basal electrical activity not only depends on the abundance of BK channels but also on the RMP and dynamics (e.g., spiking vs. nonspiking) of electrical events. 2) Agonist-induced responses may alter the ability of ChTX to bind to BK channels by modifying membrane potential (e.g., resting potential or the dynamics of active events) via BK channels or any other conducting channel present in the membrane. 3) The apparent discrepancies between ChTX binding using patch-clamp techniques on isolated cells and channels vs. effects on tissues may depend on the dynamics of spontaneous electrical activity and specific voltage-

clamp protocols. 4) In the presence of ChTX and other slow blockers, protocols designed to measure current-voltage relationships (e.g., voltage ramps) must take into account activation/inactivation kinetics as well as voltage-dependent binding/unbinding rates. The relatively simple model presented in Fig. 4 provides a useful tool to estimate the efficacy and dynamics of ChTX binding.

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