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Probing a Ca^{2+}-activated K^+ channel with quaternary ammonium ions

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Abstract. A series of quaternary ammonium (QA) ions were used to probe the gross architecture of the ion conduction pathway in a Ca^{2+}-activated K^+ channel from rat muscle membrane. The channels were inserted into planar phospholipid membranes and the single channel currents were measured in the presence of the different QA ions. Internally applied monovalent QA ions (e.g. tetramethylammonium and analogues) induced a voltage-dependent blockade with a unique effective valence of the block equal to 0.30, and blocking potency increases as the compound is made more hydrophobic. Blockade is relieved by increasing the K^+ concentration of the internal or external side of the channel. The effective valence of block is independent of K^+ concentration. These results suggest that, from the internal side, all monovalent QA ions interact with a site located in the channel conduction system. Divalent QA ions of the type n-alkyl-bis-α,β-trimethylammonium (bisQn) applied internally also block the channel in a voltage dependent fashion. For short chains (bisQ2-bisQ5), the effective valence decreases with chain length from 0.41 to 0.27, it remains constant for bisQ5 to bisQ6 and increases up to 0.54 for bisQ10. This dependence of block with chain length implies that 27% of the voltage drop within the channel occurs over a distance of ≈ 1 nm. Externally applied monovalent QA ions also block the channel. The site is specific for tetraethylammonium; increasing or decreasing the side chains in one methylene group decrease potency by about 400-fold. It is concluded that the Ca^{2+}-activated K^+ channel has wide mouths located at each end and that they are different in molecular nature.

Key words: Ca^{2+}-activated K^+ channel – Lipid bilayers – Quaternary ammonium ions – Blockade

Introduction

Quaternary ammonium ions have shown to be rather specific blockers of K^+ channels (Stanfield 1983). Armstrong (1969, 1971, 1975a, b) used these organic cations to investigate the gross architecture of the squid axon delayed rectifier. He proposed that the delayed rectifier channel contains, at its inner end, a mouth wide enough to accommodate tetraethylammonium (TEA; 0.8 nm in diameter). This internal site, at which a series of QA compounds bind, appears to be highly hydrophobic since increasing the alkyl chain length enhances blocking potency (Armstrong 1971; French and Shoukimas 1981; Swenson 1981). Armstrong has pointed out that the ability of TEA to block K^+ channels resides in the similarity in diameters of TEA and the hydrated K^+ ion. Assuming that the mouth ends in a constriction, K^+ can continue permeating into the channel because it can lose the hydration water and get solvated by polar groups on the pore walls. On the other hand, potassium channels of the node of Ranvier possess internal and external TEA receptors. The internal receptor in this channel is similar to the one existing in the squid axon delayed rectifier, but the external site is much more selective for TEA and substitution of even one methylene group reduces the binding constant (Armstrong and Hille 1972; Hille 1967). The large unitary conductance Ca^{2+}-activated K^+ channel present in a large variety of cells (for a review see Latorre 1986) is also blocked by internal and external TEA (Latorre et al. 1982; Vergara 1983; Vergara et al. 1984; Blatz and Magleby 1984; Yellen 1984a).

To investigate the characteristics of the internal and external TEA receptors in the large unitary conductance Ca^{2+}-activated K^+ channel we have studied in detail the blockade induced by a series of TEA analogues. We show here that, from the internal side of the channel, all QA compounds are able to block the channel by binding at a site located at 30% of the total potential drop and that the site is hydrophobic in nature. The external site behaves as the external TEA receptor of the Ca^{2+}-activated K^+ channel of *Aplysia* neurons (Hermann and Gorman 1981), i.e., it is very specific for TEA. Furthermore we have extracted the block and unblock rates for the external TEA blockade from the shape of the distribution of current amplitudes during channel flicker induced by this QA ion. We have also used a series of bis-quaternary ammonium blockers in an attempt to probe the spatial dependence of the electric potential through the pore (Miller 1982). We found that the voltage dependence of the blocking reaction varies with methylene chain length suggesting that 30% of the total potential drop inside the channel occurs over a distance equivalent to six CH_2 groups.

Materials and methods

Bilayer formation and channel incorporation. Single channel currents were recorded by incorporating purified transverse tubule vesicles into planar bilayers composed either of phosphatidylcholine (PC) or a mixture of 70% phosphatidyl-

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ethanolamine (PE) and 30% phosphatidylserine (PS). The phospholipids were dissolved in decane to a final concentration of 14 mg/ml and were obtained from Avanti Polar (Birmingham, AL, USA). Preparation of transverse tubule vesicles has been described (Moczydlowski and Latorre 1983). Transverse tubule vesicles were added to one side of the membrane only, which correspond to the cytoplasmic or internal side in the cell. The opposite "external" side is defined as zero voltage. Aqueous solutions used were composed of 100 mM KCl, 5 mM MOPS, pH 7, unless otherwise indicated. Calcium concentrations used ranged from 10 to 100 μM.

Monovalent QA compounds were obtained from Eastman Kodak (Rochester, NY, USA) and bis-quaternary ammonium ions were synthesized according to the method described by Miller (1982). For the bis-quaternary ammonium compounds we will follow the nomenclature used by Miller (1982). bisQn will refer to an n-chain alkane with a trimethylammonium residue at each end. For example bisQ10 refers to n-decane-1,10-N,N,N,N′,N′,N′-hexamethyl-bis-ammonium. Other compounds will be called by their proper names.

Electrical measurements. Lipid bilayers were usually formed in a small hole (300 μm in diameter) in a Teflon partition (25 μm in thickness) that separates the two chamber compartments. In some experiments where low current noise was desired smaller holes (≈ 100 μm in diameter) were made. Membrane capacitance varied from 300 – 500 pF for bilayers made in the large holes and was 50 – 100 pF for those made in the small holes. The current passing through the membrane was measured with a two electrode voltage clamp. Connections were made through Ag/AgCl bridges connected to 3 M KCl agar bridges. One side of the chamber (internal) was connected to a waveform generator and the other to a current-to-voltage converter (Burr-Brown OPA 101 operational amplifier, Burr-Brown, Tucson, AZ, USA) with a feedback resitor of 10 GΩ. The output of this stage was amplified and the overall response of the system improved with a frequency booster (Sigworth 1983). Under these conditions the time constant of the amplifier was 100 μs. Current noise for membranes of 150 pF was 1 pA peak-to-peak, after a 1 kHz first order low pass filter.

Data analysis. In a simple model, in which the channel is either conducting or blocked by the QA compound, the time-average channel current, I, in the presence of a charged blocker, with valence z is given by (Woodhull 1973)

$$I = I_0(1 + [B]/K(0)\exp(-z\delta FV/RT))^{-1}, \quad (1)$$

where I_0 is the value of the unblocked channel current, and [B] is the concentration of the blocker, K(0) is the zero-voltage dissociation constant and δ the fraction of the total potential drop at the binding site. The quantity $z\delta$ measures the voltage dependence of the block and is usually called the "effective valence" of the blocking reaction. The apparent dissociation constant K_d is $K(0)\exp(-z\delta FV/RT)$. The effective valence of the blocking reaction and the dissociation constant were found by fitting Eq. (1) to the experimental data using the nonlinear least square method of Gauss-Newton.

External TEA induces a flickering noise of the open channel current (see Fig. 9). However, transitions between open and blocked states of the channel cannot be resolved. To find the rate constant that describe the external TEA blockade we used the amplitude distribution analysis method (FitzHugh 1983; Yellen 1984a). We assume here that the blocking and unblocking reaction is a two state process with a blocking rate constant α and an unblocking rate constant β. The probability density function of the current amplitude is a function of α, β and the time constant of the current detection system. In order to correct for unrelated noise (noise coming from the membrane capacitance and from the I – V converter), the probability density function was convolved with a Gaussian distribution with a standard deviation obtained by fitting the amplitude distribution of either the closed or open channel noise, in the absence of blocker. The rate constants of the blocking reaction were found using a nonlinear curve fitting procedure which searches for the α and β parameters that give a probability density function of the same shape as the experimental amplitude histogram.

The procedure for collecting and processing the amplitude histograms was as follows: Sections of single channel records were digitized at 100 μs per point and the amplitude divided by the average current passing through the open channel in the absence of blocker. Thus, the open channel has amplitude 1 and the closed channel amplitude 0. All the points corresponding to a given amplitude were added and this number was divided by the total area of the histogram. Individual amplitude histograms contained contributions from 200 to 3000 ms of open channel flicker. In order to set the histogram limits, the average amplitude of the current when the channel is closed was measured, and this value defined as amplitude 0. Amplitude 1 was defined as the difference between this amplitude and that of the open channel in the absence of blocker.

Results

Internal monovalent QA ions induce fast and flickering block

When TEA analogues are added to the internal side, two types of blockade can be distinguished, as seen in Fig. 1: a "quiet" (fast) blockade induced by the shorter alkyl chain compounds (e.g. TMA, TEA), and a flickering blockade promoted by the longer chain compounds (e.g. nonyltrimethylammonium). In the first case the blocking events are too fast to be resolved by the current measuring system and the apparent open state current corresponds to a time-averaged value determined by the rapid blocking and unblocking processes. As predicted by Eq. (1), this time averaged current is reduced as the concentration of blocker is increased. On the other hand, a compound like nonyltrimethylammonium when applied to the inside face of a Ca^{2+}-activated K^+ channel, it produces brief interruptions of the open channel current. In this case the dwell times in the blocked and open states are long enough to be measured directly.

Characteristics of internal TEA blockade

Several groups have shown that internally applied TEA produces a voltage dependent block (Vergara 1983; Vergara et al. 1984; Blatz and Magleby 1984; Yellen 1984a). However, the detailed characteristics of the blockade induced by this QA compound have not been studied. The T-tubule

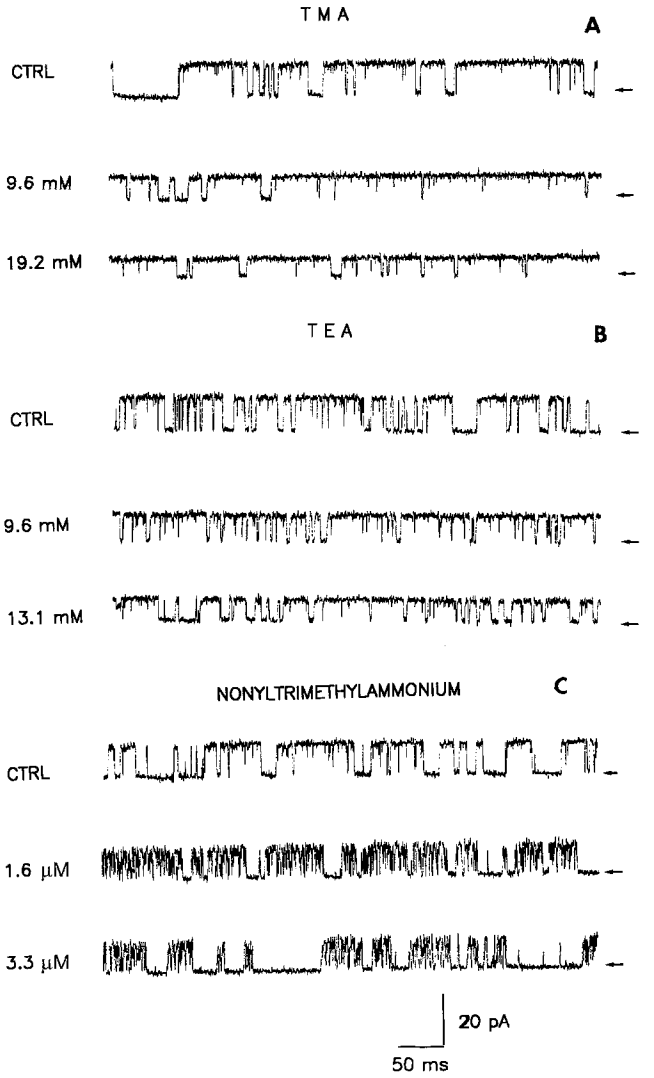


Fig. 1 A–C. Single channel block by monovalent cations. **A** Single channel current records in the absence (*ctrl*), and in the presence of TMA at the indicated concentrations in mM. **B** Current records in the absence (*ctrl*) and in the presence of TEA (mM). **C** Current records in the absence (*ctrl*), and in the presence of nonyltrimethylammonium; *arrows* indicate the closed state. Notice that TMA and TEA induce a reduction in channel current whereas nonyltrimethylammonium induces a flickering block. Channels incorporated into PE/PS = 4/1 membranes. The KCl concentration was 100 mM and the membrane voltage was + 60 mV

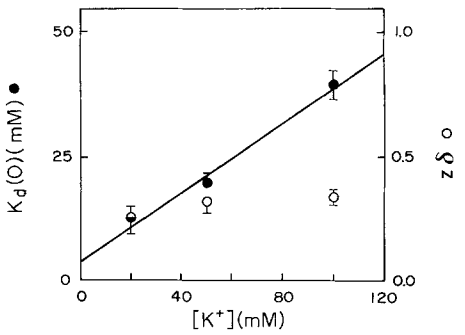


Fig. 2. TEA block is competitive with K^+. Measurements of the parameters $z\delta$ and K(0) at the indicated $[K^+]$ were done as described in methods. The least-squares regression line through the K(0) data correspond to a K(0) for TEA of 4 mM at zero K^+ concentration. In this $[K^+]$ range the $z\delta$ parameters remain constant. The concentration of TEA was 33 mM. All measurements were done in PE membranes. Each *point* is the average of five determinations and the error bars are standard error of the mean

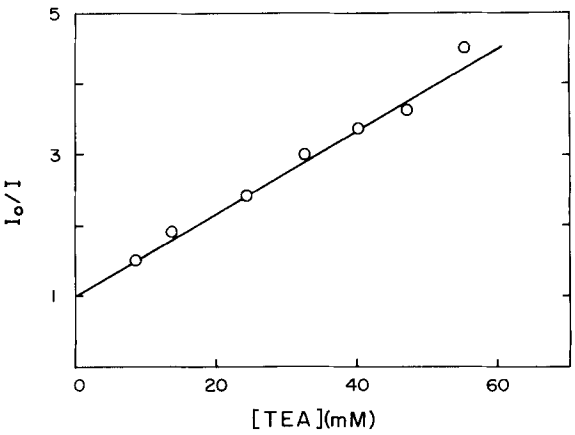


Fig. 3. Stoichiometry of the TEA blocking reaction. The ratio between channel current I_0 in the absence and in the presence, I, of blocker was plotted against [TEA]. The experimental data is well described by a regression line with an intercept of 1 and a K_d = 17 mM. Channels were incorporated into a PE/PS = 4/1 membrane at the voltage was + 60 mV. $[K^+]$ = 100 mM. Data obtained in a single membrane

channel is blocked by TEA with a $z\delta$ = 0.27 and a K(0) = 34 mM in PE/PS = 4/1 membranes (Table 1). These results are in good agreement with those obtained by Vergara (1983) in PE/PS = 7/3 membranes [$z\delta$ = 0.34; K(0) = 35 mM]. Figure 2 shows that the voltage dependence of TEA blockade is independent of $[K^+]$ as demanded by Eq. (1). Furthermore, K(0) increases from 12.5 mM at 20 mM K^+ to 40 mM at 100 mM K^+ in this experiment. It is clear, then, that TEA blockade is relieved by K^+. Extrapolation to zero $[K^+]$ yields a K(0) = 4 mM. These results are important inasmuch as they support the simple model given in methods, and that the voltage dependence of the TEA blockade arises as a sole consequence of the interaction of the probe with the electric field. It is possible that despite the fact that in many regards the Ca^{2+}-activated K^+ channel behaves as a multi-ion pore, the behavior of QA ions blockade follows that expected for one-ion pores because the probability of double occupancy in the range of $[K^+]$ tested is low (Cecchi et al. 1986). Relief of internal TEA blockade is also obtained when the $[K^+]$ is increased in the external side only. In this case the voltage dependence of block is unaffected by changes in the $[K^+]$ range tested. This result strongly suggest that K^+ ions are able to relieve the TEA block by competing with the blocker for a site located in the conduction system of the channel.

Figure 3 illustrates the concentration dependence of the reduction of the channel current by TEA at 60 mV. The solid line represents the best fit to Eq. (1) with a K_d = 17 mM. The lower value of K_d at 60 mV compared with that obtained at zero applied voltage (Table 1) reflects the voltage dependence of TEA block. Also, this result is consistent with the assumption that only one TEA molecule is needed to block the channel.

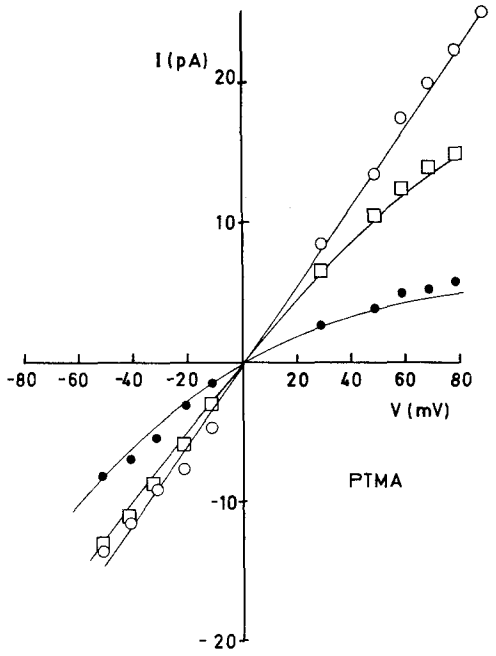


Fig. 4. Internal block induced by phenyltrimethylammonium. (○) Control, (□) 0.38 mM PTMA, (●) 3.0 mM PTMA. The *solid lines* are fit to the data using Eq. (1) with K(0) = 1.6 mM and $z\delta$ 0.28 (□), and K(0) = 2.0 mM and $z\delta$ 0.3 (●). The K^+ concentration was 100 mM. PE/PS = 4/1 membrane. Data obtained in a single membrane

Table 1. Blocking parameters for internal monovalent QA ions

Compound	K(0) mM	$z\delta$	N
Tetramethylammonium	65 ± 6	0.29 ± 0.007	5
Tetraethylammonium	34 ± 7	0.27 ± 0.06	10
Tetrapropylammonium	17 ± 5	0.29 ± 0.10	2
Tetrabutylammonium	0.5	0.30	1
Phenyltrimethylammonium	1.8 ± 0.2	0.27 ± 0.02	4
Nonyltrimethylammonium	0.008 ± 0.001	0.40 ± 0.20	8
Nonyltriethylammonium	0.004[a]	0.35[a]	
Tetrakis	29 ± 5	0.26 ± 0.03	3
		mean 0.30	

The parameters were calculated from fits of Eq. (1) to the I/I_0 vs. voltage data. The concentration of K^+ was in all cases 100 mM. Values are the mean ± SD. PE/PS = 4/1 membranes. [a] Data from Vergara (1983)

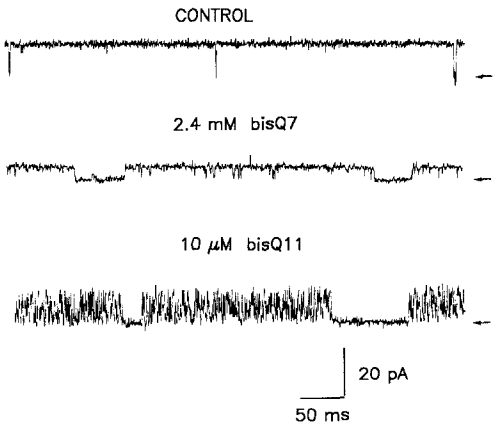


Fig. 5. Quiet and flickering block induced by internal bisQn ions. Records of channel fluctuations are shown in the absence or presence of bisQn compounds. *Top trace* is a control channel current record in the absence of blocker. The channel conductance was 225 pS. *Middle trace* is a record taken in the presence of 2.4 mM bisQ7. The channel conductance was 58 pS. The *lower trace* shows flickering block induced by 10 μisQ11. *Arrows* indicate the closed state. Membrane voltage was + 60 mV. Records were filtered at 2.5 kHz. PE/PS = 4/1 membrane. $[K^+] = 100$ mM

All QA ions bind to the same internal site

All the QA compounds tested here induced a voltage dependent block of the Ca^{2+}-activated K^+ channel and Fig. 4 shows the current-voltage curves obtained for two different concentrations of phenyltrimethylammonium. The current-voltage curves are well fitted using Eq. (1) with $z\delta$ values of 0.28 and 0.29, and K(0) of 1.6 and 2.0 mM. Thus, this QA compound has the same effective valence for the blocking reaction as TEA, but a much lower dissociation constant. We determined the parameters $z\delta$ and K(0) for a series of QA ions of different structure by fits of Eq. 1 to the current-voltage curves obtained in their presence (e.g. Fig. 4). Table 1 shows the values of these parameters for a series of QA compounds. It is apparent from Table 1 that increasing hydrophobicity of the QA ion increases the affinity for the site. Nonyltrimethylammonium is the most hydrophobic compound of the series tested and has the largest affinity. On the other hand, the $z\delta$ are very similar, indicating that all QA ions block the channel by binding to a site located at an electrical distance equivalent to ≈ 30% of the voltage drop within the channel. It is interesting to note that tetrakis (a TEA-like compound in which one of the hydrogen of each methyl group is replaced by −OH groups) with a size similar to TEA, but much more hydrophilic, blocks the channel with an affinity similar to that of TEA.

BisQn ion voltage-dependent block depends on blocker structure

Figure 5 shows that internally applied bisQ7 induces a "quiet" block. The effect of bisQ7 is to reduce the channel current and no flickering events can be seen. This type of quiet block is seen for all compounds with hydrocarbon chains of eight carbon atoms or less. On the other hand, bisQ11 causes the open channel current to flicker between an open and a blocked state. In those well-resolved blocking events it can be seen that the conductance of the blocked state is not different from that of the closed state. Other internally applied divalent quaternary ammonium (bisQn) ions also induce a voltage dependent block of the Ca^{2+}-activated K^+ channel (Fig. 6). Figure 6 shows that the data obtained in the presence internally applied bisQ2, bisQ5, and bisQ10 are well fitted by Eq. (1). However, at difference of the monovalent QA compounds, the $z\delta$ parameter for bisQn compounds is a function of hydrocarbon chain length. Actually, the voltage dependence of bisQ10 and bisQ2 block is about twice as steep as that found for bisQ5.

Figure 7 shows the $z\delta$ parameter as a function of chain length. For short hydrocarbon chains the $z\delta$ parameter is larger than the average value found for monovalent QA compounds, $z\delta$ reaches a constant value of about 0.26 for

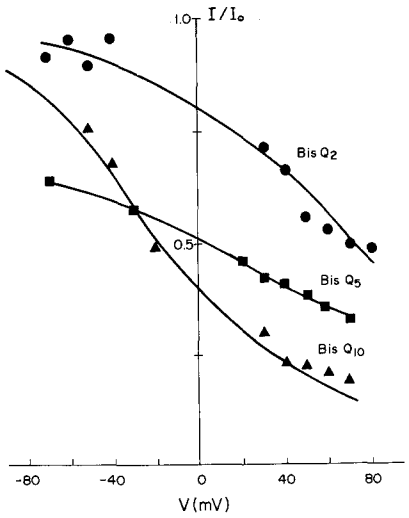


Fig. 6. Voltage dependence of internal bisQn block. Experimental points are the ratio between single channel currents in the presence (I) and in the absence of (I_0) of bisQn. The concentrations of bisQ2, bisQ5, and bisQ10 were 8.6 mM, 4.96 mM, and 53 μM respectively. *Solid curves* are drawn according to Eq. (1) with the following parameters: K(0) = 16 mM and $z\delta = 0.48$ for bisQ2; K(0) = 5 mM and $z\delta = 0.25$ for bisQ5; K(0) = 43 μM and $z\delta = 0.6$ for bisQ10. These are representative experiments. Each curve is the result of a set of measurements in one membrane

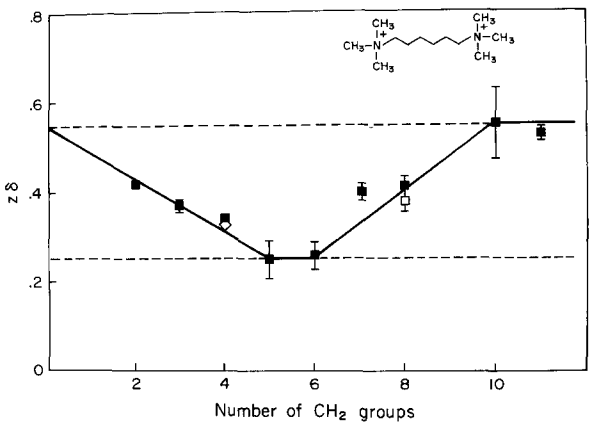


Fig. 7. Voltage dependence of bisQn compounds as a function of methylene chain length. The $z\delta$ parameter was calculated as in Fig. 6. The *lower broken line* is drawn at the mean $z\delta$ obtained for monovalent QA ions, and the *upper one* is drawn at 2 times $z\delta$. (◇) 2-butene,N,N,N,N′,N′,N′hexamethyl-1,4-bisammonium. (□) 1,8bis-guanidinium-n-octane. The K^+ concentration was 100 mM. PE/PS = 4/1 membranes. *Error bars* are standard error of the means of 3–5 experiments. *Lines* are drawn by eye

bisQ5 and bisQ6 and increases again for bisQn ions of larger chains. For example, the average $z\delta$ value for bisQ10 is 0.55. In other words, for bisQn compounds with the two charges separated by a very short or very long hydrocarbon chain $z\delta$ has a value that is close to twice the value obtained for QA ions, and for bisQ5 and bisQ6 this value is very close to the average value obtained for monovalent quaternary ammonium ions (i.e. 0.30). We note here that the rigid four-carbon analogue of bisQ4, with a C2–C3 double bond (2-butene,N,N,N,N′,N′,N′hexamethyl-1,4-bis-ammonium)

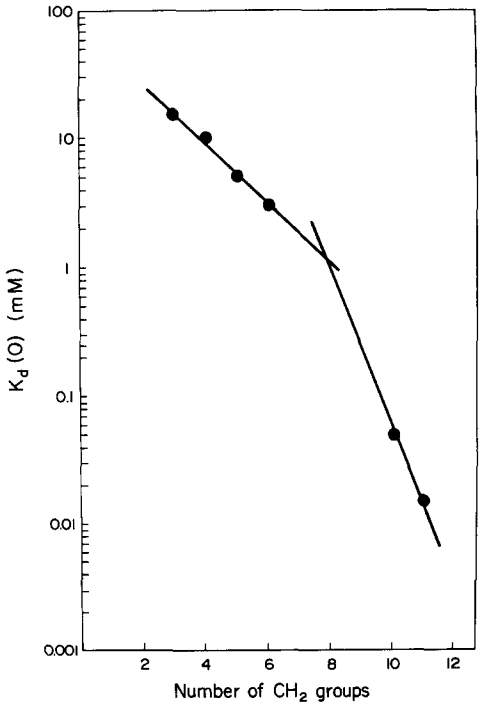


Fig. 8. Zero voltage dissociation constants for bisQn compounds. The *solid line* for quiet blockers (bisQ2–bisQ8) was drawn by eye and the slope correspond to an energy of 311 cal/mole of CH_2. This value is obtained using the equation $\Delta G^\circ = -n\,RT \ln (K_{d1}/K_{dn})$ where ΔG° is the standard free energy per mole of CH_2, K_{d1} and K_{dn} are the dissociation constants for a molecule with one and n CH_2 groups respectively, R and T have their usual meanings. For flickering blockers (bisQ10–bisQ11) the *solid line*, drawn by eye, has a slope of 720 cal/mole of CH_2.

and the eight carbon analogue of bisQ8, bisG8 (1,8bis-guanidinium-n-octane), show effective valences very similar to those obtained for bisQ4 and bisQ8 respectively.

Figure 8 shows the variation in K(0) for bisQn compounds with methylene chain length. The affinity of the site for a given bisQn compound increases as the chain length increases. Moreover, the dependence of K(0) on chain length is 311 cal/mol CH_2 for compounds with less than eight carbon atoms and increases to 720 for larger chain length bisQn compounds.

Characterization of the external QA ion binding site

External TEA is also able to block the Ca^{2+}-activated K^+ channels and it does so with a much higher affinity [K(0) ≈ 0.2 mM; Vergara 1983; Vergara et al. 1984; Yellen 1984a; Blatz and Magleby 1984]. Furthermore, Vergara (1983) showed that nonyltriethylammonium up to an external concentration of 300 μM, does not induce any appreciable block. These results suggested that the internal and external QA ion receptors are different.

Table 2 shows the blocking parameters for a series of monovalent and divalent quaternary ammonium ions added to the external side. The external QA ion site is very specific for TEA and compounds slightly larger, like tetrapropylammonium, or slightly smaller, like TMA, have binding affinities about 400-fold smaller. It is also noticeable from this table that the $z\delta$ parameter for TMA (0.2) and tetrakis (0.21) is about 2-fold larger than that for TEA or for

Table 2. Blocking parameters for external monovalent QA ions

Compound	K(0) mM	$z\delta$	N
Tetramethylammonium	43 ±9	0.20±0.05	3
Tetraethylammonium	0.12±0.01	0.13±0.05	5
Tetrapropylammonium	54 ±4	0.13±0.03	2
Tetrakis	48 ±5	0.21±0.03	3
bisQ3	6 ±1	0.48±0.09	3
bisQ4	11 ±2	0.38±0.09	2
bisQ5	71 ±3	0.22±0.01	2

The parameters were calculated from fits of Eq. (1) to the I/I_0 voltage data. The concentration of K^+ was in all cases 100 mM. Values are the mean ± SD. PE/PS = 4/1 membranes

tetrapropylammonium (0.13). Possibly the former compounds can get further into the conduction system of the Ca^{2+}-activated K^+ channel than the latter.

BisQn compounds also block the channel from the external side, but in contrast to their action from the internal side, K(0) increases as the bisQn becomes larger (Table 2). On the other hand, their $z\delta$ parameters appear to be a function of methylene chain length. The effective valence, as in the case of internal blockade, appears to increase as the methylene chain length decreases. Given the binding characteristics of the external binding site it was not possible to perform a complete study for the bisQn compounds as the one done for the internal site. Longer chain bisQn ions bind less and high concentrations of the more hydrophobic bisQn compound are required to promote channel block making the experiments very difficult to perform.

External TEA block analyzed from amplitude histograms

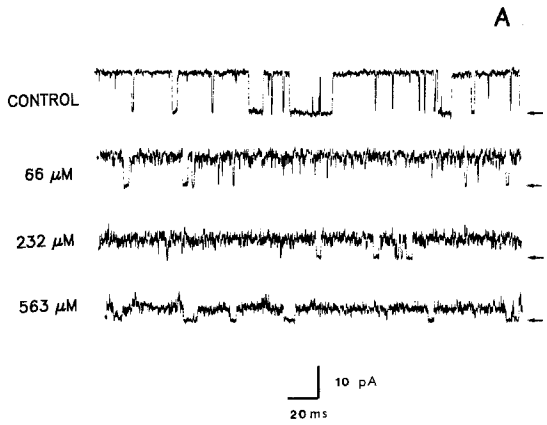
TEA added to external side of the channel causes the open channel current to decrease and appear very noisy or flickery (Fig. 9A). Even at the practical recording limit of the bilayer set-up electronics (≈ 2.5 kHz), the flicker induced by external TEA cannot be resolved into discrete opening and blocking events. However, the noisy records shown in Fig. 9A allow the possibility for analyzing the kinetics of blocking by the amplitude distribution method.

The histogram of the extreme right in Fig. 9B corresponds to that obtained in the absence of blocker. Other histograms of Fig. 9B were obtained in the presence of the indicated blocker concentration. Fig. 9B shows that the amplitude histograms shifted to the left as the concentration of TEA was increased. The blocking and unblocking rates for TEA added externally were determined from these histograms. The blocking rate constant increases linearly with blocker concentration and the unblocking rate constant is not affected by TEA concentration (data not shown). These results are consistent with a model in which TEA binds to a site in the channel and blocks the potassium current flowing through the channel.

Figure 10 shows the effect of voltage on the rate of the blocking and unblocking rate constants as determined from the amplitude distribution analysis method. Both rate constants are voltage dependent and are well described by

$$\alpha = \alpha_0 \exp(-z\delta_1 FV/RT), \quad (2a)$$

$$\beta = \beta_0 \exp(z\delta_2 FV/RT), \quad (2b)$$



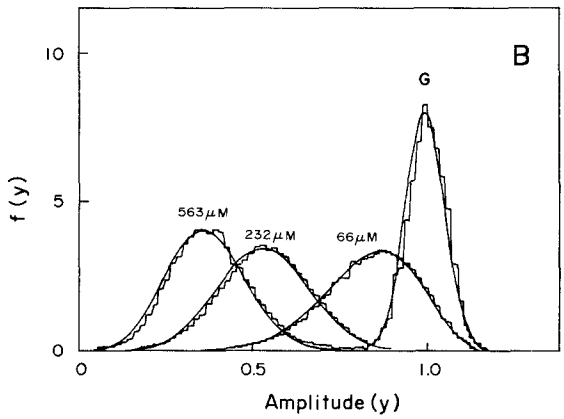


Fig. 9. A Flickery block by external TEA. Single channel records at the indicated TEA concentrations (in μM) added to the external solution were obtained at + 60 mV. The *arrows* indicate the baseline. Data were filtered at 2.5 kHz. The K^+ concentration was 100 mM. PE/PS membrane. **B** Amplitude histograms for individual channel openings in the presence and absence of external TEA. Data is plotted as the number of ocurrences, $f(y)$, vs the amplitude, y. The *histogram* labelled G was obtained in the absence of TEA and was fitted with a Gaussian distribution. Other *histogram* were fitted as described in Methods and correspond to the theoretical amplitude distributions

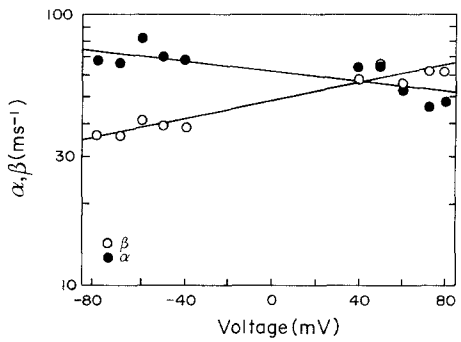


Fig. 10. Voltage dependence of TEA block and unblock rate constants. Blocking kinetic parameters were calculated as described in Methods and in Fig. 9. Both rate constants are well fitted by exponential functions (Eq. 2a and 2b) with $\alpha_0 = 63\ ms^{-1}$ and $\delta_1 = 0.1$, and $\beta_0 = 43\ ms^{-1}$ and $\delta_2 = 0.05$

where α_0 and β_0 are the blocking and unblocking rate constants at zero voltage and $\delta_1 = 0.1$ and $\delta_2 = 0.05$. Furthermore, the sum of $\delta 1 + \delta 2$ should predict the voltage dependence of K_d since $z\delta = z(\delta_1 + \delta_2)$. The parameters obtained for the equilibrium behavior of the TEA block were: $K(0) = 0.12 \pm 0.01$ mM, $z\delta = 0.13 \pm 0.01$. On the other hand, those obtained with the amplitude distribution analysis were: $K(0) = 0.30 \pm 0.01$, $z\delta = 0.15 \pm 0.01$. The agreement is reasonable good and serves as an internal check of the validity of amplitude distribution analysis.

Discussion

Potassium channels and QA ion block

The Ca^{2+}-activated K^+ channel studied here shows an internal TEA site that binds strongly the long chain quaternary ammonium ions. Furthermore, blocking potency for monovalent QA ions increased with increasing size in a way similar to that found for the squid axon potassium channel (French and Shoukimas 1981). Both French and Shoukimas (1981) and Swenson (1981) concluded that the inner mouth of the K^+ channel of the squid axon had to be larger than the 0.8 nm diameter originally proposed by Armstrong. Swenson pictured the K^+ channel as having an inner mouth of about 1.2 nm in diameter. Given the results presented here and those obtained in the delayed rectifier channel, we think that this size may be also appropriate for the inner mouth of the Ca^{2+}-activated K^+ channel.

Tetramethylammonium penetrates the squid axon K^+ channel to an electrical distance that is about twice the value obtained for the longer chain monovalent QA (French and Shoukimas 1985). In the Ca^{2+}-activated K^+ channel TMA blocks with the same voltage dependence shown by the other monovalent QA compounds. This may indicate that the inner mouth in the Ca^{2+}-activated K^+ channel ends in a constriction (≈ 0.25 nm; diameter of TMA) that does not allow TMA to go further into the conduction system.

Tetrakis penetrates further than monovalent QA ions in the squid axon delayed rectifier. Its voltage dependence is the same as that found for TMA and its K(0) falls between TMA and TEA (French and Shoukimas 1985). Therefore, it is not essential for a QA compound to have its chains terminated in hydrophobic alkyl groups to be an effective blocker. The same conclusion is reached when analyzing the results obtained here, but tetrakis blocks at the same site as the other monovalent QA ions do. As stated in results this finding is in apparent contradiction with the fact that blocking potency is increased by making the compound more hydrophobic (see Table 1). This paradox can be explained if the actual site is very near an hydrophobic pocket, but in itself is not hydrophobic. Also, Dani (1986) has argued that the size of the organic cation is an important factor in determining the binding characteristics of the channel.

Detailed studies of the external TEA site have been done by Hille (1967) in frog node of Ranvier delayed rectifier and by Hermann and Gorman (1981) in a molluscan neuron Ca^{2+}-activated K^+ channel. In both cases it was found that the site is very specific for TEA. The T-tubule Ca^{2+}-activated K^+ channel does not escape to this rule indicating once again the similarity in structure (at least of the wide mouths) of potassium channels of very different conductance. We note here that the Ca^{2+}-activated K^+ channel present in clonal anterior pituitary cells the TEA specific site is located in the cytoplasmic side of the channel (Wong and Adler 1986). This is a puzzling observation since the pituitary cell channel share many of its properties with the channel we studied here.

Models for TEA blockade in Ca^{2+}-activated K^+ channels

The $z\delta$ parameter can be interpreted as the actual electrical distance where the blocking site is located only when channel is occupied by a maximum of one ion. For a multi-ion channel the effective valence of the blocking reaction obtained through Eq. (1) cannot be directly associated with a particular position of a single discrete binding site in the electric field. In multi-ion pores the voltage dependence of a blocking ion includes contributions from the interaction of both the blocker and the permeant ions with the electric field (Hille and Schwarz 1978).

At present, evidence is mounting that Ca^{2+}-activated K^+ channels of large conductance are multi-ion pores (Vergara et al. 1984; Yellen 1984b; Eisenman et al. 1986; Cecchi et al. 1987). However, large organic cation blockade has the characteristics expected for a single-ion channel. In addition, the following results support this contention: (a) the $z\delta$ parameter is independent of $[K^+]$ in the range of concentrations tested; (b) QA ion block is competitively relieved by K^+. The relief by potassium of the blockade appears to be competitive whether we increase the concentration of this ion in the internal, external, or at both sides simultaneously (cf. Danko et al. 1986).

Why are we in the presence of a "well behaved" type of block despite the fact that the channel under study is a multi-ion channel? The reason for this behavior cannot be understood completely at present, but our modelling for conduction of the Ca^{2+}-activated K^+ channel (Cecchi et al. 1986; Latorre 1986) produces an energy barrier picture in which the channel can be only occupied by a single K^+ in the range of $[K^+]$ tested here. The same result has been obtained by Villarroel and Eisenman (1987) by doing a careful examination of the current-voltage relationship for the open channel in a wide range of $[K^+]$.

Relation between electrical and physical distances

We will follow the analysis used by Miller (1982) to interpret the variation of $z\delta$ with methylene chain length for bisQn compounds in the sarcoplasmic reticulum channel. In this case, the voltage dependence for bisQn compounds must be given by the sum of the $z\delta$ for each charge.

Assuming that one of the charges is always interacting with a site located at a $\delta = 0.27$, a measurement of the voltage dependence of a given bisQn ion allows us to determine δ for the other charge. We expect a decrease in $z\delta_{\text{bisQn}}$ as the chain length increases until it reaches a constant value when the second charge is completely outside of the electric field.

Because bisQ5 behaves as a monovalent QA ion ($z\delta = 0.26$) we conclude that the second charge of this compound must be outside of the field. The length of bisQ5 is 0.96 nm and, therefore, $\approx 27\%$ of the voltage drops over a distance of ≈ 1.0 nm. Compounds larger than bisQ7 can block in bent-over conformation as suggested by Miller (1982). For bisQ10 it appears that both charges are interacting with the site inasmuch as this compound shows a $z\delta = 0.55$.

The results obtained with the bisQn ions show that the energy of transfer from the internal solution to the site is 311 cal/mol of CH_2. This value applies up to methylene chains of seven carbon atoms, and is very similar to the one obtained by Miller (1982) when using bisQn compounds to probe the access of the sarcoplasmic reticulum K^+ channel. For larger compounds this energy is 720 cal/mol of CH_2. Thus, bisQn's are also interacting with the hydrophobic moeity located at the channel mouth. The classic hydrophobic effect predicts an energy of transfer of 800 cal/mol of CH_2 transferred from water to a liquid hydrocarbon. This value is very close to that found for long-chain blockers, but is 2-fold larger than the one obtained for bisQn ions with chains shorter than bisQ7. One possible explanation for this difference can be that for long-chain compounds the bent-over conformation favors the hydrophobic interactions with the hydrophobic pocket located in the internal entrance to the channel. For the more rigid compounds only part of the methylene chain would be able to interact with this site.

Mechanism of external TEA blockade

In analysing the external TEA blockade with the amplitude distribution method (Yellen 1984a) it was assumed that block is a simple process, in which the channel is either blocked or open and that the transition between states is described by a two-state Poisson process. The first assumption is reasonable since, as predicted by the model, the rate of entering the channel varies linearly with [TEA] and the unblocking rate constant is independent of [TEA].

The voltage dependence of the blocking rate constant is larger than the voltage dependence of the rate constant for unblocking. One conclusion that can be extracted from these results is that the energy profile for the interaction of external TEA with the channel is asymmetric. The value of the rate constants at zero voltage are 2.1×10^8 $M^{-1}s^{-1}$ for the blocking rate constant and 6.3×10^4 s^{-1} for the unblocking rate constant. These values indicate that the entry of TEA to the channel from the external site is very close to a diffusion limited process, but that it is difficult for TEA to leave the site once in the blocking position.

QA ion block and channel structure

Several conclusions can be extracted from the present experiments from which we can obtain an idea about the gross architecture of the Ca^{2+}-activated K^+ channel. The results show (a) the channel has wide entrances at each side, (b) at least part of the internal mouth is hydrophobic, (c) the external mouth is specific for TEA and is not hydrophobic in nature, and appears to select monovalent QA ion by their sizes, (d) the block induced by mono- and divalent QA ions appears to follow a simple model in which the channel is either open or blocked, (e) the analysis of the data obtained with the bisQn compounds allowed us to estimate the physical distance from the place where the voltage drop begins to the blocking site and thus obtain a relation between physical and electrical distance in this part of the channel. This distance is about 1 nm, (f) the amplitude distribution analysis of the channel flicker induced by external TEA allowed us to obtain the rate constant for this block and the shape of the energy barrier for this ion.

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