

Voltage-dependent Block of Fast Chloride Channels from Rat Cortical Neurons by External Tetraethylammonium Ion

DOROTHEA Y. SANCHEZ and ANDREW L. BLATZ

From the Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

ABSTRACT Tetraethylammonium ion (TEA) and its longer chain derivatives have been used extensively to block currents through K-selective ion channels. Substantial information has been gained about the structure and gating mechanisms of K and other cation channels from the analysis of the blocking interactions of TEA and other quaternary ammonium ions. We now present an analysis of blocking interactions between single Cl-selective ion channels from acutely dissociated rat cortical neurons and externally applied TEA. TEA applied to the extracellular membrane surface (TEA_o) blocked Cl channels in a voltage-dependent manner, with hyperpolarizing potentials favoring block. The voltage dependence of block could be adequately fit assuming that TEA enters the channel pore and binds to a site located $\sim 28\%$ of the way through the membrane electrical field. The dose-response relationship between fractional current and $[TEA]_o$ at a fixed holding potential of -40 mV was well fit to a simple model with two blocking sites with dissociation constants (K_d) of ~ 2 and 70 mM. The dose-response relationship could also be fit by a mechanism where TEA only partially blocks the channels. At the bandwidth used in these experiments ($1-2$ kHz), both the mean open duration (composed of the open and blocked durations) and burst duration (composed of open, blocked, and short lifetime shut durations) increased with increased $[TEA]_o$. This is expected if TEA_o can bind and unbind only when the channel is in the open kinetic state. These results suggest that the structure of the permeability pathway of these anion-selective channels may be very similar to that of other channels that are blocked by TEA. Additionally, these results caution that a blocking effect by TEA cannot, by itself, be used as sufficient evidence for implicating the participation of K channels in a particular process.

INTRODUCTION

TEA has been used for many years as a biophysical probe of K channel properties as well as to implicate the presence of K-selective ion channels in a variety of tissues and physiological processes (Armstrong, 1975; Stanfield, 1983; Hille, 1984). Although many K channels are not blocked by TEA, and other non-K cation-selective channels

Address reprint requests to Dr. Andrew L. Blatz, Department of Physiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9040.

are blocked by TEA, this drug is still widely used to assess the participation of K-selective ion channels. We now report that rat neuronal Cl-selective ion channels are blocked by TEA at concentrations similar to those that are used to block K channels. The mechanism of Cl channel block by $[\text{TEA}]_o$ appears to be very similar to TEA block of K channels in a variety of tissues (for reviews see Stanfield, 1983; Hille, 1984).

“Fast” Cl channels are found in tissue-cultured rat skeletal muscle (Blatz and Magleby, 1985, 1986*b*; Weiss and Magleby, 1990) and in acutely dissociated rat cortical neurons (Blatz, 1991). These channels exhibit a mean open duration on the order of 1–2 ms and a permeability ratio (based on reversal potential shift measurements) of ~ 0.2 for P_K/P_{Cl} (Blatz and Magleby, 1985; Blatz, 1991). Cl channels with similar overall characteristics have been described (Franciolini and Nonner, 1987; Franciolini and Petris, 1988) in tissue-cultured fetal rat hippocampal neurons. A Cl channel with much smaller single channel conductance has been described by Lukács and Moczydlowski (1990) from lobster walking nerve. These lobster channels, when incorporated into lipid bilayers, also are blocked by TEA. Manning and Williams (1989) have reported on an anion-selective channel from human platelet membranes incorporated into lipid bilayers which has many properties similar to those of muscle and neuronal fast Cl channels.

Portions of these studies have been reported in preliminary form (Blatz, Sanchez, and Sigler, 1991; Sanchez and Blatz, 1991).

METHODS

Acutely Dissociated Rat Cortical Neurons

Dissociated neurons were obtained using methods described by Kay and Wong (1986). Briefly, brains from young rats (4–14 d old) were removed and sliced into sections 0.5 mm thick. Plugs (1 mm diam) were removed from cortex areas using a glass pipette and placed into PIPES-saline containing (mM): 120 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 25 glucose, and 20 PIPES (piperazine-*N,N'*-bis(2-ethanesulfonic acid)). The plugs were stirred slowly at 35°C for ~ 1 h, after which they were washed several times with PIPES-saline and held at room temperature until needed. When neurons were needed, a plug was triturated in PIPES-saline through a fire-polished Pasteur pipette three to five times until the plug was dissociated into single cells. Neurons obtained in this manner exhibited characteristic morphologies and could be easily differentiated from glial cells and debris.

Single Channel Recording

Currents through single fast Cl channels were obtained using the inside-out configuration of the patch clamp technique on surface membranes of cortical neurons (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981; Blatz, 1991). Single channel currents were recorded with an Axopatch I-B (Axon Instruments, Inc., Burlingame, CA) on FM or VCR tape (model VR-10; Instrutech, Elmont, New York). All solutions contained 5 mM TES (*N*-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid) buffer and 1 mM EGTA and were at pH 7.00. Internal solutions contained, in addition, 1,000 mM KCl. External solutions contained 140 mM KCl and sufficient amounts of TEA as the bromide salt to give the desired concentrations. The pH buffer, TES, at the concentrations used in these experiments does not block fast Cl channel currents (Blatz, A. L., unpublished observations). Other pH buffers, such as HEPES, have been

shown to block certain Cl-selective channels in other preparations (Yamamoto and Suzuki, 1987; Manning and Williams, 1989). Internal solutions were continuously superfused over the inside-out membrane patch using the microchamber described by Barrett, Magleby, and Pallotta (1982). Experiments were performed at room temperature (20–22°C).

Data Analysis

The single channel currents were filtered at 1.4–6 kHz (–3 dB, Bessel), digitized at 4–180 $\mu\text{s}/\text{pt}$ with 12- or 14-bit accuracy, and analyzed off-line using DEC 11-73 or Dell 386 computers and homemade programs. PClamp (Axon Instruments, Inc.) and SigmaPlot version 4.1 (Jandel Scientific, Corte Madera, CA) were used to plot raw current records. Analysis of dose–response relationships was performed using the least-squares–based curve-fit routine contained in SigmaPlot. For kinetic analysis, currents were sampled every 4 μs and a 50% threshold criterion was used to determine the durations of open and closed events (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986b). The collected open and closed intervals were log-binned as in McManus, Blatz, and Magleby (1987). Sums of exponential components were fit to the distributions of open and closed interval durations using a maximum likelihood method (Colquhoun and Sigworth, 1983; Blatz and Magleby, 1986b). The number of significant exponential components required to describe the experimental data was determined using the likelihood ratio test (Rao, 1973; Horn and Lange, 1983; Blatz and Magleby, 1986b; McManus and Magleby, 1988).

Methods detailed in Blatz and Magleby (1986a), Clay and DeFelice (1983), and Magleby and Weiss (1990) were used to simulate single channel interval durations, taking into account the effects of limited bandwidth. To simulate the effects of the apparent reduction of current amplitude caused by the rapid on and off rates for TEA block, rate constants were chosen that would generate open and blocked intervals with durations much shorter than intervals due to normal channel gating (Spruce, Standen, and Stanfield, 1987). These short open and blocked interval durations would be time-averaged and detected as open intervals with a lowered single channel amplitude. Since the method of simulation used calculates the observed amplitude of single channel currents and then measures durations at the 50% threshold, it is important to set the threshold to 50% of the *observed* single channel amplitude in the presence of fast blockers, rather than to 50% of the *true* amplitude. The effects of noise were not explicitly taken into account, but under the recording conditions used in these experiments (filter cutoff, 2 kHz) noise effects should not significantly influence the results.

The variance of the open and closed single channel current noise was calculated from digitized current records. Currents were played back from the FM tape recorder into the computer at an effective sampling interval of 4 μs after filtering at 6 kHz. Closed channel current variance was calculated from records containing no channel activity and was subtracted from the open channel variance. Open channel variance was measured from portions of the open channel current that did not contain any obvious gating transitions. This method erroneously includes some gating transitions with the open channel noise, but this should not affect the results because these transitions would be present in the TEA as well as the control patches.

RESULTS

External TEA Reduces Single Channel Current Amplitudes

The blocking effect of 10 mM TEA_o on fast Cl channel currents is shown in Fig. 1. In the presence of 1 M intracellular KCl and 140 mM extracellular KCl, neuron and muscle fast Cl channels have an unblocked single channel conductance of ~ 140 pS

(Blatz and Magleby, 1986b; Weiss and Magleby, 1990; Blatz, 1991). When single fast Cl channel currents were measured at a holding potential of -40 mV in separate patches with increasing concentrations of TEA at the formerly extracellular membrane surface (pipette solution), single channel current amplitudes were reduced in a dose-dependent manner. As shown in Fig. 1, the major blocking action of TEA_o appears as a reduction of unitary current levels rather than a reduced percentage of time the channel is open. This observation suggests that the binding and unbinding rate constants for TEA block are rapid enough that the mean lifetime of the blocked kinetic state is less than can be resolved in these experiments. Thus, TEA_o block of fast Cl channels is manifested as a reduction in single channel current amplitude due to the time-averaging of the open and blocked kinetic states. In addition to the reduction in single channel current amplitude, the overall appearance of the kinetic activity of these channels is altered by external TEA. Although the current traces in

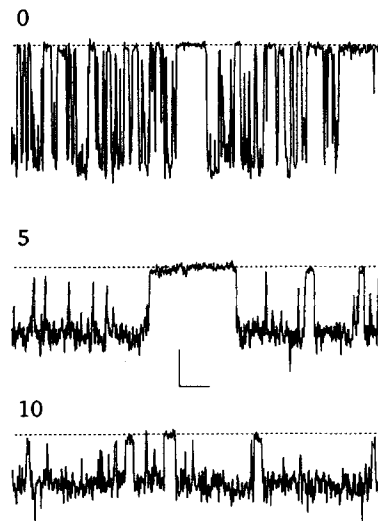


FIGURE 1. Fast Cl channel block by TEA. [TEA]_o blocks neuronal fast Cl channels in a dose-dependent manner by reducing unitary conductance amplitude. Current traces are from three separate inside-out membrane patches each containing a single fast Cl channel. As the concentration of TEA in the pipette solution was increased from 0 to 10 mM, single channel current amplitudes were reduced and the durations of the apparent open intervals and burst durations increased (Bessel, -3 dB = 1.4 kHz). Horizontal and vertical calibrations are 10 ms and 3.0 pA, respectively.

Fig. 1 were selected to show amplitudes, it is apparent that the bursting behavior of these channels is affected by TEA_o. Bursts will be quantitatively defined below, but simply by inspection of Fig. 1 it is clear that the duration of bursts of channel activity is increased by external TEA.

Dose-Response Relationship of External TEA Block

TEA blocked fast Cl channels in a dose-dependent manner when applied to the extracellular membrane surface. The concentration of TEA_o required to block neuronal Cl channels was similar to that required to block a variety of K channels (Stanfield, 1983). The dose-response relationship for fractional Cl channel current measured at a holding potential of -40 mV is shown in Fig. 2. The fractional currents were adequately fit by a single site blocking model (dotted line in Fig. 2) at [TEA]_o < 20 mM, although the reduction in fractional current by higher concentra-

tions of TEA was less than predicted. The fractional current remaining after application of TEA_o was much better fit by a two-site model (solid line in Fig. 2) where $K_{d1} = 2.0 \pm 0.4$ mM and $K_{d2} = 70.0 \pm 15.3$ mM ($n = 3$). In both of these models, when TEA binds and unbinds to the channel, it is assumed that the single channel conductance fluctuates between a fully open and a completely closed configuration. An alternative mechanism, which is difficult to rule out, is that when TEA binds and unbinds, the channel fluctuates between a fully open and a partially open configuration (i.e., "subconducting state"). The dashed line in Fig. 2 is the best fit of a partial-blocking mechanism with the blocked channel current equal to 28% of the unblocked current amplitude to the experimental data points and is at least as good a fit as the two-site model (solid line). Preliminary results (Sanchez and Blatz, 1991; Sanchez, Phillips, Breedlove, and Blatz, 1992) show that in the presence of

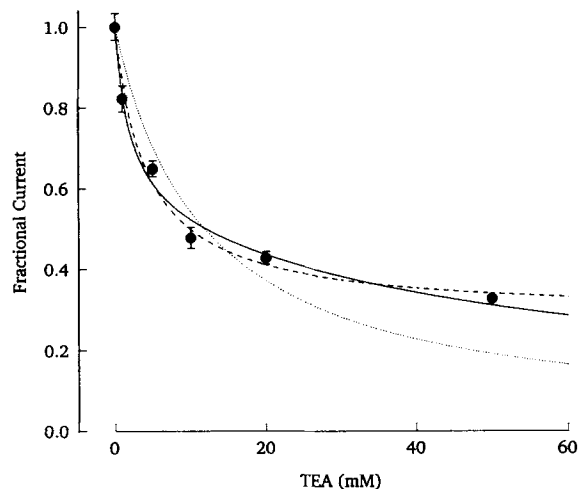


FIGURE 2. Dose-response relationship between $[\text{TEA}]_o$ and fractional current through neuronal fast Cl channels. The fractional current (current amplitude in the presence of TEA_o divided by current amplitude without blocker) through Cl channels decreases with increasing concentrations of TEA. The solid line is the best fit (least-squares criteria) of a two-site model (fractional current = $0.5 \cdot \{K_{d1}/(K_{d1} + [\text{TEA}])\} + \{K_{d2}/(K_{d2} + [\text{TEA}])\}$) with $K_{d1} = 2.0 \pm 0.4$ mM and $K_{d2} = 70.0 \pm 15.3$ mM. The dotted line is the

best fit to a single-site model (i.e., $K_{d1} = K_{d2} = 11.8$ mM). The dashed line is the best fit of a single-site model in which TEA_o reduces single channel current amplitude only partially (to 28% of normal in this case).

longer chain derivatives of TEA, such as tetrabutylammonium ion (TBA), these channels fluctuate between fully open and fully nonconducting blocked states. Assuming that the mechanism of block by TEA_o is similar to that of the longer chain derivatives, we suggest that when TEA binds to the channel, the single channel current is completely blocked. The deviation from a one-site model at high $[\text{TEA}]_o$ was not the result of filtering, as decreasing or increasing the filtering cut-off frequency did not change the shape of the dose-response relationship.

Voltage Dependence of External TEA Block

TEA block of many (although not all) K channels is voltage dependent, particularly when applied internally, as if the binding site for TEA is located within the electrical field of the membrane (reviewed by Stanfield, 1983). Single fast Cl channel currents

in the presence of 0 and 10 mM $[\text{TEA}]_o$ at positive and negative holding potentials are presented in Fig. 3. Single channel currents were reduced at negative membrane potentials in the presence of TEA_o . Under the conditions of this study, TEA_o had very little blocking effect on currents at depolarizing potentials. For example, at -60 mV with 20 mM TEA_o , single Cl channel current amplitudes were reduced by 69%, whereas at $+60$ mV they were reduced by only 23%. Current-voltage relationships obtained from similar currents are presented in Fig. 4 *A*. As expected from inspection

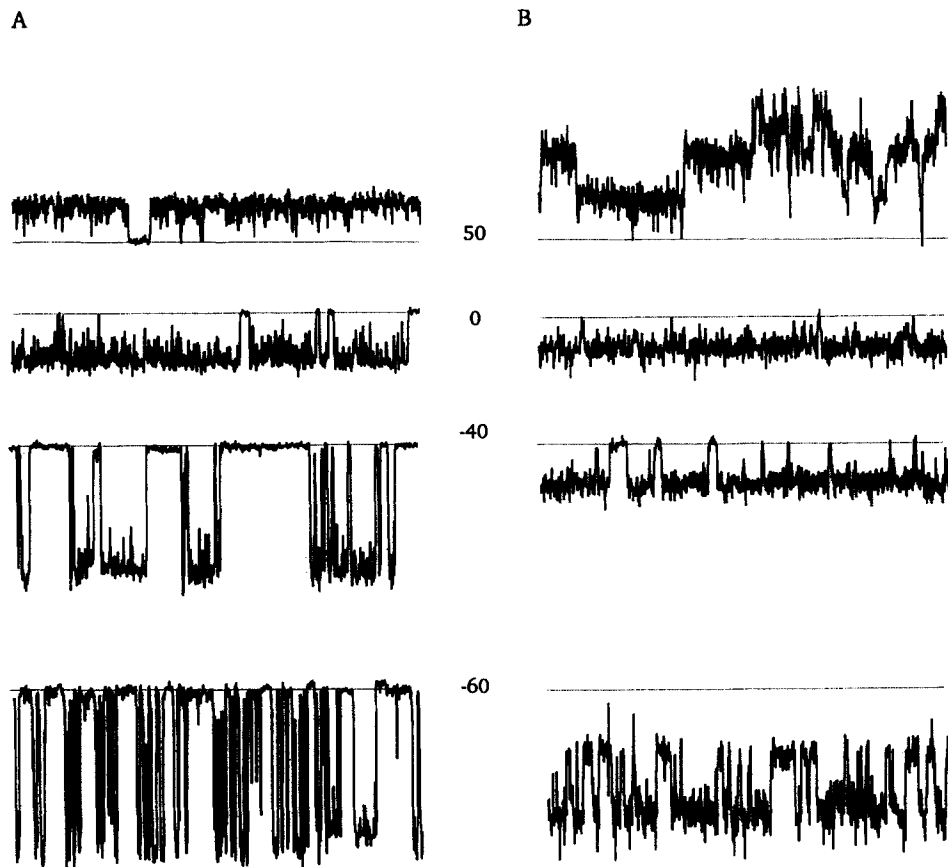


FIGURE 3. Voltage-dependent TEA_o block of fast Cl channel currents. Single channel currents from two separate excised, inside-out neuronal membrane patches. The current traces shown in *A* were recorded in the presence of zero $[\text{TEA}]_o$ and the currents in *B* were recorded in the presence of 10 mM $[\text{TEA}]_o$. The patch shown in *A* contained a single Cl channel, while the patch shown in *B* contained at least three channels. Membrane potentials listed between the two series of current traces are absolute voltages (not relative to "rest"). Inward currents are depicted as downward deflections and represent Cl ions flowing from the formerly intracellular membrane surface to the formerly extracellular membrane surface. Current records were chosen to represent the change in amplitude and may not reflect changes in kinetic activity. Closed channel current level for each voltage is indicated by the dotted lines (Bessel, -3 dB = 1.4 kHz). Vertical and horizontal calibration bars are 5 pA and 30 ms, respectively.

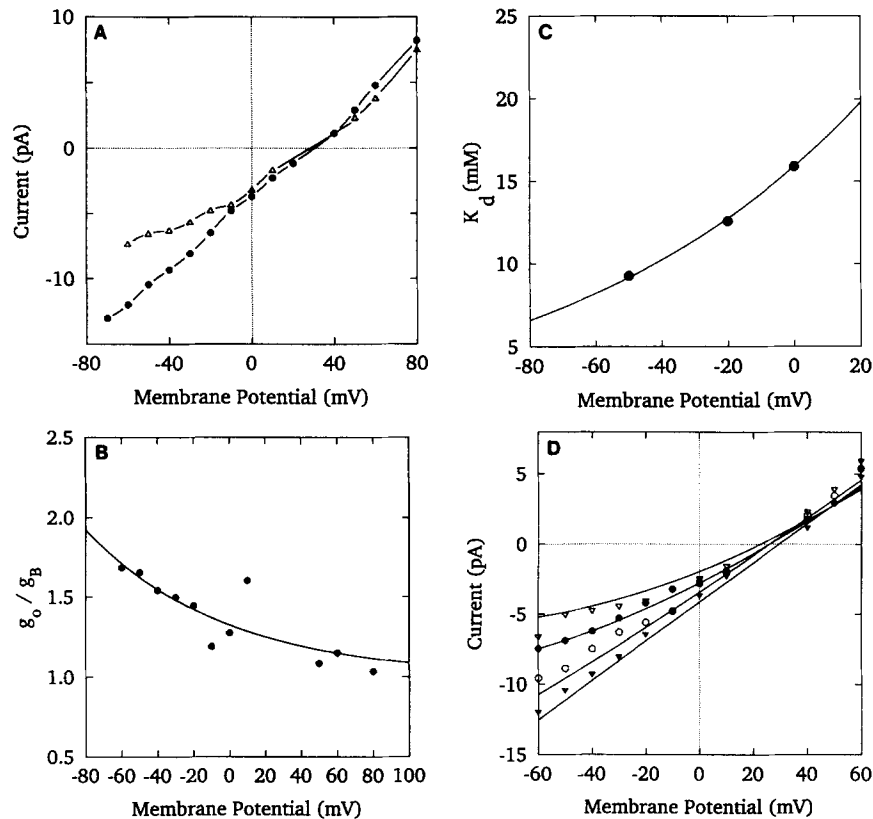


FIGURE 4. Voltage dependence of Cl channel block by TEA. (A) Current–voltage relationships (single channel current amplitude plotted against holding potential) for 0 mM $[\text{TEA}]_o$ (filled circles) and 10 mM $[\text{TEA}]_o$ (open triangles) are shown. $[\text{TEA}]_o$ preferentially blocks inward current with less effect on outward current. Lines connecting points were drawn by eye. (B) Conductance ratio of unblocked channels to channels blocked by 10 mM $[\text{TEA}]_o$ plotted against membrane potential. Solid line was calculated using Eq. 1 with the parameters: $K_B(0) = 30.88$ mM and $d = 0.34$. (C) Voltage dependence of the apparent dissociation constants, K_B 's, were calculated by separately fitting dose–response curves for fractional currents against $[\text{TEA}]_o$ obtained at -50 , -20 , and 0 mV. The solid line represents the best fit of the equation $K_B = K_B(0) \exp(zdVF/RT)$ to the points obtained experimentally with the parameters $K_B(0) = 15.9$ mM and $d = 0.28$. (D) Current–voltage relationships in the presence of 0, 1, 5, and 10 mM $[\text{TEA}]_o$. Solid lines were calculated using Eq. 1 with values for $K_B(0)$ of 15.9 mM and for d of 0.28 and converting conductances to currents.

of the raw current records, the current–voltage relationship in the presence of $[\text{TEA}]_o$ overlaps that of the unblocked channels at depolarized potentials and becomes sublinear as the membrane potential is made more negative. One explanation for this voltage-dependent reduction of currents is that TEA binds to a blocking site within the electric field of the membrane (Armstrong, 1969, 1975; Armstrong and Hille, 1972; Woodhull, 1973; Hermann and Gorman, 1981; Coronado and Miller, 1982; Blatz and Magleby, 1984). If this is the case, the fraction of the membrane

potential through which the blocking ion must pass to access this binding site is given by Woodhull (1973):

$$g_o/g_b = 1 + b/K_b(0) \exp(zdVF/RT) \quad (1)$$

where g_o/g_b is the ratio of single channel conductance in the absence and presence of extracellular blocker, b is the blocker concentration, K_b is the dissociation constant at 0 mV, z is the valence of the blocker, d is the location of the blocking site in terms of the fraction of the electrical field, V is the membrane potential, and F , R , and T have their usual thermodynamic definitions. Since the dose–response relationship between $[\text{TEA}]_o$ and fractional current was adequately fit at the lower concentrations used in most of these experiments, we will consider only a single binding site for these calculations. The ratio of the unblocked to blocked single channel conductances calculated from the currents at 10 mM $[\text{TEA}]_o$ shown in Fig. 3 is plotted in Fig. 4 *B* against membrane potential. The solid line is the best fit of Eq. 1 to these data with values of $K_b(0)$ and d of 15.9 mM and 0.282, respectively. The large scatter of the conductance ratios near the reversal potential is probably due to the difficulty in the measurement of the small currents through these channels at low driving forces. In terms of Woodhull's (1973) interpretation, voltage-dependent block is manifested as a voltage dependence of the dissociation constant. Fig. 4 *C* presents the results of separately fitting dose–response curves to current amplitudes measured between -50 and 0 mV to demonstrate that the apparent dissociation constant becomes smaller with increased negative membrane potentials. The solid line in Fig. 4 *C* was calculated with:

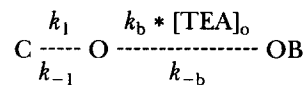
$$K_b = K_b(0) \exp(zdVF/RT) \quad (2)$$

using 15.9 mM for $K_b(0)$ and 0.28 for d .

The result of a similar analysis of currents through four separate channels in the presence of 0, 1, 5, and 10 mM $[\text{TEA}]_o$ is shown in Fig. 4 *D*. The solid lines in this figure were calculated from Eq. 1 with values of $K_b(0)$ and d of 15.9 mM and 0.282, respectively, after converting conductances into currents.

TEA_o Modifies Single Channel Gating Kinetics

A simple scheme for the block of the open kinetic state of a channel is given by:



Scheme 1

where O represents the open kinetic state and OB represents the TEA-blocked state. The compound closed kinetic state C represents the six or seven closed states known for these channels (Blatz, 1991). If Scheme 1 is correct, then with increasing concentrations of TEA the true mean open lifetime will decrease as the channel enters the blocked state more often. The true mean closed and blocked lifetimes should remain unchanged with increasing $[\text{TEA}]_o$. Since it is already known that TEA block of these channels appears as a reduction in single channel current amplitude, the values of rate constants k_b and k_{-b} must be quite large so that the mean lifetimes

of the open state O and the blocked state OB are short enough to be time-averaged into a state of partial conductance in the presence of TEA. If this is the case, then the compound state consisting of O and OB will appear kinetically as a single open state with a reduced single channel conductance and a lifetime that increases with $[\text{TEA}]_o$. If nearly 100% of the transitions between the open and blocked kinetic states are missed due to filtering and threshold detection, then the relationship derived by Neher and Steinbach (1978) for burst duration in the presence of increasing concentrations of a rapid blocker should describe the effects of increasing $[\text{TEA}]_o$ on the measured mean open interval duration. If $K_B = k_{-b}/k_b$, then mean open duration (actually blocked burst duration) would be:

$$\text{mean open time} = \{1 + ([\text{TEA}]_o/K_B)\}/k_{-1} \quad (3)$$

The observed effect of increasing $[\text{TEA}]_o$ on the measured mean open interval duration is shown in Fig. 5. The mean open interval increases with $[\text{TEA}]_o$ in a

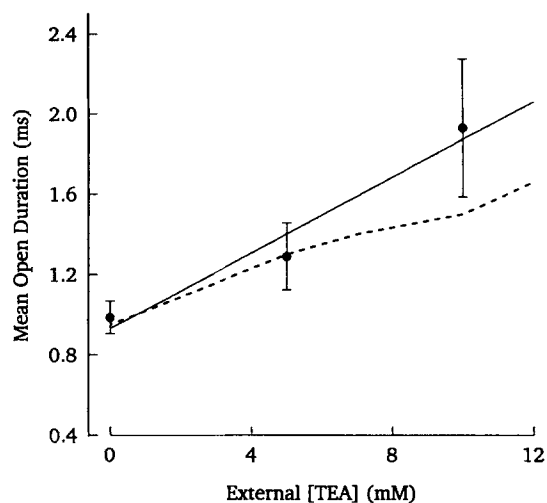


FIGURE 5. Effect of increasing $[\text{TEA}]_o$ on mean open interval duration (actually blocked burst duration). Mean open durations were measured at -47 mV using a 50% threshold method after filtering currents at 2 kHz and sampling at 4 $\mu\text{s}/\text{pt}$. The solid line was calculated with Eq. 3 and the parameters $k_{-1} = 1,013.2 \text{ s}^{-1}$ and $K_B = 11.82 \text{ mM}$ (the best fit single-site model from dose-response curves). The dashed line was calculated by simulating, filtering, sampling, and detecting 10,000–100,000 open and closed intervals as described in the text.

manner consistent with Eq. 3 (solid line in Fig. 5). For this calculation, k_{-1} was estimated as the inverse of the mean open interval measured with zero $[\text{TEA}]_o$.

The analysis of blocked burst duration by Neher and Steinbach (1978) does not take into account the effects of filtering on the detection of open and closed intervals. We also applied the technique of Magleby and Weiss (1990), which explicitly accounts for the effects of filtering and missed events, to the simulation of open and closed interval durations from the fast Cl channel in the presence of TEA_o . The dashed line in Fig. 5 is the prediction of the simulation for the lengthening of the measured mean open duration by increasing $[\text{TEA}]_o$. The simulation fits the lower TEA concentrations well but does not predict the large increase in measured mean open duration seen experimentally at the higher blocker concentrations.

TEA_o Increases Burst Duration

An alternative test of the validity of the blocking mechanism implied by Scheme 1 is to measure the durations of bursts of channel activity (defined below) at increasing [TEA]_o. Such an analysis should be much less affected by the known complications of missed events, filtering, and noise (Colquhoun and Hawkes, 1983; McManus et al., 1987). The method of Magleby and Pallotta (1983) was used to determine that the critical duration of closed event durations within a burst, during fast Cl channel activity, would be 3.2 ms.

Using 3.2 ms as the critical duration for defining a gap between bursts, burst duration was measured by fitting a single exponential component to the distributions of burst durations at 0 and 5 mM [TEA]_o. As expected from Scheme 1, mean burst duration increased from 19.7 ± 6.7 ms ($n = 5$) with zero [TEA]_o to 36 ± 8.4 ms ($n = 4$) in the presence of 5 mM [TEA]_o. It should be pointed out that the relationship between burst duration as defined in this section and [TEA]_o is not as simple to represent as the burst durations defined by Neher and Steinbach (1978) (i.e., our Eq. 3). This is the case because the bursts defined by Neher and Steinbach (1978) contain transitions only between the open and the blocked kinetic states, while bursts defined here contain these transitions as well as those between the open state and the shortest shut states. Other methods of determining the critical time to separate bursts gave qualitatively equivalent results.

Open Channel Variance Does Not Increase with [TEA]_o

Given the simple kinetic reaction of Scheme 1, it might be expected that the current noise when the channel is open would increase in the presence of [TEA]_o to reflect the blocking and unblocking of the channel by the drug. We calculated open channel variance as described in Methods and found that this was not the case. The open channel variance in the absence of TEA at -40 mV was 0.6 pA² and in the presence of 5 mM TEA_o was 0.4 pA². These measurements are complicated by the fact that with fast Cl channels it is virtually impossible to isolate current records that do not contain transitions into the short-lived closed kinetic state and so the variance measurements will contain a contribution from the gating kinetics. In the present case this should not represent a major problem, as these short-lived transitions should be present in the presence as well as the absence of TEA.

Fast Cl Channels Are Not Blocked by All K Channel Blockers

Another compound commonly used to block certain types of K channels, 4-aminopyridine, did not reduce single channel currents through fast Cl channels at concentrations up to 5 mM (not shown). Other quaternary ammonium compounds with carbon chains longer than the ethyl groups of TEA also blocked fast Cl channels, but with higher affinities and apparently slower kinetics (Sanchez and Blatz, 1991). Tetramethylammonium ion (TMA) reduced single Cl channel currents, but only at concentrations > 100 mM (not shown).

DISCUSSION

These results clearly demonstrate that the classical K channel blocker, TEA, when applied extracellularly, blocks neuronal Cl channels in a dose-dependent and voltage-sensitive manner.

Other Anion-selective Ion Channels Are Blocked by TEA

Little information is available concerning the block of other Cl-selective channels by quaternary ammonium ion derivatives. In most experiments, TEA is used to reduce contamination of currents by K-selective channels and so effects on anion channels could be overlooked. One other Cl-selective channel, that of lobster walking nerve, has been definitively shown to be blocked by millimolar concentrations of TEA (Lukács and Moczydlowski, 1990). An anion-selective ion channel from human platelets reconstituted into lipid bilayers (Manning and Williams, 1989) that has many properties in common with the neuronal fast Cl channels may also be blocked to some extent by TEA. Interestingly, the gating kinetics of the platelet Cl channel may also be affected in the same manner as the neuronal channel, as Fig. 7 B of Manning and Williams (1989) seems to indicate an increase in burst duration in the presence of internal and external TEA. Comparisons between lipid bilayer results and ours are difficult as, in the bilayer experiments, the currents are necessarily filtered at 300 Hz and so much of the rapid kinetic information is lost. For example, what would be interpreted as "open duration" by Manning and Williams (1989) would be interpreted in our experiments as the durations of clusters of bursts of open and closed intervals.

Comparison of the dose-response relationship between $[TEA]_o$ and fractional fast Cl channel current and the limited data available for other anion-selective channels suggests that the neuronal channels are more sensitive to $[TEA]_o$ than either the lobster walking nerve (Lukács and Moczydlowski, 1990) or the platelet (Manning and Williams, 1989) anion channels.

Voltage Dependence of $[TEA]_o$ Block Suggests a Cation-binding Site within the Channel Pore

The results of the analysis of the voltage dependence of $[TEA]_o$ block strongly suggest that this blocking ion can enter the channel pore and bind to a site located ~28% of the way through the membrane electrical field. Franciolini and Nonner (1987) proposed a mechanism for the selectivity of a Cl-selective channel from tissue-cultured rat hippocampal neurons that involves a cation binding site located within the channel pore. In this mechanism, selectivity is determined by interactions between the cation bound at this selectivity site and anions attempting to pass through the channel. The location of the cation binding site of Franciolini and Nonner's (1987) model was, for simplicity, not specified as a free parameter, but was placed half-way through the membrane electrical field. Because of this, we cannot equate the TEA binding site suggested by our results to the cation binding site proposed by Franciolini and Nonner (1987). Based on electrical distance through the electrical field, there is no reason to believe that the selectivity and blocking sites are

separate. The predictions of the selectivity mechanism proposed by Franciolini and Nonner (1987) should not be adversely affected by moving the cation binding site a short distance within the membrane electrical field.

TEA_o Binds to the Open Channel

It is often assumed that TEA blocks delayed rectifier K channels by binding preferentially to the open kinetic state (Armstrong, 1969, 1971), although single channel kinetic analysis suggests that TEA block of delayed rectifier K channels in skeletal muscle is independent of channel gating (Spruce et al., 1987). We attempted to determine if external TEA block of neuronal Cl channels was consistent with a kinetic model which allows the binding and unbinding of TEA only to the open kinetic state. Scheme 1 is a simplified model for fast Cl channel kinetic activity recorded at a bandwidth of 2 kHz in the presence of external TEA, assuming that TEA can bind only to the open kinetic state (the buzz, subconductance, and inactivated kinetic modes are ignored, and at this level of filtering the channel exhibits only one open kinetic state [Blatz, 1991]).

It could be argued that measurements of mean open duration will be complicated by the known complexity of channel gating in fast Cl channels. For example, higher resolution measurements show that at least six exponential components are required to fit adequately the distribution of shut interval durations, implying that these channels enter at least six closed states during normal kinetic activity (Blatz, 1991). One of these shut kinetic states has a mean lifetime of $\sim 20 \mu\text{s}$, meaning that most events from this state do not reach the 50% threshold, are not detected, and therefore corrupt the measured duration of open intervals. Methods exist for compensating for these missed short shut events (Roux and Sauve, 1985; Wilson and Brown, 1985; Blatz and Magleby, 1986a) but all are approximations and rely on a detailed knowledge of the underlying kinetic mechanism. Measurement of the effect of $[\text{TEA}]_o$ on burst duration is an alternative test of the validity of Scheme 1 which would be much less influenced by the effects of filtering and missed events. The observed increase in burst duration with $[\text{TEA}]_o$ provides additional consistent evidence that external TEA blocks the open kinetic state.

Open Channel Noise Is Not Increased by $[\text{TEA}]_o$

Although the blocking and unblocking rates were too fast to be distinguished by kinetic analysis, they may still have been slow enough to influence the open-level noise. This was tested by measuring the variance of the open-level current both with and without TEA present. We found that the noise did not increase, and in fact both the open channel and closed channel noise actually decreased slightly with the addition of TEA. This suggests that the on and off blocking rate constants are so large that the excursions between the unblocked and blocked kinetic states are completely time-averaged to appear as an open state with a reduced single channel conductance. The decrease in open channel current variance measured in the presence of TEA_i could represent the block by TEA of small conductance channels (K or Cl selective) that are active throughout the experimental records. This would be consistent with the observation that both the open and the closed channel current noise variances are decreased by TEA_i . No open-closed transitions can be observed

in the experimental records that would reflect activity in these alleged channels. This observation constrains the maximum single channel currents of these channels under these conditions to be <0.1 pA (the limit of our resolution). "Baseline" ion channels with currents of this magnitude have been reported to be present in tissue-cultured rat myotubes, although in that case the small conductance channels were not sensitive to 5 mM TEA_o (Blatz and Magleby, 1986c).

TEA Block of Cl Channels Suggests Structural Similarities between Cation and Anion Channels

These results demonstrate that TEA blocks currents through mammalian neuronal Cl channels at concentrations that are used to block neuronal K channels and that the mechanism of Cl channel block by [TEA]_o is similar to that proposed for K channel block. A similar block of Cl and K channels by TEA suggests that significant structural similarities may exist between the pore regions of these two channel types and that ion permeation mechanisms may be quite similar. This is not an entirely unexpected result, as many Cl channels, including the fast Cl channels described here and the anion-selective channels from platelets, are permeable to cations as well as to anions (Blatz and Magleby, 1985; Franciolini and Nonner, 1987; Manning and Williams, 1989). The Cl channel from lobster walking nerve, on the other hand, is not measurably permeable to cations (Lukács and Moczdlowski, 1990). We predict that substitutions of amino acids in the membrane-spanning regions of fast Cl channels (when these channels are cloned) will have profound effects on TEA block, as has been shown for K channel TEA block (MacKinnon and Yellen, 1990; Yellen, Jurman, Abramson, and MacKinnon, 1991).

The authors thank Monya Phillips for performing the initial TEA blocking experiments.

This work was supported by NIH grants GM-39731 and HL-07360.

Original version received 3 January 1992 and accepted version received 11 May 1992.

REFERENCES

- Armstrong, C. M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. *Journal of General Physiology*. 54:553–575.
- Armstrong, C. M. 1971. Interactions of TEA ion derived with K channel of giant axons. *Journal of General Physiology*. 58:413–437.
- Armstrong, C. M. 1975. Ionic pores, gates and gating currents. *Quarterly Review of Biophysics*. 7:179–210.
- Armstrong, C. M., and B. Hille. 1972. The inner quaternary ammonium ion receptor in potassium channels in the node of Ranvier. *Journal of General Physiology*. 59:388–400.
- Barrett, J. N., K. L. Magleby, and B. S. Pallotta. 1982. Properties of single Ca-activated K channels in cultured rat muscle. *Journal of Physiology*. 331:211–230.
- Blatz, A. L. 1991. Properties of single fast chloride channels from rat cerebral cortex neurons. *Journal of Physiology*. 441:1–21.
- Blatz, A. L., and K. L. Magleby. 1984. Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. *Journal of General Physiology*. 84:1–23.
- Blatz, A. L., and K. L. Magleby. 1985. Single chloride-selective channels active at resting membrane potentials in cultured rat skeletal muscle. *Biophysical Journal*. 47:119–123.

- Blatz, A. L., and K. L. Magleby. 1986a. Correcting single channel data for missed events. *Biophysical Journal*. 49:967–980.
- Blatz, A. L., and K. L. Magleby. 1986b. Quantitative description of three modes of activity of fast chloride channels from rat skeletal muscle. *Journal of Physiology*. 378:141–174.
- Blatz, A. L., and K. L. Magleby. 1986c. Single apamin-blocked Ca-activated K channels of small conductance in cultured rat skeletal muscle. *Nature*. 323:718–720.
- Blatz, A. L., D. Y. Sanchez, and M. L. Sigler. 1991. Voltage-dependent block of neuronal fast chloride channels by tetraethylammonium ion. *Biophysical Journal*. 59:268a. (Abstr.)
- Clay, J. R., and L. J. DeFelice. 1983. Relationship between membrane excitability and single channel open-close recording. *Biophysical Journal*. 42:151–157.
- Colquhoun, D., and A. G. Hawkes. 1983. The principles of stochastic interpretation of ion-channel mechanisms. In *Single Channel Recording*. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 135–176.
- Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. In *Single Channel Recording*. B. Sakmann, and E. Neher, editors. Plenum Publishing Corp., New York. 191–264.
- Coronado, R., and C. J. Miller. 1982. Conduction and block by organic cations in a K-selective channel from sarcoplasmic reticulum incorporated into planar phospholipid bilayers. *Journal of General Physiology*. 79:529–547.
- Franciolini, F., and W. Nonner. 1987. Anion and cation permeability of a chloride channel in rat hippocampal neurons. *Journal of General Physiology*. 90:453–478.
- Franciolini, F., and A. Petris. 1988. Single chloride channels in cultured rat neurons. *Archives of Biochemistry and Biophysics*. 261:97–102.
- Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv*. 391:85–100.
- Hermann, A., and A. L. F. Gorman. 1981. Effects of tetraethylammonium on potassium currents in molluscan neuron. *Journal of General Physiology*. 78:87–110.
- Hille, B. 1984. *Ionic Channels of Excitable Membranes*. Sinauer Associates Inc., Sunderland, MA. 426 pp.
- Horn, R., and K. Lange. 1983. Estimating kinetic constants from single channel data. *Biophysical Journal*. 43:207–223.
- Kay, A. R., and R. K. S. Wong. 1986. Isolation of neurons suitable for patch-clamping from adult mammalian central nervous systems. *Journal of Neuroscience Methods*. 16:227–238.
- Lukács, G. L., and E. Moczydlowski. 1990. A chloride channel from lobster walking leg nerves. Characterization of single-channel properties in planar bilayers. *Journal of General Physiology*. 96:707–734.
- MacKinnon, R., and G. Yellen. 1990. Mutations affecting TEA blockade and ion permeation in voltage-activated K channels. *Science*. 250:276–279.
- Magleby, K. L., and B. S. Pallotta. 1983. Burst kinetics of single Ca-activated, potassium channels in cultured rat muscle. *Journal of Physiology*. 344:605–623.
- Magleby, K. L., and D. S. Weiss. 1990. Estimating kinetic parameters for single channels with simulation. A general method that resolves the missed event problem and accounts for noise. *Biophysical Journal*. 58:1411–1426.
- Manning, S. D., and A. J. Williams. 1989. Conduction and blocking properties of a predominantly anion-selective channel from human platelet surface membrane reconstituted into planar phospholipid bilayers. *Journal of Membrane Biology*. 109:113–122.

- McManus, O. B., A. L. Blatz, and K. L. Magleby. 1987. Sampling, log binning, fitting, and plotting durations of open and shut intervals from single channels and the effects of noise. *Pflügers Archiv.* 410:520–553.
- McManus, O. B., and K. L. Magleby. 1988. Kinetic states and modes of single large-conductance calcium-activated potassium channels. *Journal of Physiology.* 402:79–120.
- Neher, E., and J. H. Steinbach. 1978. Local anesthetics transiently block currents through single acetylcholine-receptor channels. *Journal of Physiology.* 277:153–176.
- Rao, C. R. 1973. *Linear Statistical Inference and Its Applications.* John Wiley & Sons, Inc., New York. 625 pp.
- Roux, B., and R. Sauve. 1985. A general solution to the time interval omission problem applied to single channel analysis. *Biophysical Journal.* 48:149–158.
- Sanchez, D. Y., and A. L. Blatz. 1991. Tetraethylammonium and related quaternary ammonium ions block chloride channels in cerebral cortical neurons. *Society for Neuroscience Abstracts.* 17:1521. (Abstr.)
- Sanchez, D. Y., M. L. Phillips, K. E. Breedlove, and A. L. Blatz. 1992. Kinetics of TEA block of fast chloride channels in rat cortical neurons. *Biophysical Journal.* 61:A255. (Abstr.)
- Spruce, A. E., N. B. Standen, and P. R. Stanfield. 1987. The action of external tetraethylammonium ions on unitary delayed rectifier potassium channels of frog skeletal muscle. *Journal of Physiology.* 393:467–478.
- Stanfield, P. R. 1983. Tetraethylammonium ions and the potassium permeability of excitable cells. *Review of Physiology, Biochemistry, and Pharmacology.* 97:1–67.
- Weiss, D. S., and K. L. Magleby. 1990. Voltage dependence and stability of the gating kinetics of the fast chloride channel from rat skeletal muscle. *Journal of Physiology.* 426:145–176.
- Wilson, D. L., and A. M. Brown. 1985. Effect of limited interval resolution on single channel measurements with application to calcium channels. *IEEE (Institute of Electrical and Electronics Engineers) Transactions on Biomedical Engineering.* 32:786–797.
- Woodhull, A. M. 1973. Ionic blockage of sodium channels in nerve. *Journal of General Physiology.* 61:687–708.
- Yamamoto, D., and N. Suzuki. 1987. Blockage of chloride channels by HEPES buffer. *Proceedings of the Royal Society of London B.* 230:93–100.
- Yellen, G., M. E. Jurman, T. Abramson, and R. MacKinnon. 1991. Mutations affecting internal TEA blockade identify the probable pore-forming region of a K channel. *Science.* 251:939–942.