J. Membrane Biol. 164, 275–281 (1998)

The Journal of
Membrane
Biology

The Kinetics of Quinine Blockade of the Maxi Cation Channel in the Plasma Membrane of Rye Roots

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Received: 20 January 1998/Revised: 1 May 1998

Abstract. The maxi cation channel from the plasma membrane of rye (*Secale cereale* L.) roots was studied following its incorporation into planar phosphatidylethanolamine bilayers. Current recordings were made in the presence of 100-mM KCl containing quinine on both sides of the bilayer. Quinine produced voltage- and concentration-dependent blockade of the channel, reducing its apparent unitary current and open probability. The voltage-dependence suggested that blockade was effected from the cytoplasmic side by cationic quinine. Blockade was modelled using a kinetic scheme with two independent blocked states termed *B1* and *B2* ($B1 \leftrightarrows O \rightleftarrows B2$). Rate constants promoting fast kinetics (k_1 and k_{-1}) were found to be several orders of magnitude greater than those promoting slow kinetics (k_2 and k_{-2}). Analysis of the fast kinetics indicated that the rate constants for blockade of the open channel at the first site (k_1) and its clearance (k_{-1}) had voltage-dependencies ($z\delta_p$) of 0.41 and −0.71, respectively, and that the equilibrium dissociation constant for the binding site ($K_d(0)$) was about 1 mM. Analysis of the slow kinetics indicated that the rate constants for blockade of the open channel at the second site (k_2) and its clearance (k_{-2}) had $z\delta_p$ values of 0.12 and −1.27, respectively. The $K_d(0)$ value for the second binding site was about 10 mM.

Key words: Blockade — Calcium — Channel kinetics — Maxi cation channel — Quinine — Rye (*Secale cereale* L.)

Introduction

The maxi cation channel has been characterized following the incorporation of plasma membrane fractions from rye (*Secale cereale* L.) roots into planar lipid bilayers (PLB; White, 1993, 1997). This channel is permeable to a wide variety of monovalent and divalent cations and exhibits high unitary conductances in single-salt solutions. Its kinetics are dominated by voltage-dependent inactivation, and both the time-constant for channel inactivation and the probability of the activated channel being in an open state are reduced as the membrane potential is displaced from the zero-current (reversal) potential of the channel. Under physiological ionic conditions, the channel would be activated by plasma membrane depolarization and mediate Ca^{2+} influx into the root cell (White, 1993, 1997). Thus, it has been argued that the maxi cation channel may have a role in cell signalling (White, 1997, 1998). It will open in response to stimuli which depolarize the plasma membrane, contributing to a rise in cytoplasmic Ca^{2+} concentration and the initiation of a physiological response.

The maxi cation channel is inhibited by ruthenium red, diltiazem, verapamil and quinine at micromolar concentrations and by tetraethylammonium (TEA^+) at millimolar concentrations (White, 1996). The pharmaceuticals with highest affinity have complex effects on channel activity. Both ruthenium red and diltiazem appear to stabilize a variety of subconductance states, while verapamil and quinine exhibit multiple, rapid rate constants for binding and unbinding to produce ''fast'' or ''flickery'' blockade. Initially, this precluded a detailed analysis of the interactions between these pharmaceuticals and the channel.

A computational technique has now been developed which allows rapid rate constants for multistate kinetic models to be estimated from current-amplitude frequency distributions of single channel electrical recordings (White & Ridout, 1998). This technique has already enabled the complex interactions between verapamil and the maxi cation channel to be dissected (White & Ridout, 1998) and here it is used to analyze the interactions be-

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tween quinine and the maxi cation channel. It is concluded that quinine produces flickery kinetics by interacting with the channel from the cytoplasmic side in a voltage-dependent manner with an equilibrium dissociation constant (K_d) of about 1 mM at zero millivolts. This resembles the effect of quinine on susceptible cation (K^+, Na^+ and Ca^{2+}) and Cl^- channels in the membranes of both plant and animal cells (Cooke & Quast, 1990; Hille, 1992; Weiser & Bentrup, 1993; Lesage et al., 1996; White, 1997). Quinine also interacts with the maxi cation channel from the cytoplasmic side in a voltage-dependent manner at a second site, to produce slow kinetics. The K_d value for the latter binding site is about 10 mM at zero millivolts.

Materials and Methods

Plant Material and Plasma Membrane Isolation

Rye (*Secale cereale* L. cv. Rheidol) was grown hydroponically in a complete nutrient medium containing 400-μM K^+, as described by White (1996). Plasma-membrane vesicles were obtained by aqueous-polymer two-phase partitioning of a microsomal fraction derived from roots harvested 14 days after sowing (White, 1996). Plasma-membrane vesicles were resuspended at a concentration of 1-mg protein ml^{-1} in 5-mM N-tris-[hydroxymethyl]-methyl-2-aminoethane sulfonic acid (Tes), titrated to pH 7.5 using N-methyl-D-glucamine (NMDG) and stored at −20°C.

Ion Channel Recordings

Electrical recordings of ion channel activity were obtained following the incorporation of plasma-membrane vesicles into bilayers composed of 30-mM synthetic 1-palmitoyl-2-oleoyl phosphatidylethanolamine dispersed in *n*-decane, as described by White (1996). Vesicles were added to the *cis* side of the bilayer and plasma-membrane ion channels were assumed to become orientated with their cytoplasmic face exposed to the *trans* chamber (White & Tester, 1994). Current was monitored under voltage-clamp conditions using a low noise operational amplifier with frequency compensation, connected to the bilayer chambers by calomel electrodes and 3-M KCl salt bridges. To conform with the physiological convention (Bertl et al., 1992), membrane potentials were recorded *trans* with respect to *cis*, which was held at ground. Thus, the sign of the membrane potential is opposite to that used in the earlier studies of White (1993, 1996), but conforms to that used by White (1997) and White & Ridout (1998).

Experiments were performed with 100-mM KCl, buffered with 5-mM Tes titrated to pH 7.5 using NMDG, on both sides of the bilayer. Quinine is membrane permeant and, to ensure a constant quinine concentration throughout the experiment, equimolar quinine was added to the solutions in both chambers either from an aqueous stock solution (containing 1-mM quinine, 100-mM KCl and 5-mM Tes titrated to pH 7.5 with NMDG) or, to obtain concentrations above 100-μM, from a 100-mM stock in ethanol. The presence of ethanol affected neither the gating of the maxi cation channel nor the stability of the bilayer. The KCl concentration was maintained at 100 mM by addition of KCl from a 3-M stock solution made up in 5-mM TES titrated to pH 7.5 with NMDG. Experiments were performed at room temperature (20°C).

Analysis of Single-Channel Electrical Recordings

Experimental data were stored on digital audiotape (DTC1000ES; 44.1 kHz per channel; Sony, Japan). The apparent unitary current was estimated from current recordings replayed and filtered at 100 Hz using an 8-pole low pass Bessel filter (902LPF, Frequency Devices, Haverhill, MA). For other analyses, recordings were replayed and filtered, with an effective filtering of 2 kHz (White & Ridout, 1998), then sampled by computer after digitization using a CED 1401plus (Cambridge Electronic Design, Cambridge, UK) at 11 kHz. The CED Patch and Voltage Clamp Software (Version 6.0) was used to estimate the mean amplitude and standard deviation of the current when the channel was in the closed or blocked state and to generate current frequency histograms. Although the maxi cation channel exhibited at least two closed states (White, 1993), shorter closures were rare and unlikely to influence current-frequency distributions greatly, and lengthy closures were identified in current recordings and excluded form current-amplitude histograms (*cf.* Fig. 1). Periods when the channel was in a subconductance state were also excluded from current-amplitude histograms.

Estimates of rapid rate constants for the transitions between the open and shortlived (*B1*) blocked states were obtained from current-amplitude frequency distributions, generated with the exclusion of long closed and blocked (*B2*) states, using the computer program FIT© (HRI, Wellesbourne, UK) as described by White & Ridout (1998). The proportional area beneath the current frequency distribution attributed to the lengthy closed and *B2* states was used to estimate the apparent probability of finding the channel in bursting activity (P_b, defined here as including the open, shorter closed and blocked states). The mean dwell time for the lengthy blocked state (τ_{B2}) was estimated from current recordings at voltages at which, in the absence of quinine, no closures were observed. Regression lines were fitted by least squares using GENSTAT (NAG, Oxford, UK).

Results

The effects of quinine on the activity of the maxi cation channel were categorized using a nomenclature based on the speed with which it dissociates from the channel protein (Hille, 1992). The term blockade is used here to describe any reduction in channel unitary conductance or open probability induced by quinine. In current recordings, slow blockade is manifest by extended periods at the current amplitude of the blocked state, while fast blockade appears as a flat decrease in the apparent unitary current without transitions to the current amplitude of either the open or blocked states.

Quinine was reported to inhibit the maxi cation channel with complex, fast kinetics when present at equimolar concentrations in the solutions in the *cis* and *trans* chambers (White, 1996). Inhibition was more pronounced as the voltage was driven to more positive voltages, which is consistent with quinine interacting with the maxi cation channel in its cationic form from the cytoplasmic side. The analyses reported here suggest that these kinetics can be generated by transitions between the open channel and a single, shortlived blocked state. This blocked state will be referred to as *B1*. On closer inspection of the current recordings, a slower ki-

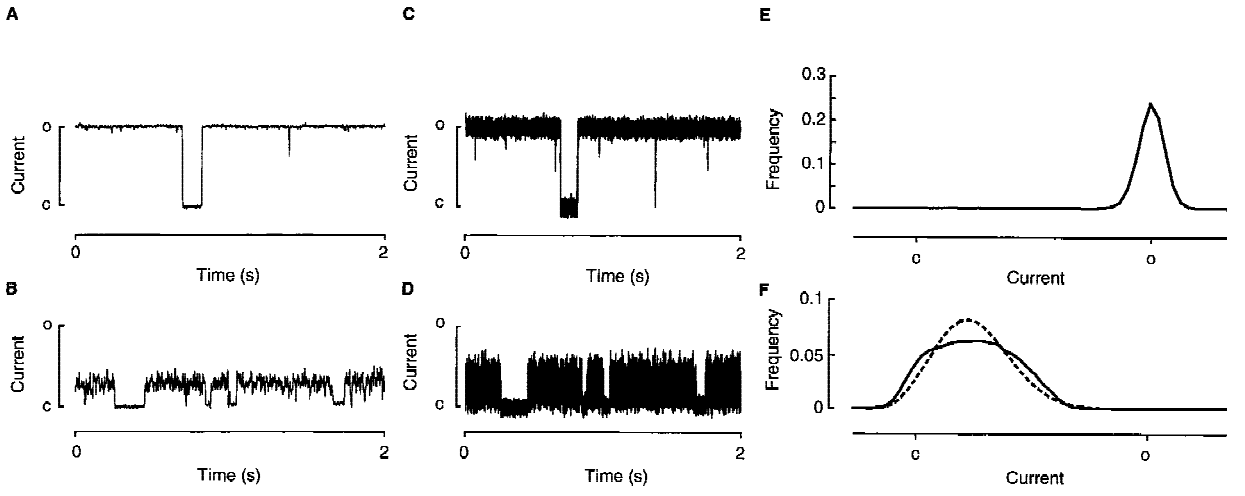

Fig. 1. (*A–D*) Electrical recordings of the maxi cation channel from the plasma membrane of rye roots obtained following its incorporation into a planar PE bilayer. Current was recorded at 40 mV in the presence of 100-mM KCl and in the absence (*A* and *C*) or presence (*B* and *D*) of 300 μM quinine. The effective filtering of the recordings was 100 Hz (*A* and *B*) or 2 kHz (*C* and *D*). (*E* and *F*) The observed current frequency distribution obtained either including (E) or excluding (F) the contribution from the lengthy closed or blocked states (—), is compared with that estimated for a two-state model for blockade using the program FIT© (---) for the data presented in panels *C* and *D*, respectively. The current amplitude of the open channel (*O*) and closed or blocked channel (*C*) is indicated. The unitary current through the channel was 19.9 pA.

netic component was also apparent in the interactions between quinine and the maxi cation channel (Fig. 1). This blocked state will be referred to as *B2*. Since *B2* was rarely observed, and its mean duration (τ_{B2}) was sufficiently lengthy to be noticed in current recordings and excluded from current-amplitude frequency histograms, it was possible to investigate the fast transitions between the open and *B1* states independently.

Analysis of Fast Kinetics

Initial estimates of the equilibrium binding constant (K_d) and voltage-dependence of quinine blockade were obtained from the apparent reduction in unitary current when the recording was filtered at 100 Hz in the presence of quinine. This was expressed as the ratio of the apparent unitary current in the presence (I_q) and absence (I_o) of quinine (Fig. 2*A* and *B*). Quinine was assumed to effect blockade by entering the channel pore across an energy barrier and binding at a site at an electrical distance δ across the electric field of the membrane (Woodhull, 1973; Moczydlowski, 1992). This leads to the equations:

$$I_q/I_o = K_d(V)/\{K_d(V) + [Q]\}$$
$$K_d(V) = K_d(0)\exp(-z\delta FV/RT)$$

where $K_d(0)$ is the value of K_d at zero millivolts, $K_d(V)$ is the value of K_d at voltage V, $[Q]$ is the concentration of quinine, z is the charge valence of quinine (assumed to be 1: for quinine $pK_1 = 5.1$ and $pK_2 = 9.7$), F is Faraday's constant, R is the gas constant and T is absolute temperature. These equations can be combined to give:

$$I_q/I_o = 1/\{1 + [Q]\exp(RT/z\delta FV)/K_d(0)\}$$

Values for $K_d(0)$ of 704 ± 54 μM and $z\delta$ of 0.71 ± 0.034 ($n = 131$) were estimated. However, since the apparent current was not a constant amplitude, but flickered toward zero current (e.g., Fig. 1*B*), the duration of channel blockade is likely to be underestimated (especially at higher quinine concentrations and more positive voltages) in this analysis, causing I_q/I_o and $K_d(0)$ to be overestimated.

The rate constants for transitions between the open and *B1* states were estimated from current-amplitude frequency distributions using the computer program FIT© (White & Ridout, 1998; Fig. 1*E* and *F*). The standard deviation of current noise, determined when the channel was in the closed state, was 1.03 ± 0.020 (mean ± SE; $n = 83$ determinations in the presence of 0, 30, 100, 300 and 1,000 μM quinine) and the dependence of the unitary current of the unblocked channel on voltage (Fig. 2*A*) was similar to previous studies (White, 1993, 1996). The data were analyzed assuming a single blocked state, since goodness of fit criteria did not merit the inclusion of a second blocked state:

$$O \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B1$$

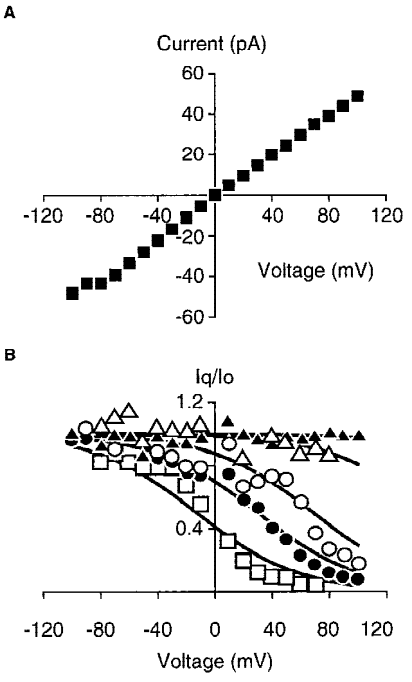

Fig. 2. (*A*) The relationship between unitary current and voltage for the maxi cation channel from the plasma membrane of rye roots assayed in the presence of symmetrical 100-mM KCl. (*B*) The voltage-dependence of the apparent unitary current obtained in the presence of quinine (expressed relative to that observed in the absence of quinine I_q/I_o). Data were estimated from current recording filtered at 100 Hz. For clarity, data are shown only for quinine concentrations of 1 (▲), 10 (△), 100 (○), 300 (●) and 1,000 μM (□). Regression lines were fitted to the data using the equation $I_q/I_o = 1/\{1 + [Q] \exp(RT/z\delta FV)/K_d(0)\}$. Parameter estimates were $K_d(0) = 704 \pm 54$ μM and $z\delta = 0.71 \pm 0.034$ ($n = 131$).

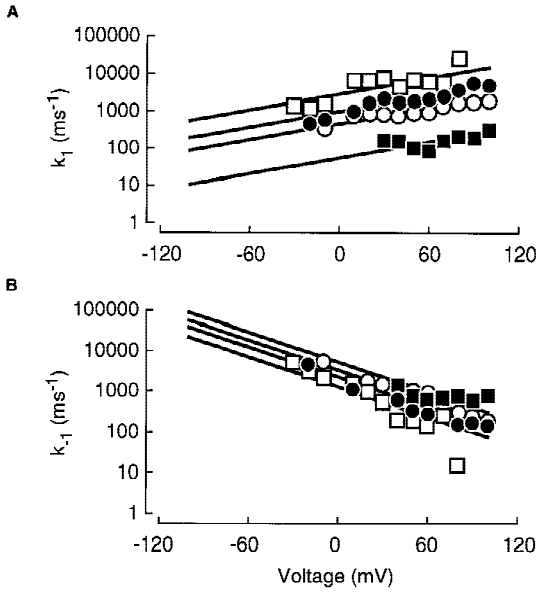

Fig. 3. The voltage-dependence of the rate constants for the transitions between open and B1 states of the maxi cation channel in the presence of 30 (■), 100 (○), 300 (●) and 1,000 (□) μM quinine. The rate constant for blockade of the open channel is indicated by k_1 and the rate at which the channel is unblocked by k_{-1}. The fitted lines are parallel regression lines. The natural logarithms of the intercepts for k_1 were 4.008 (30 μM), 6.119 (100 μM), 6.887 (300 μM) and 7.949 (1000 μM), and the slope ($z\delta_p F/RT$) was 0.0164. The natural logarithms of the intercepts for k_{-1} were 8.563 (30 μM), 8.112 (100 μM), 7.721 (300 μM) and 7.156 (1000 μM), and the slope was −0.0282.

Although neither k_1 (the rate at which the open channel is blocked) nor k_{-1} (the rate at which quinine leaves the channel) could be estimated individually with much precision (White & Ridout, 1998), some confidence in the estimates can be taken from the clear relationships between each rate constant and both voltage and quinine concentration. Moreover, the ratio of the rates will be estimated with reasonable precision (White & Ridout, 1998).

The relationships between k_1 and k_{-1} and voltage (Fig. 3; Table) were estimated at concentrations of 30, 100, 300 and 1000 μM quinine using the Boltzmann equation:

$$k(V) = k(0) \exp(z\delta_p FV/RT)$$

where $k(0)$ is the rate at zero millivolts, and is dependent on quinine concentration, $k(V)$ is the rate at voltage V and $z\delta_p$ is the apparent electrical distance between the outside (for k_1) or the quinine binding site (for k_{-1}) and the energy peak. The value of $k_1(0)$ increased with increasing quinine concentration and $z\delta_p$ was 0.41 ± 0.038, consistent with entry of the cation from the cytoplasmic side. The value of $k_{-1}(0)$ appeared to decrease with increasing quinine concentration, perhaps indicating an allosteric inhibition of quinine unbinding by quinine in the solution. The value of $z\delta_p$ was −0.71 ± 0.046, suggesting unbinding of the cation to the cytoplasmic side. The unequal values of $z\delta_p$ for k_1 and k_{-1} suggest that the energy barrier for the interaction of quinine and the maxi cation channel is asymmetrically related to the electrical profile (Tikhonov & Magazanik, 1998). Summation of the two $z\delta_p$ values suggests an electrical distance of 1.12 ± 0.060 between the cytoplasmic solution and the quinine binding site. This is likely to be more accurate than the value of 0.71 calculated from the apparent reduction in unitary current (Fig. 1). The $K_d(0)$ for the *B1* binding site was about 1 mM, as estimated from the ratio of the unimolecular unblocking rate and the bimolecular blocking rate ($k_{-1}[Q]/k_1$).

Analysis of Slow Kinetics

The rate at which transitions occur between the lengthy blocked state (*B2*) and the bursting state of the channel (defined here as including the open, shorter closed and

Table. The relationship between the rate constants for the *B1* ⇄ *O* ⇄ *B2* kinetic model and voltage at quinine concentrations of 30, 100, 300 and 1,000 μM.

Rate constant	Parameter	Quinine concentration (μM)			
		30	100	300	1,000
k_1	$k(0)$	55	454	979	2833
	$z\delta_p$	0.41 ± 0.038			
k_{-1}	$k(0)$	5234	3334	2255	1282
	$z\delta_p$	−0.71 ± 0.046			
k_2	$k(0)$	0.97	0.63	2.98	19.84
	$z\delta_p$	0.12 ± 0.091			
k_{-2}	$k(0)$	0.330	0.072	0.108	0.137
	$z\delta_p$	−1.27 ± 0.190			

The rate constants for blockade of the open channel by quinine are indicated by k_1 (msec^{-1} = 10^3 sec^{-1}) and k_2 (sec^{-1}) and the rates at which the channel is unblocked by k_{-1} and k_{-2} (both msec^{-1}), the subscripts referring to the two independent blocked states *B1* and *B2*, respectively. Estimates for $k(0)$, the rate at zero volts, and $z\delta_p$, the apparent electrical distance between the energy peak and the outside (for k_1 and k_2) or the quinine binding site (for k_{-1} and k_{-2}), were obtained by fitting parallel regression lines to of the data presented in Fig. 3 (for k_1 and k_{-1}) and Fig. 4 (for k_2 and k_{-2}).

blocked states) can be estimated from the reciprocal of the mean dwell time for the lengthy blocked state ($\tau_{B2} = 1/k_{-2}$). Unfortunately, the *B2* and closed states cannot be distinguished. However, an estimate of τ_{B2} was obtained from current recordings at voltages between −50 and 30 mV assuming that the long closed state was absent (Fig. 4). This is justified since no closures were observed at these voltages in the absence of quinine. The relationship between k_{-2} and voltage was estimated at concentrations of 30, 100, 300 and 1,000 μM quinine using a Boltzmann equation (Table). It was not possible to estimate k_{-2} precisely, since excursions to the *B2* state were lengthy and rare, which limited the sample size. It was also difficult to distinguish the *B2* state at extreme positive voltages in the presence of millimolar quinine. However, the value of $k_{-2}(0)$ appeared to be independent of quinine concentration and $z\delta_p$ was −1.27 ± 0.190, which suggests that the cation left the binding site to the cytoplasmic side.

To estimate k_2, it was assumed that quinine interacted with the maxi cation channel at two distinct sites, that these sites could not be accessed except though the open channel and that the sites were not occupied simultaneously. The kinetic scheme is:

$$B1 \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} O \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} B2$$

This scheme was chosen since the alternative scheme *O* ⇄ *B1* ⇄ *B2* yielded inappropriate estimates of k_2, which

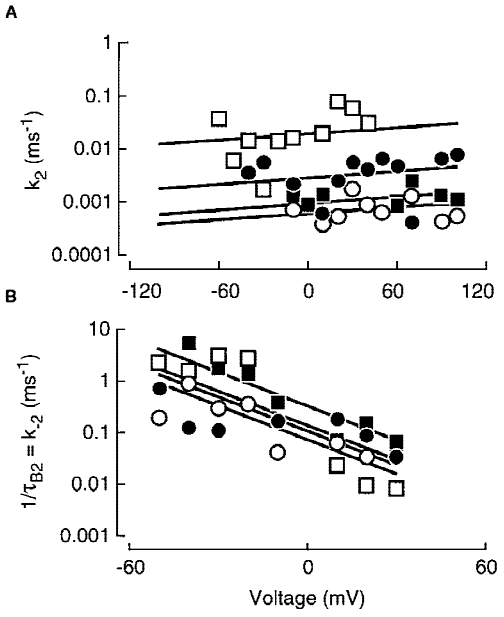

Fig. 4. The voltage-dependence of (*A*) the rate constants for blockade of the open channel (k_2) and (*B*) the reciprocal of the mean dwell time for the lengthy blocked state ($1/\tau_{B2} = k_{-2}$) in the presence of 30 (■), 100 (○), 300 (●) and 1,000 (□) μM quinine. The fitted lines are parallel regression lines. The natural logarithms of the intercepts for k_2 were −6.937 (30 μM), −7.364 (100 μM), 5.817 (300 μM) and 3.920 (1000 μM), and the slope was 0.0046. The natural logarithms of the intercepts for k_{-2} were −1.110 (30 μM), 2.627 (100 μM), 2.226 (300 μM) and 1.983 (1,000 μM), and the slope ($z\delta_p F/RT$) was −0.0504.

exhibited both a negative voltage-dependence and a dependence on quinine concentration.

The relationships between k_2 and voltage were estimated at concentrations of 30, 100, 300 and 1,000 μM quinine from the probability of the channel being in the *B2* state ($P_{B2} = 1\text{-}P_b$) and the fitted rates k_1, k_{-1} and k_{-2} using the relationship (Colquhoun & Hawkes, 1992):

$$P_{B2} = \frac{(k_{-1}/k_1)\,(k_2/k_{-2})}{1 + (k_{-1}/k_1)\,(1 + k_2/k_{-2})}$$

The value P_b was calculated as the relative time the channel exhibited bursting activity in the presence (P_{bq}) and absence (P_{bo}) of quinine (P_{bq}/P_{bo}; Fig. 5). The value of $k_2(0)$ increased with increasing quinine concentration and $z\delta_p$ was 0.12 ± 0.091 (Table), which suggests that the cation approached the binding site from the cytoplasmic side. Summation of the $z\delta_p$ values for k_{-2} and k_2 suggests an electrical distance of 1.39 ± 0.211 between the cytoplasmic solution and the quinine binding site. From the ratio of the unimolecular unbinding rate and the bimolecular binding rate ($k_{-2}[Q]/k_2$) the $K_d(0)$ for the *B2* binding site was estimated to be approximately 10 mM. The apparent paradox that at extreme positive voltages the predicted P_b declines as the quinine concentration is

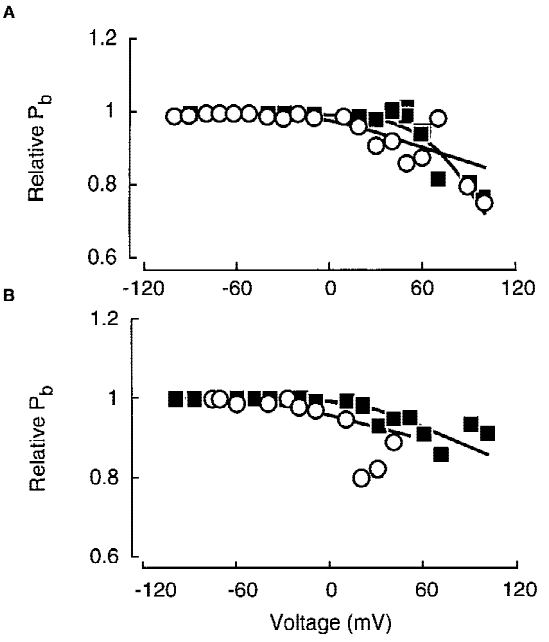

Fig. 5. The relationship between the length of time the channel exhibited bursting kinetics (P_b) in the presence of quinine (expressed relative to that observed in the absence of quinine P_{bq}/P_{bo}) and voltage. Experiments were performed in the presence of (*A*) 30 (■) and 300 μM-(○) quinine and (*B*) 100 (■) and 1,000 (○) μM-quinine. Regression lines were fitted to the equation $P_b = 1 - ((k_{-1}/k_1)(k_2/k_{-2}))/(1 + ((k_{-1}/k_1)(1 + k_2/k_{-2})))$ using k_1, k_{-1}, k_2 and k_{-2} values calculated from their dependencies on voltage and quinine concentration as presented in the Table.

raised above 30 μM (Fig. 5*A*) is a consequence of the channel occupying the *B1* state for the majority of the time.

Discussion

The results presented here suggest that quinine blocked the maxi cation channel from the cytoplasmic side at two distinct binding sites (*B1* and *B2*). The $K_d(0)$ values for these sites were approximately 1 and 10 mM, respectively. These values compare with $K_d(0)$ values of between 0.1 and 1 mM for susceptible K^+, Na^+, Ca^{2+} and Cl^- channels in animal cell membranes (Cooke & Quast, 1990; Hille, 1992) and for other K^+ channels in plant cell membranes (Weiser & Bentrup, 1993; Lesage et al., 1996; White, 1997). The $K_d(0)$ values obtained for the maxi cation channel do not offer much scope to use quinine either as an affinity matrix to purify the protein (*cf.* Paliwal, Costa & Diwan, 1992), or as a ligand to label the channel during its purification. Furthermore, due to its lack of specificity, and its membrane permeability, it is unlikely that quinine will prove to be a useful pharmaceutical to identify channel activities underlying electrical responses of plant membranes in vivo.

Quinine dissociated rapidly from the binding site with highest affinity (*B1*), resulting in fast or flickery kinetics, and more slowly from *B2*, resulting in slow kinetics. Fast or flickery kinetics are exhibited by all channels which are susceptible to quinine inhibition. The kinetics of quinine blockade have been observed at single-channel resolution for several K^+ channels in animal cell membranes. These include various Ca^{2+}-activated K^+ channels (Guggino et al., 1987; Mancilla & Rojas, 1990; Segal & Reuss, 1990; Fatherazi & Cook, 1991; Pavenstädt et al., 1991; Banderali & Roy, 1992; Hayashi, Young & Cook, 1996; Takeuchi & Irimajiri, 1996), Ca^{2+}-activated cation channels (Takeuchi et al., 1995), ATP-dependent K^+ channels and delayed-rectifier K^+ channels (Bokvist, Rorsman & Smith, 1990; Fatherazi & Cook, 1991). Unfortunately, although the potency of quinine blockade of these channels has been determined, few estimates of its voltage-dependency and rates of blockade or clearance are available for comparison (but *see* Bokvist et al., 1990; Mancilla & Rojas, 1990; Segal & Reuss, 1990). Open-channel blockade of the Ca^{2+}- and depolarization-activated maxi K^+ channel by cytoplasmic quinine exhibited a $K_d(0)$ of 100 to 200 μM and only slight voltage-dependence (Mancilla & Reuss, 1990; Segal & Reuss, 1990). It is interesting to note that the maxi K^+ channel, like the maxi cation channel in the plasma membrane of plant roots, is not only inhibited by quinine but also by TEA^+, verapamil and diltiazem (Pavenstädt et al., 1991; Ishikawa & Cooke, 1993; Takeuchi & Irimajiri, 1996).

The selectivity filter of the maxi cation channel, which is expected to correspond to a decrease in the diameter of the pore and therefore prevent quinine movement, is likely to be situated towards the extracellular end of the pore. The apparent electrical distance from the cytoplasmic solution to both quinine binding sites in the maxi cation channel was greater than unity, when calculated as the summed voltage-dependence of the binding and unbinding rates. This might be the result of the inability to estimate these parameters precisely, but it could also indicate that the maxi cation channel has a complex pore structure, which can accommodate molecular interactions. For example, the voltage-dependent blockade of the maxi K^+ channel in the vacuolar membrane of *Chara* by both Cs^+ (Draber & Hansen, 1994) and divalent cations (Laver, 1992) has been interpreted in terms of a multi-ion pore. It would be interesting to investigate the K^+ concentration-dependence of quinine blockade of the maxi cation channel to assess the magnitude of interactions between K^+ and quinine within the pore and to determine whether K^+ and quinine compete for specific intrapore binding sites (Tikhonov & Magazanik, 1998). The possibility that molecular interactions do occur within the pore of the maxi cation channel is supported by the observations that (i) conductance and permeability ratios differed, and were critically depen-

dent upon the ionic composition of the experimental solutions (White, 1997) and (ii) that the blockade of the maxi cation channel by verapamil had a voltage-dependence considerably in excess of unity (White & Ridout, 1998). Acting upon this evidence, it is now important to investigate, and model, the permeation properties of the maxi cation channel and to understand the relationships between its biophysical characteristics, molecular structure and physiological function.

This work was supported by the Biotechnology and Biological Sciences Research council (UK). I thank my colleagues at HRI, in particular Mr. M.S. Ridout and Prof. M.A. Venis, for helpful discussions.

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