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Mechanism of Asymmetric Block of K Channels by Tetraalkylammonium Ions in Mouse Neuroblastoma Cells

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Summary. Experiments were performed to compare the mechanism of block of voltage-dependent K channels by various short and long alkyl chain tetraalkylammonium (TAA) ions at internal and external sites. Current through single channels was recorded from excised membrane patches of cultured neuroblastoma cells using the patch-clamp technique. All of the TAA derivatives tested blocked the open channel when applied to either side of the membrane. Tetraethylammonium (TEA) reduced the amplitude of current through the open channel. Tetrabutylammonium (TBA) and tetrapentylammonium (TPeA) reduced the open time as a function of the concentration. An additional nonconducting state was observed when TBA or TPeA was applied internally or externally, due to the presence of a drug-bound and blocked state of the channel. The closing rate under control conditions was similar to that in the presence of external tetramethylammonium (TMA), suggesting that channel closing is independent of external drug binding. The concentration for half maximal block of the channel by external TEA was 80 μM. The channel was less sensitive to internal TEA, which half blocked the channel at 27 mM. The dissociation rate of long alkyl chain TAA ions from the channel was slower when applied to the inside, compared to external application, suggesting the presence of distinct internal and external receptors. Long alkyl chain TAA derivatives, such as TBA had a faster association rate with the open channel when applied to the inside of the membrane than when applied to the outside.

Key Words potassium channel · tetraethylammonium · patch clamp

Introduction

Quaternary ammonium ions block a variety of K channels, including voltage-dependent and Ca-activated varieties (reviewed by Stanfield, 1983). Classic experiments performed on squid axon have shown that compounds such as tetraethylammonium (TEA) or nonyl-triethylammonium (C9) reduce the outward current with a delay after depolarization when applied to the internal surface (Armstrong, 1969, 1971). The time constant for block and the fraction of K current not blocked in the steady state decreases with concentration and membrane depolarization. Recovery from block is promoted by hyperpolarization and K ions flowing inward following repolarization. In the model developed by Armstrong from these observations, the compounds block the channel only after it has opened. Block is time and voltage dependent following a depolarization because the channel must open before the ions can block. Evidence also suggested that the channel can close when previously blocked.

In squid axon, TEA does not block K current when applied to the external surface in squid axon (Armstrong & Binstock, 1965). However, TEA blocks K current in frog node of Ranvier from either side of the membrane (Armstrong & Hille, 1972). However, the characteristics of the block are dependent on the side of application. Block by internal quaternary ammonium ions was found to be fundamentally similar to that in squid axon, exhibiting a time dependency following depolarization. For external ions, the channel was less sensitive to long chain derivatives, such as pentyl-triethylammonium, and block was not time dependent. These data were interpreted to mean that two distinct receptors exist in this preparation, and that the activation gate need not be open for block to occur from the outside.

Structural evidence indicates that multiple receptors exist on voltage-dependent K channels. Alterations of the amino acid compositon in the S5–S6 linker region of either of two types of K channels were made using molecular biological techniques. These alterations change the sensitivity to extracellular TEA and the conductance of the open channel (MacKinnon & Yellen, 1990; Hartmann et al., 1991). Differences in this region could also underlie the differences in sensitivity to external TEA of potassium channels derived from mammalian brain (RCK-type, Stühmer et al., 1989). A second region

which alters sensitivity to internally applied, but not externally applied, TEA has recently been identified (Yellen et al., 1991). These two regions likely form TEA receptors on opposite sides of the membrane. However, in one case a fivefold change in sensitivity to external TEA seems to be produced by an alteration in the amino-acid composition on the intracellular side (Stocker et al., 1990).

The present study was initiated to compare the mechanism and kinetics of block of a voltage-dependent K channel in neuroblastoma cells by internal and external tetraalkylammonium (TAA) ions. Little has been done on the single channel level to test the hypothesis that TAA ions only block the open channel, or to compare the kinetics of block by long alkyl chain derivatives at internal and external receptors. Single channel analysis was restricted to the 35 pS channel in N1E-115 cells (FK type, *see* Quandt, 1988). The delayed rectifier in other preparations is primarily identified by its relatively slow inactivation during a voltage step, and high sensitivity to 4-aminopyridine (*see* Dubois, 1983; Clay, 1985*a*; Rudy, 1988). The 35 pS channel has properties similar to the delayed rectifier of other preparations.

Block by internal and external TAA ions was found to be kinetically different. The sensitivity of the open channel to block by TEA was over two orders of magnitude greater when TEA was applied to the outside. However, for tetrabutylammonium (TBA) the association rate was greater and the apparent dissociation rate was less when applied on the inside. These data suggest that fundamental asymmetries in the receptors on the two sides of the membrane account for the difference of the time course of the macroscopic currents between internal and external blockers. Some of these results have been presented in an abstract (DeCoursey et al., 1989).

Materials and Methods

Cell Culturing

N1E-115 neuroblastoma cells were used in patch-clamp experiments. The methods used for growing the cells in tissue culture were similar to those previously described (Quandt & Narahashi, 1984), except cells were grown in 5% fetal calf serum. In order to differentiate the cells prior to their use in experiments, 1.5% dimethylsulphoxide was added to the growth media and the serum level was reduced to 2.5% for three days to one week.

Patch Clamp and Recording Methods

Excised membrane patches were used to record currents through single K channels. Inside-out, or outside-out configurations were employed so that the internal, or the external solution, respectively, could be altered during an experiment by bath perfusion. Unless noted elsewhere, the frequency response of the recording system was brought to 1 kHz (−3 dB) with the use of an eighth-order Bessel filter (Frequency Devices 902 LPF). Other aspects of the patch-clamp methods and recording conditions are given in Quandt (1987). Experiments to record single channel currents were done at 10 to 12°C, unless noted otherwise. The temperature was controlled in any one experiment to within 1°C. Test potentials are noted in the legends if not equal to 0 mV. The holding potential in each experiment was −80 mV unless given in the legend. The channel type used in this study was identified by measuring the slope conductance. The conductance was calculated as the change in current through the open channel, for a 20 or 40 mV change in the membrane potential.

The normal external solution contained (in mM): NaCl, 125; KCl, 5.5; $CaCl_2$, 3.0; $MgCl_2$, 0.8, HEPES, 20; dextrose, 25. Sucrose was added to bring the osmolarity to 330 mOsmol, and the pH was brought to 7.3 by the addition of 1N NaOH. The normal internal solution contained (in mM): Kglutamate, 150; EGTA, 20; HEPES, 20. The pH was adjusted to 7.25 with 1N KOH. TetraethylammoniumCl (minimun purity 98%), tetrapropylammoniumCl, tetrabutylammoniumBr, tetrapentylammoniumBr, and tetrahexylammoniumBr were obtained from Eastman Kodak (Rochester, NY) and were not purified further. Tetramethylammoniumcl (minimum purity 97%) was obtained from Aldrich (Milwaukee, WI).

Analysis

The open and closed time histograms plotted in the figures are cumulative, so that the probabilities are those that the event has a duration greater than the time on the abscissa. Multi-exponential distributions were determined using a program which minimized the square of the difference between the fit and the data (vanMastright, 1977). The number of exponentials was selected to maximize the probability using a Chi squared test. Fits to other functions were generated using a commercially available program (Minsq, Micromath Scientific Software, Salt Lake City, UT).

Results

Mechanism of Block by TAA Derivatives

The mechanism of action of long alkyl chain derivatives of tetraethylammonium (TEA), such as tetrapentylammonium (TPeA) was studied by measuring single channel currents. TPeA reduced the open time of the channel and led to a nonconducting state of long duration. The action any of the TAA ions used in this study was readily reversible following washout of the drug from the solution when excised patches were used. Records from a typical experiment are shown in Fig. 1*A*. Internal tetrapropylammonium (TPrA), as well as tetrabutylammonium (TBA) and tetrahexylammonium also blocked the channel in a manner similar to TPeA. In contrast, tetramethylammonium (TMA), TEA, or external TPrA reduced the amplitude of current through the

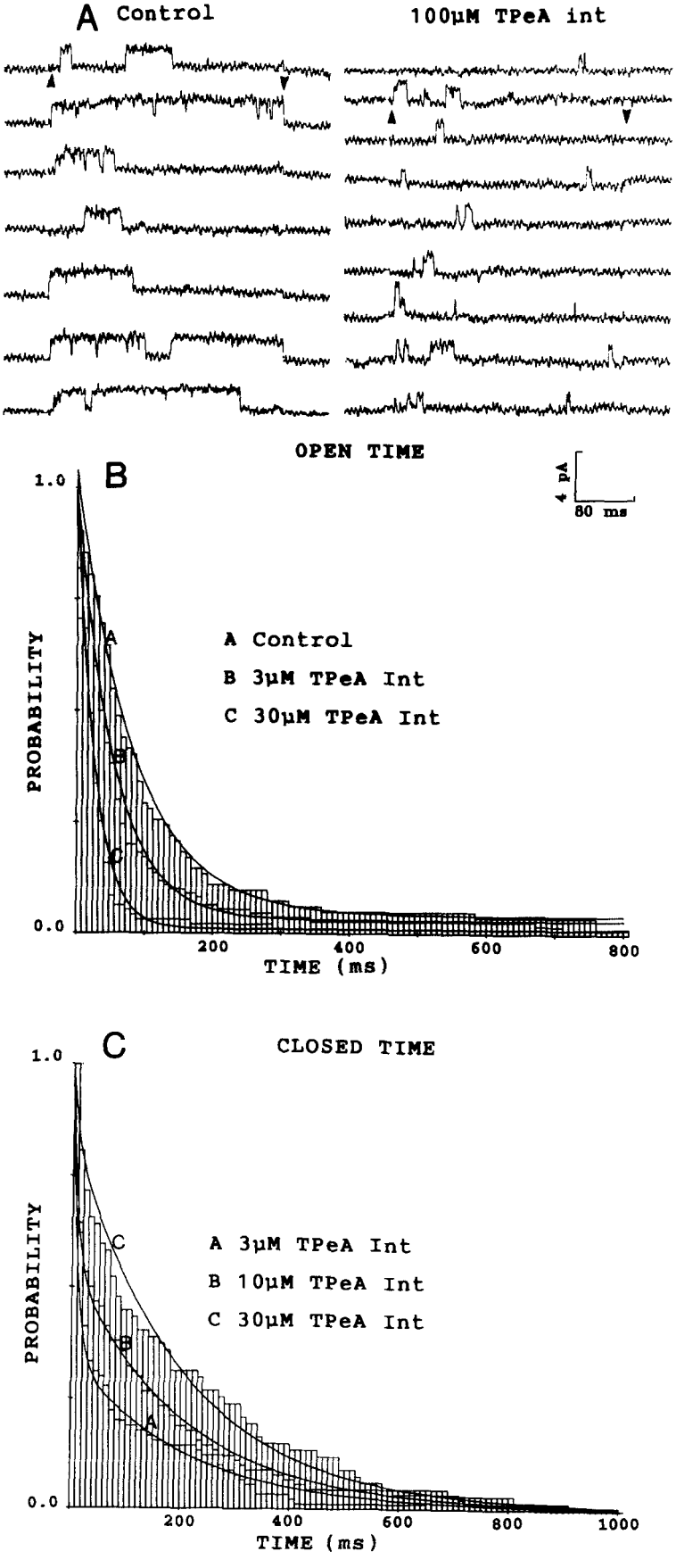
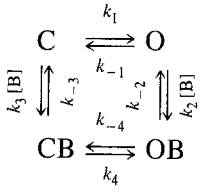


Fig. 1. Characteristics of open channel block by internal TPeA. (*A*) Representative currents in response to step depolarizations before (left panel) or after (right) application of TPeA are shown (+20 mV, 500 Hz filter). Upward deflections are outward currents. The onset and offset of the depolarizations are marked by upward and downward arrows, respectively. (*B*) The open time histograms are superimposed for control conditions, and in the presence of 3 or 30 μM TPeA. The continuous curves give the best fit to a single exponential probability distribution. Mean open times: control, 82 ms; 3 μM, 55 ms; 30 μM, 25 ms. Temperature 8°C. (*C*) Closed time histograms from this experiment are superimposed. The continuous curves each mark the best fit to a distribution having two exponentials. The form of the equation was $P(t) = P_1 \exp(-t/T_1) + P_2 \exp(-t/T_2)$. These two components had mean durations of 10 (T_1) and 200 (T_2) ms. The proportion of 200 ms events ($P_2 = 1 - P_1$) increased with TPeA: 3 μM, 0.35; 10 μM, 0.55; 30 μM, 0.84. Histograms represent measurements of a variable number of open or closed events (n): control, $n = 85$; 3 μM, $n = 90$; 10 μM, $n = 90$; 30 μM, $n = 67$.

open channel (see below). The difference in the time course of block between TEA and TPeA would occur if blocking and unblocking kinetics slowed as the alkyl chain length was increased. The kinetics of the interactions of TAA ions with the channel were obtained by examining the concentration dependence of the open and closed times. Scheme I illustrates the general mechanism for a channel blocker. O represents the open state in which the channel is gated open and unblocked. C represents the closed and unblocked state of the channel. States OB, and CB are the open-blocked, and closed-blocked states respectively. Only state O is conducting. [B] is the concentration of the blocker.

Scheme I



For a blocker which can bind to the open channel, the duration of the state in which the channel is conducting (gated open and unblocked) would be reduced as the concentration of blocker is increased, due to reaction k_2 in Scheme I. Three histograms of open times in different concentrations of TPeA applied to the internal solution are superimposed in Fig. 1*B*. The continuous curves give the fits for a single open state. The open time was reduced as the concentration of TPeA was increased, as expected for a blocking reaction which follows channel opening.

The association rate for block of the open channel by TPeA increased as the membrane was depolarized. For example, in one typical experiment the mean open time under control conditions was 15.6 ms at 0 mV and 24.5 ms at +40 mV (16°C). In the presence of 10 μM TPeA, the mean open time decreased to 8.5 ms at 0 mV, and 8.2 ms at +40 mV. Calculation of the forward rate constants from these data indicates that the blocking rate increased from 5.3 ms^{-1} mM^{-1} at 0 mV to 7.7 ms^{-1} mM^{-1} at +40 mV.

For the general blocking scheme, three nonconducting states can occur in the presence of the blocker (C, OB, and CB). The duration of C should decrease as the concentration of the blocker is increased, due to k_3. The actual time in CB or OB should be independent of the concentration, since either is determined by the dissociation rate constants (k_{-2} and k_{-3}). If the dissociation rate is independent of the gated state of the channel, the duration of these two states could be different, since exit from CB is influenced by the opening rate (k_4), while exit from OB is dependent on the closing rate (k_{-4}). However, the average time in the two blocked states would only be dependent on the dissociation rate.

If the drug does not bind to the closed state of the channel to form state CB, the time in C and OB would not change as a function of the concentration. However, the fraction of the total number of nonconducting events associated with the OB state would increase with concentration, relative to the fraction of the nonconducting events associated with state C.

Histograms of the duration of nonconducting events could not be fit by a single state, as shown in Fig. 1*C*. The component associated with the shorter duration (10 ms) was dominant in normal saline. This state then represents the duration of the normal closed state. In the presence of TPeA, longer duration events were readily observed associated with one or more blocked states. The superimposed curves represent the best fit to a two nonconducting state model having a single blocked state (OB) and a single closed state (C). In this case there are two components. The fraction of events with a mean duration of 10 ms decreased, and the frequency of a second nonconducting state with a mean duration of 200 ms increased, as the concentration of TPeA was increased although the mean duration of each component did not. This pattern of changes in the histogram of nonconducting states is similar to that predicted for block of the open channel only.

The open time was also decreased when TPeA was applied to the external solution (*see* Fig. 4). However, in this case the analysis of the nonconducting events indicated that the blocked time was similar to the closed time in the absence of the blocker.

One additional test of the state dependency of interaction between the blocker and the channel is to examine the ability of the channel to close when blocked by TAA ions. If the blocked channel cannot close, the conducting state (O) will appear in a burst pattern, due to repetitive O and OB states prior to the O to C transition. The average of the sum of the open and open-blocked states prior to channel closing will be greater than that for the average duration of the open state in the absence of the blocker (Neher & Steinbach, 1978). In order to determine the duration of such a burst, the duration of OB must be different from that of C, so that the closing event can be distinguished from the blocking event. TEA and TMA reduced the amplitude of the open channel apparently due to rapid association and dissociation kinetics. Since the duration of the OB state in the

presence of short alkyl chain length compounds is much shorter than the shortest duration closed state, the burst duration in the presence and absence of TEA or TMA was compared to the normal open time.

A representative experiment with TMA applied to an outside-out patch is shown in Fig 2*A*. Fractional block, calculated from the reduction in amplitude of current through the open channel was 31%. The mean open time was slightly reduced in the presence of TMA. The comparable experiment showing the action of TEA on an inside-out patch is shown in Fig 2*B*. Fractional block in the presence of TEA was 40%. In either type of experiment, we were unable to detect the required increase in the duration of the burst in the presence of the blocker compared to the normal open state for a model in which the channel is unable to close when blocked. The burst duration in the presence of TMA was actually shorter than the control open time. However, the mean burst duration in the presence of internal TEA was in fact greater than the normal open time, but only by 22%. This result indicates that the gating and blocking reactions are independent for external application. The closing rate is slowed when TEA blocks the channel from the inside.

TEA Action on Internal and External Receptors

The blocking action of TEA at internal and external receptors was compared. The action of TEA on K channels when applied to the external surface of an outside-out patch is shown in Fig. 3*A*. The figure shows currents generated by the opening of single voltage-dependent K channels in response to step depolarizations before and after application of the blocker. TEA reduced the level of current through the open channel. Association and dissociation rates are fast, so that discrete blocking events were not resolved. The current through the open channel is then an average value given by the difference between the blocked and unblocked current levels multiplied by the fraction of time the channel is not blocked. This situation is similar to that previously examined for TEA block of Ca-activated K channels (Yellen, 1984; Spruce et al., 1987) and ATP-sensitive K channels (Davies et al., 1989).

A similar action of TEA on K currents was obtained when this compound was applied to the internal membrane. Although TEA also reduced the amplitude of the single channel current, a much higher concentration was required to suppress the current when applied from the inside. A graph comparing the effect of external application of TEA with that for internal application is shown in Fig. 3*B*. The blocked fraction (F) was determined by calculating the fractional decrease in the amplitude of the single channel current, which is given by:

$$F = (I_{\text{control}} - I_{\text{TEA}})/I_{\text{control}}.$$

I_{TEA} and I_{control} are the single channel currents in TEA and in control solutions, respectively. The average concentration for half maximal block of the single channel current by external TEA in the two experiments shown was found to be 80 μM. In the example experiment, the K_i for block of the single channel current by internal TEA was found to be 27 mM. The channel is over two orders of magnitude more sensitive to external TEA than internal TEA.

Action of Long Alkyl Chain Derivatives on External and Internal Receptors

Block of the K channel at internal and external sites by TAA ions having a long alkyl chain length was compared by determining the association and dissociation rates for internal application and those for external application. Association rates were determined in excised patches of membrane by measuring the open time as a function of the concentration of blocker. Currents from a typical experiment, in this case recorded from an outside-out patch of membrane under control conditions and in the presence of TBA added to the external solution, are shown in Fig. 4*A*. The association rate constant is given by the slope of the inverse of the open time as a function of the concentration (Neher & Steinbach, 1978). This relationship is plotted for three experiments in which TBA was applied to the internal surface in Fig. 4*B*. The average association rate constant for TBA was found to be 0.48 ms^{-1} mM^{-1} (13°C, 0 mV).

The apparent association rate constant for block by externally applied TBA was much smaller than that for internal application of the same compound. Effects of the addition of TBA to an outside-out patch are shown in Fig 4*C*. The calculated rate constant for TBA in this typical experiment was 0.006 ms^{-1} mM^{-1}.

A comparison of the rate of dissociation of TBA from the channel can be estimated by examining the nonconducting times. The dissociation rate was found to be faster for the external receptor than for the internal receptor. Histograms of the nonconducting times in the presence of TBA, for example inside-out and outside-out patches, are shown in Fig. 4*D*. The mean duration of the component of nonconducting events which increased in the presence of TBA, representing the blocked state, was 110 ms when

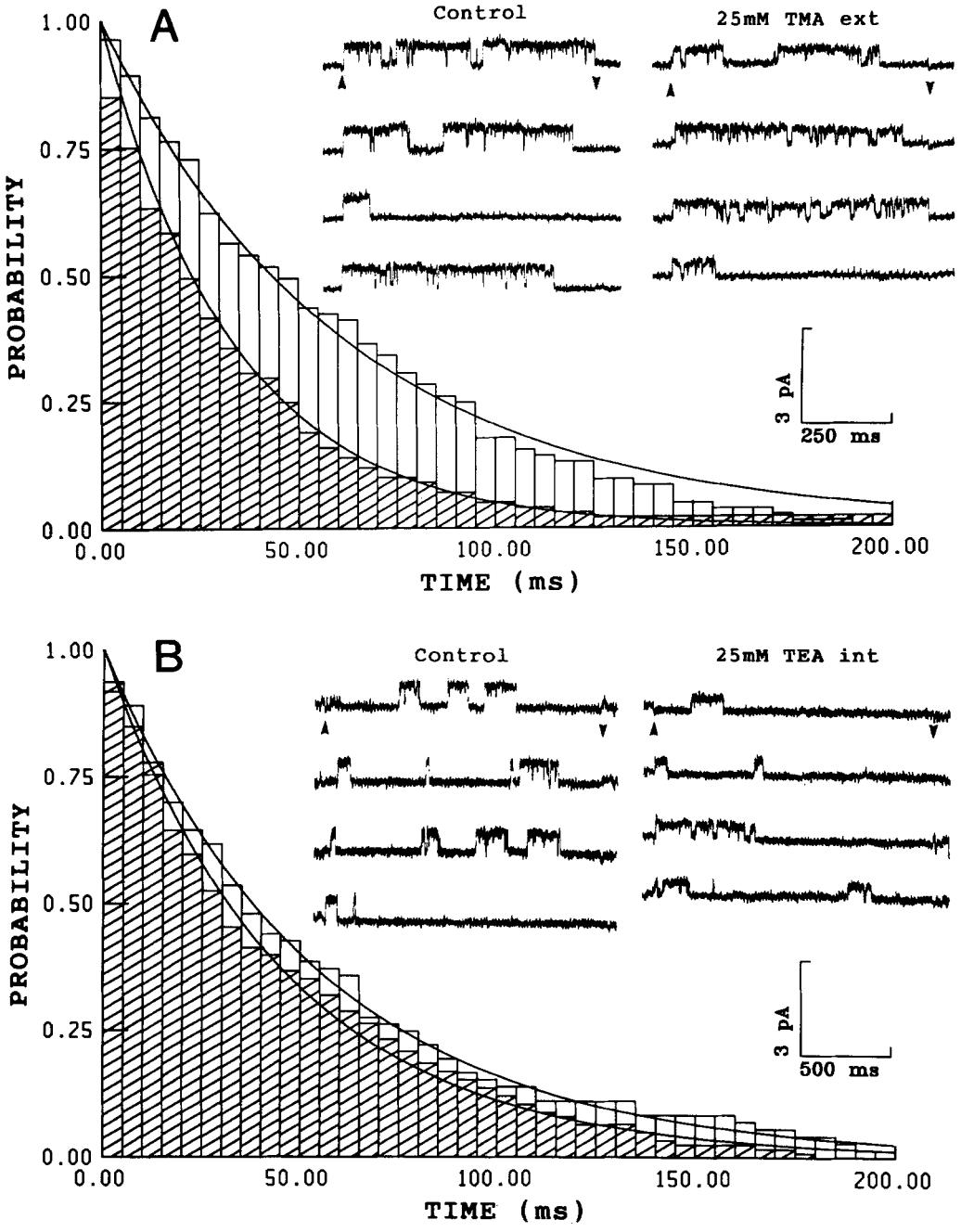


Fig. 2. Effects of TAA ions on the apparent closing rate. (*A*) The probability distribution is shown for the duration of the open state in normal solutions (unhatched), and the burst duration in the presence of 25 mM TMA added to the external solution (hatched). An outside-out patch of membrane was utilized (15.2° C). The insert shows representative currents under the two conditions. The curves are given by: control, $P(t) = \exp(-0.016t)$, $n = 82$; TMA, $P(t) = \exp(-0.030t)$, $n = 86$, t in ms. (*B*) Data obtained from an inside-out patch of membrane to show the effects of internal 25 mM TEA (+20 mV, 14.3° C, 500 Hz). Representative records are shown in the insert. The probability distribution of open times under control conditions (hatched) is given by $P(t) = \exp(-0.022t)$, $n = 118$; and in the presence of TEA (unhatched) $P(t) = \exp(-0.018t)$, $n = 67$. In both *A* and *B*, currents were digitally filtered to 200 Hz prior to identifying the threshold crossings to determine the open and closed times.

TBA was applied to the inside. The duration of the blocked state was only 10 ms when TBA was added to the external solution. Such a comparison indicates that the dissociation rate is dependent on the side of the membrane to which the drug is applied, being much higher for external TBA. Distinct receptors would be expected to have dissimilar dissociation rates. This result then suggests the presence of distinct internal and external receptors for TAA ions.

The association rate for TPeA block of the open

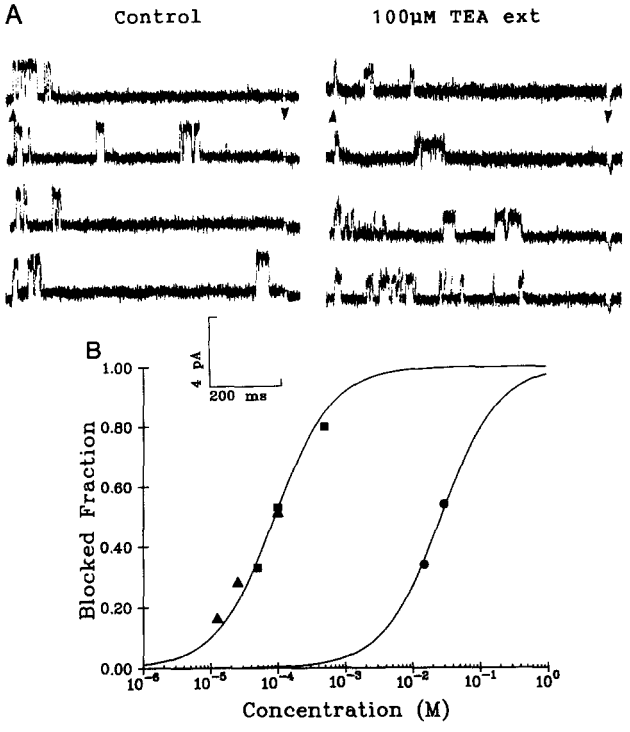


Fig. 3. Comparison of block by TEA ions at internal and external sites. (*A*) Current was recorded from an outside-out patch of membrane, in response to a step depolarization, before (left) or after (right) the addition of the blocker to the bath. Test potential +10 mV, temperature, 14°C. (*B*) Dose response curves were determined by measuring the amplitude of single channel current as a function of the concentration of TEA. Data are shown from two patches in the presence of external TEA (triangles, squares) and one in the presence of internal TEA (circles). The superimposed curves were fit to the points using an equation of the form: $F = \text{TEA}/(\text{TEA} + K_i)$, where F is the blocked fraction (defined in the text) and K_i is the concentration of TEA for half inhibition. The concentration for half maximal block was smaller for external TEA (K_i = 80 μM) than for internal TEA (K_i = 27 mM). Test potential +10 mV, temperature 20°C.

channel was also found to be substantially greater for internal application, compared to external application. For example, in one experiment on an inside-out patch the association rate was found to be 0.94 ms^{-1} mM^{-1} when TPeA was applied to the internal solution (8°C). The calculated association rate for TPeA block when added to the outside solution during an experiment on an outside-out patch was 0.005 ms^{-1} mM^{-1}.

Discussion

Comparative Pharmacology of K Channels

The sensitivity of different types of voltage-dependent K channels to TAA ions is highly variable. The K_i for block by internal TAA ions can be obtained directly from the fractional block for TEA and from the association and dissociation rates for long alkyl chain derivatives. The value of the K_i for internal TBA (19 μM), as well as the rate constants for association and dissociation are within a factor of five of those for block of the delayed rectifier in squid axon (French & Shoukimas, 1981). One difference in the kinetic values for block of K channels in neuroblastoma cells, compared to squid axon is that the concentration for half block of K channels in squid axon by internal TEA is 0.4 to 0.5 mM. This parameter appears to have a value which is greater than an order of magnitude higher in neuroblastoma cells (27 mM).

The characteristics of block of K channels in neuroblastoma cells by external TAA derivatives is similar to that for amphibian node of Ranvier. Hille (1967) reported that the K_i for block of the K current in frog node of Ranvier by external TEA was 0.4 mM. The sensitivity of K current in neuroblastoma cells to external TEA is slightly greater (80 μM). K current in the node of Ranvier has a reduced sensitivity to long chain TAA ions, such as TBA. TBA was found to have a K_i of 60 mM (Hille, 1967; *see also* Kristbjarnarson & Arhem, 1982). The K_i for block of the K channel in neuroblastoma cells at the external receptor is estimated to be 16 mM.

General properties of the block of the voltage-dependent K channels in neuroblastoma cells by TAA ions are similar to those for Ca-activated K channels (Blatz & Magleby, 1984; Yellen, 1984; Vergara et al., 1984; Villarroel et al., 1988). The affinity of the external receptor for TEA is higher than that for the internal receptor. As the alkyl chain length is increased, the affinity of the external receptor for TAA ions decreases, but the affinity for the internal receptor increases.

Time Dependence and Open Channel Block vs. Voltage-Dependent Block

In squid axon or node of Ranvier block by internal TBA and TPeA develops with a delay but block by TEA is more rapid. Results presented here support previous ideas based on macroscopic data from the axonal preparations that time-dependent block is associated with compounds having relatively slow rates of association and dissociation. Our data, as well as those in a paper utilizing single channel analysis applied to the delayed rectifier in frog muscle (Spruce et al., 1987), described a reduction in the amplitude of current through open K channels by external TEA. The kinetics are therefore quite rapid. The long chain TAA derivatives have slower kinetics which predict that block would be time dependent following a step depolarization when applied to the

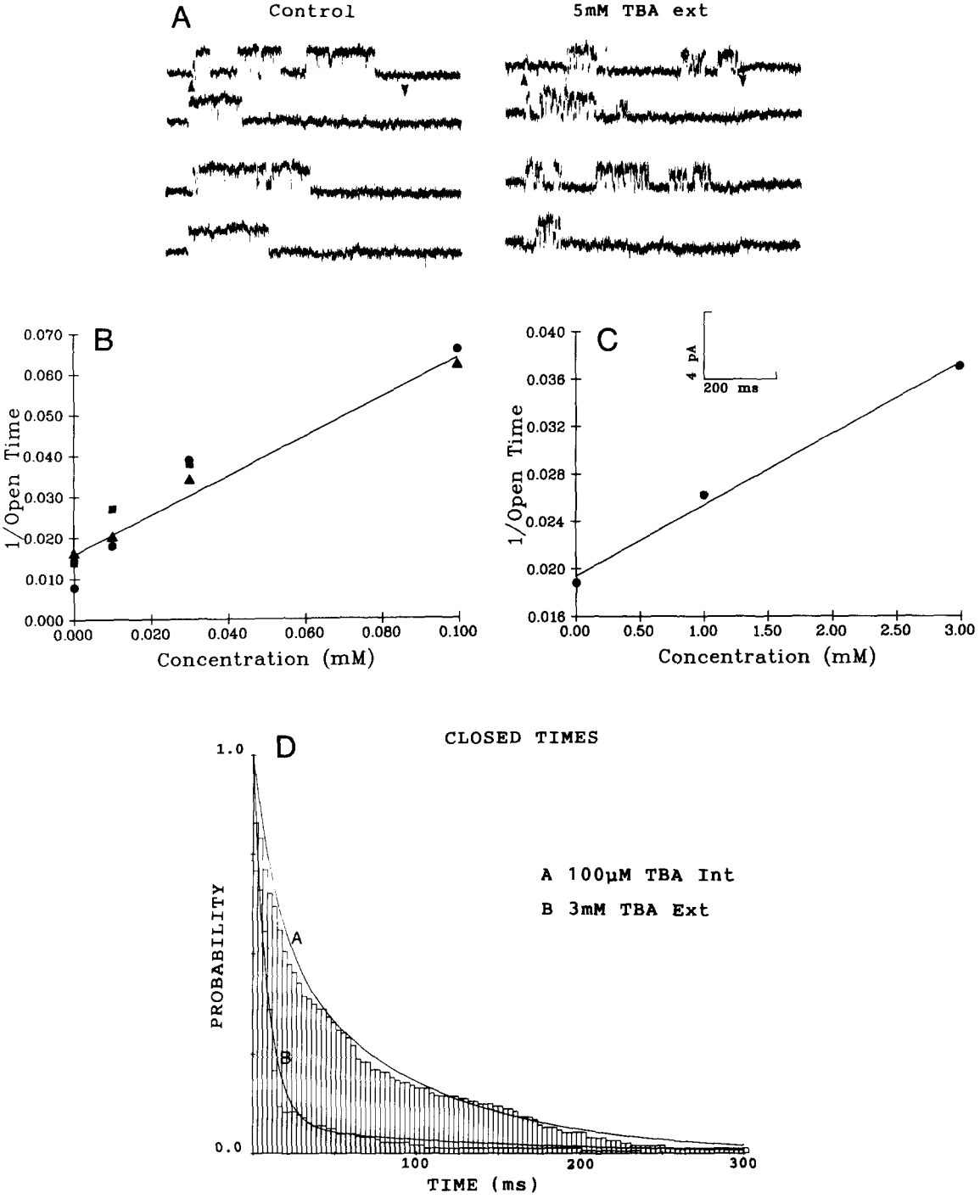


Fig. 4. Comparison of the kinetics of TBA block with internal or external application. (*A*) Currents recorded from an outside-out patch of membrane under control conditions, and in the presence of TBA added to the external solution. Test potential +30 mV, holding potential −50 mV. (*B*) The reciprocal of the mean open time as a function of the concentration of TBA is plotted for three inside-out patches of membrane (using different symbols). The mean duration at each concentration was fit to the equation: 1/Open Time = (k_2 TBA) + k_{-1}; where k_{-1} is the mean closing rate of the channel in the absence of TBA. The slope of this relationship gives the association rate for the blocker (k_2). For the curve plotted, k_2 = 0.48 ms^{-1} mM^{-1}, and k_{-1} = 0.016 ms^{-1}. (*C*) The inverse of the open time for TBA applied to the external solution is shown for an outside-out patch of membrane. The best fit line is plotted, having k_2 = 0.006 ms^{-1} mM^{-1}, and k_{-1} = 0.019 ms^{-1}. (*D*) Histograms of closed times for two experiments are superimposed. The continuous curves are best fits to two exponential distributions, and are given by: external TBA, $P(t) = 0.93 \exp(-0.11t) + 0.07 \exp(-0.007t)$, n = 102; internal TBA, $P(t) = 0.52 \exp(-0.07t) + 0.48 \exp(-0.009t)$, n = 126, t in ms. The holding potential for experiments in *B-D* was −100 mV.

inside. The decay of macroscopic K curent due to block by 1 mM TBA in the cytoplasmic solution would be expected to have a time constant of about 2 ms, based on single channel data. This value is in agreement with that found for TBA block of K current in squid axon (French & Shoukimas, 1981). In contrast, TEA has rather fast association and dissociation rates which would reduce the time dependence of block.

Armstrong (1971) hypothesized that internal TAA ions block with a delay since they bind to the channel only after it has opened when applied to the inside of squid axon. However, Clay (1985*b*) has argued that at least for TEA, the data support the view that the rate for channel block is increased by depolarization and is independent of the configuration of the channel. The effect of these blockers on currents measured through single channels indicate that interactions with the open channel dominate during the step depolarizations employed in our study. First, as expected for open channel block, we have found that the open time is reduced as the concentration of internal TAA ions having a long alkyl chain length is increased. Second, it is possible to identify two components in closed time histograms in the presence of these compounds. As expected, the proportion of the component having a mean duration similar to that found in the absence of blocker decreases with concentration. However, channels can close when blocked by internal TEA ions, although the closing rate is reduced. In addition, block of the open channel for internally applied TAA ions is voltage dependent. The association rate for block of the open channel was found to increase with depolarization.

Hille & Armstrong (1972) have found that time-dependent block of the K channel in the node of Ranvier is observed for internal long alkyl chain quaternary ammonium derivatives, but is absent when the same compounds are applied externally. They suggested that the absence of time-dependent block in the latter case is due to free access to the channel from the external mouth of the channel due to an interior location of the activation gate. TPeA and TBA block the macroscopic K current measured from neuroblastoma cells in a time-dependent manner when applied to the external solution. However, the time dependence of block occurs because these compounds can accumulate inside the cell when the whole-cell patch-clamp recording condition is utilized (Quandt & Im, 1992). The single channel analysis indicates that TAA ions acting externally do block the open channel, and channel gating is independent of drug binding. However, our data indicate that the difference in time dependence of block at the external receptor occurs because externally applied TAA ions reach this site slowly and dissociate rapidly.

Implications for Channel Structure

The experiments presented in this paper have revealed additional evidence supporting distinct external and internal receptors for TAA ions in the delayed rectifier-like channel of neuroblastoma cells. The different sensitivity of external and internal TAA ions could be due to a difference in the access to a single receptor from the internal route compared to an external route. However, the dissociation rate is independent of the access to the channel, and a single receptor would likely have a dissociation rate for any one derivative which is independent of the side of the membrane to which the blocker was applied. The dissociation rate for internal and external TBA was clearly different, pointing to distinct internal and external receptors for TAA ions.

Single channel analysis has shown that the dissociation rate for TBA is higher for the external receptor than for the internal receptor, and the association rate is slower. It is known that quaternary ammonium ions with long alkyl chains exhibit hydrophobic interactions for stabilization of the binding reaction, since the addition of a hydroxyl group to C9 greatly increases the dissociation rate (Swenson, 1981). Hydrophobic interactions are apparently not as extensively involved in stabilization of the blocker at the external receptor. The reduced association rate with the external receptor may be due to restricted access for larger molecules to the exterior mouth of the channel.

In contrast to the long alkyl chain length TAA ions, the sensitivity to TEA is higher for external application than for internal application. The association rate may be high for interactions between TEA and the external receptor or the dissociation rate may be low when compared to the internal receptor. Future experiments might systematically examine the alteration in kinetics with chain length. Perhaps the association rate with the external receptor increases dramatically as the chain length is reduced.

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