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Pharmacology of the SV Channel in the Vacuolar Membrane of *Chenopodium rubrum* Suspension Cells

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Abstract. Single channel performance and deactivation currents have been analyzed in the presence of cation channel blockers to reveal pharmacological properties of the slow-activating (SV) cation-selective ion channel in the vacuolar membrane (tonoplast) isolated from suspension cells of *Chenopodium rubrum* L. At a holding potential of −100 mV, the SV channel showed half-maximal inhibition with 20 mM tetraethylammonium (TEA), 7 μM 9-amino-acridine, 6 μM (+)-tubocurarine, 300 nM quinacrine, and 35 μM quinine, respectively. The SV channel is also blocked by charybdotoxin (20 nM at −80 mV) but not by apamine. 9-Amino-acridine, (+)-tubocurarine and quinacrine act in a voltage-dependent fashion, binding to the open channel and to different sites along the transmembrane voltage profile according to Woodhull (*J. Gen. Physiol.* **61**:687-708, 1973). No binding site could be specified for charybdotoxin, which binds to the closed channel, and for quinine. Except for quinine, all tested blockers were effective only if added to the cytoplasmic side of the tonoplast. A structural relationship between the SV channel and Maxi-K channels in animal systems is inferred.

Key words: *Chenopodium* — SV channel — Vacuolar membrane (tonoplast) — Charybdotoxin — 9-Amino-acridine — Quinine

Introduction

The vacuolar membrane of all plant tissues tested so far contains a calcium-dependent, voltage-activated ion channel permeable to monovalent cations and, to a lesser extent, also to anions. This slowly activating (SV) channel is strongly inward rectifying (Hedrich & Neher, 1987; Hedrich et al., 1988). Calcium seems to control this channel via calmodulin (Weiser, Blum & Bentrup, 1991). Although this channel was the first ion channel studied in the tonoplast (Hedrich, Flügge & Fernandez, 1986), little is known about its pharmacology (Hedrich & Kurkdjian, 1988). Due to its remarkably broad permeability, the SV channel cannot easily be assigned to established animal ion channel categories. Therefore, we deemed it instructive to investigate its pharmacology and search for inhibitors of sufficient affinity which could serve as a tool for further analysis. Additionally, we try to elucidate the inhibitory mechanism of a given drug in terms of binding and channel characteristics.

Previously we have communicated some data concerning the action of (+)-tubocurarine and charybdotoxin on the SV channel in suspension cells of *Chenopodium rubrum* (Weiser & Bentrup, 1990, 1991).

Materials and Methods

Media

The standard test solution contained (mM) 100 KCl, 2 $MgCl_2$, 0.1 $CaCl_2$, 300 mannitol and 5 Tris/Mes pH 7.2. It was used in the bath and in the patch pipette. Test solutions were filtered (0.2 μm, Schleicher and Schuell) just before use. All chemicals were of analytical grade; charybdotoxin was obtained from Latoxan. Stock solutions of 9-amino-acridine and quinacrine were prepared using methanol; control media then contained equivalent concentrations of this solvent. The stock solution of charybdotoxin was prepared with 200 mM KCl. For the experiment on deactivation

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currents, inhibitors were applied at concentrations about ten times as high as the EC_{50} for −100 mV, except for TEA, where because of the low affinity of the drug, 50 mM was applied.

Vacuole Preparation

Vacuoles were prepared from protoplasts isolated from a suspension culture of *C. rubrum* as described elsewhere (Bentrup et al., 1986). About 100 μl of the protoplast suspension (approx. 10^6/ml) was transferred to the perfusion chamber. Within minutes the protoplast sedimented and stuck to the glass surface. Perfusion broke the protoplasts and liberated the vacuoles which then were immediately accessible to the electrophysiological experiments.

Patch-Clamp Measurements

Patch pipettes were pulled from borosilicate glass (Hilgenberg) on a two-stage puller (L/M 3PA, List Electronics) and heat-polished. Experiments were performed as described elsewhere (Weiser & Bentrup, 1990). Data were stored on videotape after digitizing (VR 10, Instrutech) and analyzed on an AT-compatible computer using the pCLAMP 5.5 software (Axon). All quoted potentials refer to the pipette. In experiments performed in the whole-vacuolar and outside-out mode, downward deflections represent opening of channels. In inside-out experiments, downward deflections represent channel closing. In experiments concerning the voltage dependence of inhibitor action, applied voltages were mathematically corrected for the influence of the given access resistance. Given values represent the mean of at least five experiments.

Results

The application of negative voltage pulses to vacuoles of *C. rubrum* evoked large, slowly saturating inward directed currents. This macroscopic current is largely mediated by a single type of ion channel (Hedrich et al., 1988). This SV channel-mediated conductance is reduced in the presence of various substances. Figure 1 shows the effects of different concentrations of quinine, and (+)-tubocurarine, respectively. Obviously, both drugs reduce the trans-tonoplast current in a dose-dependent manner. Single channel recordings on outside-out patches demonstrate that both substances act on an intermediate time scale and induce a "flickering" block (Hille, 1984); that is, the open time of the channel events is reduced. This mechanism of channel inhibition also occurs in the presence of the pH-sensitive fluorescence dyes 9-amino-acridine and quinacrine, respectively (Fig. 2). The latter dye shows a reasonable affinity to the channel protein, resulting in a half maximum inhibition at about 300 nM, whereas 9-amino-acridine is effective only at micromolar concentrations.

Two other drugs, TEA and the peptide charybdotoxin known as inhibitors of Maxi-K-channels (Benham et al., 1985; Lucchesi et al., 1989; Dreyer, 1990) are able to block the SV channel with quite different efficacy (Fig. 3). About 20 mM of TEA is necessary to block the vacuolar current by 50%, whereas 20 nM of charybdotoxin is sufficient for the same degree of inhibition. Single channel recordings demonstrated that the mode of action of the two substances likewise is different: TEA induces a "fast" block with an apparent reduction of the channel's unitary conductance, whereas charybdotoxin acts on the "slow" time scale.

Figure 4 shows concentration-response curves of the inhibitors of SV channel activity presented in this study. Obviously, the range of efficacy is fairly large between the different substances, whereas the steepness of the dose responses is comparable for all drugs tested.

All channel blockers were only effective when applied to the cytoplasmic side of the tonoplast—except for quinine: Figure 5 shows the protocol of an experiment with 20 μM quinine being applied to an inside-out patch of a *Chenopodium* vacuole. In this patch, two SV channels were active (Fig. 5*A*). Quinine from the intravacuolar side not only evoked a "flickering" block, but additionally induced a subconductance level (Fig. 5*B*; arrowheads). The amplitude histogram of the experiment described above demonstrates this more evidently. In the presence of quinine, a single "substate" of about 20% of the normal conductance is induced (Fig. 5*B*; arrowhead). Quinine added from the cytoplasmic side did not show this effect.

Voltage Dependence of Inhibition

Since all inhibitors tested in this study are charged molecules, we performed experiments to elucidate the voltage dependence of blockage. From such experiments, the relative position of the binding site within the transmembrane voltage profile (δ) and the inhibitor affinity in the absence of a voltage, Kd_{0mV}, respectively, can be derived as described by Eq. (1) (*cf.* Woodhull, 1973):

$$\ln (g_o/g - 1) = \ln ([B]/Kd_{0mV}) + z\delta FV/RT, \qquad (1)$$

where g_o = conductance of the control, g = conductance in the presence of the inhibitor, [B] = inhibitor concentration, and V = voltage. R, T, F and z have their usual meanings. The fractional distance δ ranges between 0 (cytoplasmic side) and 1 (vacuolar side of the tonoplast).

Pertinent data for 9-amino-acridine and quinacrine are presented in Fig. 6. Kd_{0mV} values can be

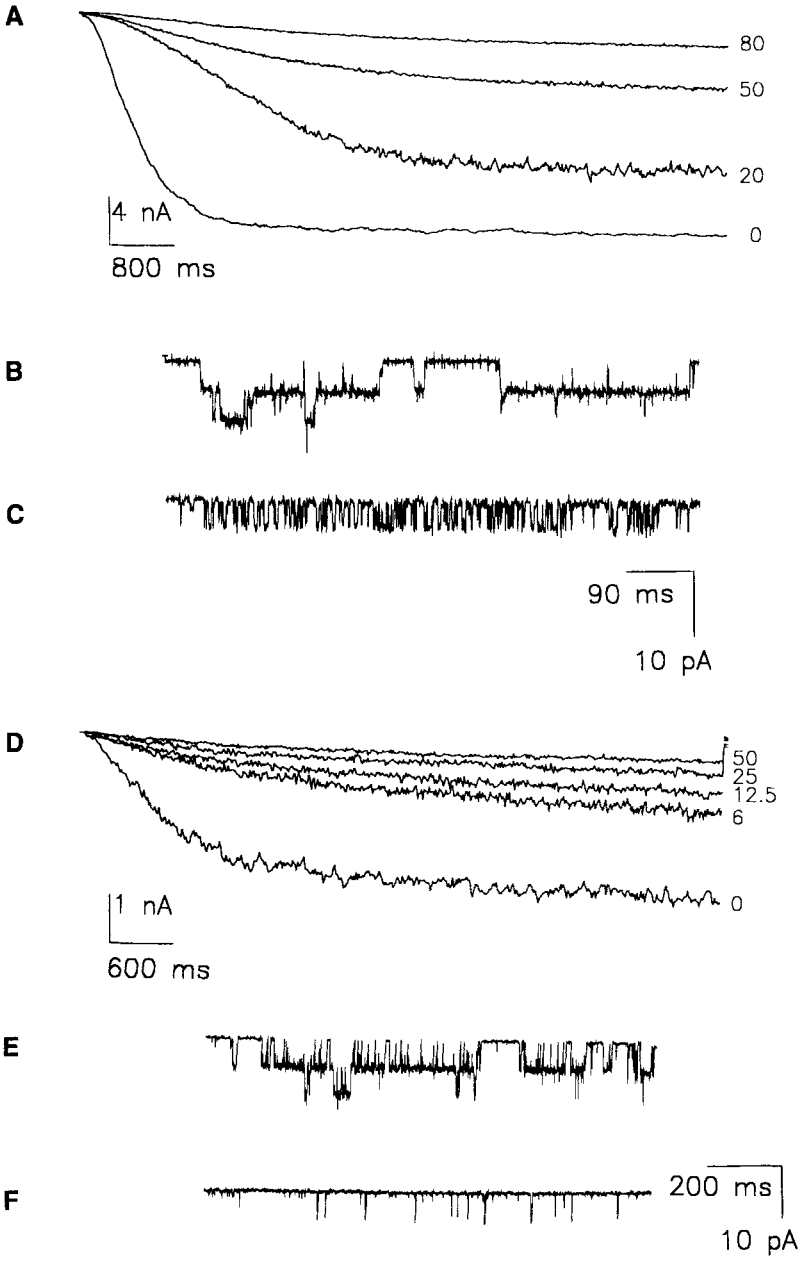

Fig. 1. Response of SV channels to quinine (*A–C*) and (+)-tubocurarine (*D–F*), respectively. Quinine reduces the current across the tonoplast (*A*, whole-vacuole-mode). Quinine (20 μM) on the cytoplasmic side induces "flickering" of single channels (*C*), compared to the control (*B*). (+)-Tubocurarine (50 μM) blocks the transtonoplast current (*D*), and induces "flickering" (*F*), compared to the control (*E*). Applied voltage was −100 mV.

derived from the intercepts, $z\delta$ values from the slopes of the graphs. $z\delta$ values of about 0.175 and 1.244 can be derived for 9-amino-acridine and quinacrine, respectively. Since 9-amino-acridine is a monovalent, quinacrine a bivalent cation, corresponding δ values are 0.175 and 0.675, respectively. Apparently, the binding site for quinacrine lies deeper within the transtonoplast voltage profile. The corresponding Kd_{0mV} values are 25 μM and 15 μM.

The Kd_{0mV} and δ data are summarized in the Table, which also includes data for (+)-tubocurarine and TEA abstracted from Fig. 7. The channel blocking effect of (+)-tubocurarine likewise is voltage dependent, with $z\delta$ of 0.62 and a Kd_{0mV} of 60 μM. Since this molecule bears two positive charges, the binding site is located at about 0.31 within the transtonoplast voltage profile. Although the effect of TEA on potassium channels is reported to be voltage dependent (i.e., Armstrong, 1966; Benham et al., 1985), Figure 7*B* clearly shows that TEA inhibition of the SV channel in the tonoplast of *Chenopodium* is not so.

Channel Deactivation

Another finding, which seems puzzling at first glance, is the observed differential influence of the tested substances on channel deactivation. Current deactivation can be described by the expression

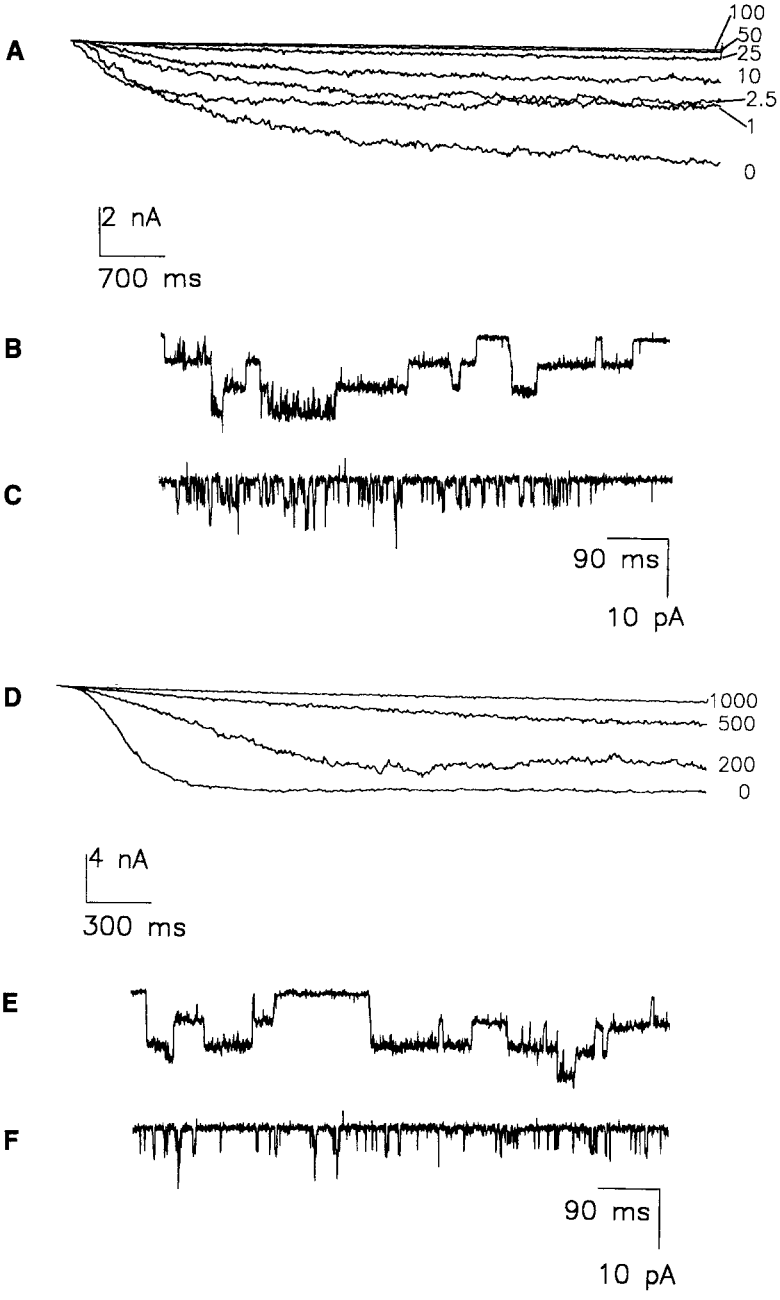

Fig. 2. Inhibition of the vacuolar current by the fluorescent dyes 9-amino-acridine (*A–C*), and quinacrine (*D–F*). *A* and *D* show whole-vacuolar currents in the presence of the different concentrations, numbers in μM (*A*) and nM (*D*), added to the cytoplasmic side. 9-Amino-acridine (10 μM) (*C*) and 100 nM quinacrine (*F*) block single channels on an intermediate time scale (controls in *B* and *E*, respectively). Applied voltage was −100 mV.

$$A_t = A_o + A_1 \cdot \exp(-t/\tau_1) + \ldots + A_n \cdot \exp(-t/\tau_n) \quad (2)$$

where A_o = the initial offset current amplitude, τ_n = the deactivation time constant, and t = time.

Schroeder (1989) found the decay of an outward potassium current in *Vicia faba* protoplasts to follow approximately a single exponential. Our SV channel-mediated currents partly corroborate Schroeder's observation. For instance, if 35 μM quinine is added to the cytoplasmic side, the time course of the deactivation currents can be approximated by a single exponential function; quinine obviously reduces the time constant tau of channel deactivation (Fig. 8*A*). By contrast, with 50 μM (+)-tubocurarine added to the cytoplasmic side, the time course of the "tail" currents is drastically altered (Fig. 8*B*). Compared to the control, the initial amplitude is increased, and the form of the currents cannot be approximated by a single exponential function.

A similar tail-current analysis has been carried

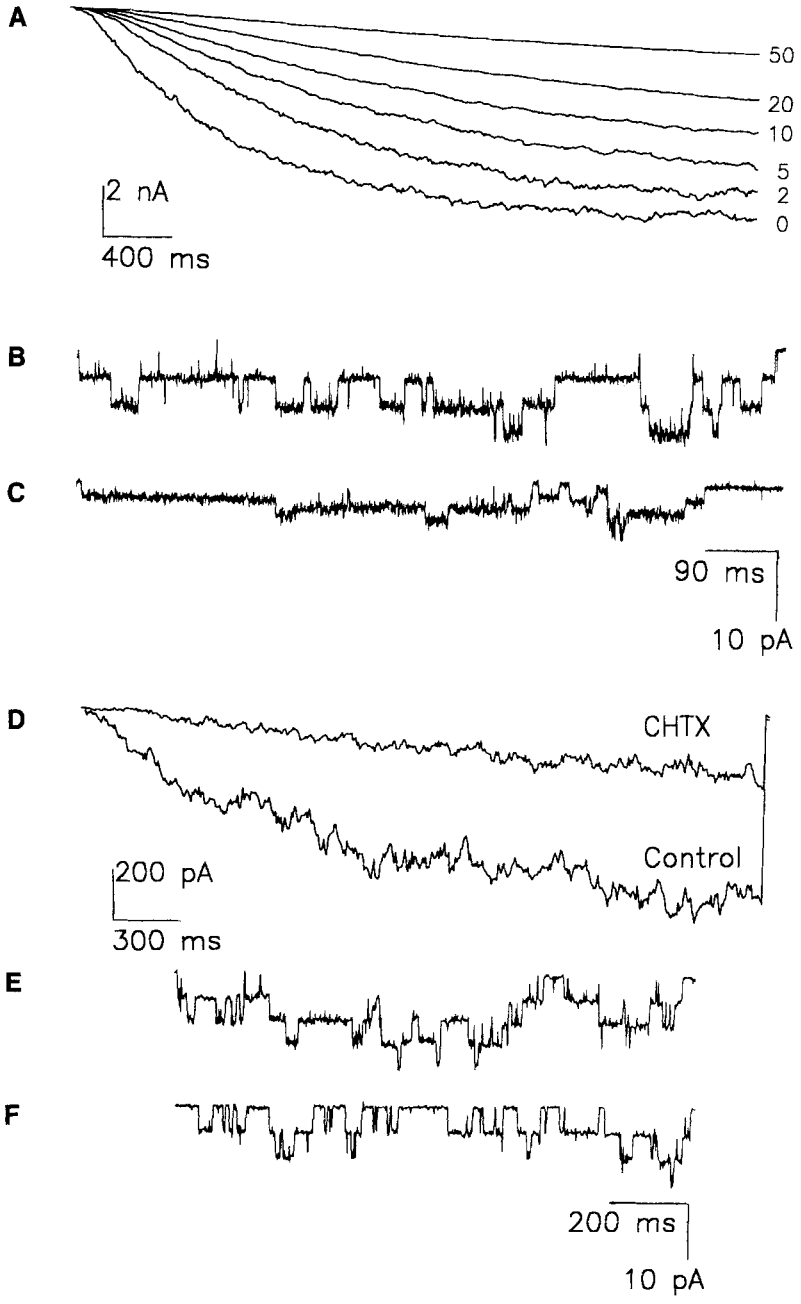

Fig. 3. Effect of TEA (*A–C*, concentrations in mM) and charybdotoxin (*D–F*) on the SV channel. TEA (2.5 mM) on the cytoplasmic side induces a "fast" block (*C*) compared to the control (*B*). Applied voltage was −100 mV. Charybdotoxin (50 nM) added to the cytoplasmic side of a large outside-out patch causes a "slow" block on single channels (*F*) compared to the control (*E*). Holding potential was −80 mV.

out with 70 μM 9-amino-acridine, and 3 μM quinacrine, respectively (Fig. 9). With both substances, the decay of the currents is altered and differs markedly from a single exponential; but in the presence of 9-amino-acridine the initial amplitude is reduced compared to the control, whereas it is slightly increased with quinacrine.

Figure 10*A* shows that 50 mM of TEA does not alter the deactivation currents significantly, whereas 200 nM charybdotoxin clearly reduces the initial tail-current amplitude (Fig. 10*B*); these latter tail currents decay according to a single exponential with comparable time constants for both charybodotoxin and the control (Fig. 10*B*).

Discussion

The differential activity of a variety of established animal channel blockers on the vacuolar SV channel introduced by the present study provides novel insights into the performance of this plant vacuolar channel. The well-known inhibitor of the acetylcholine receptor in the neuromuscular endplate, (+)-

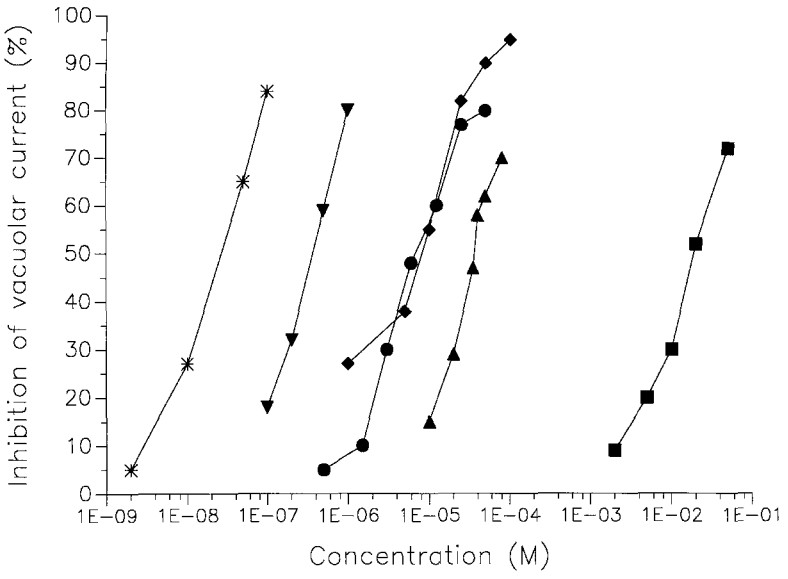

Fig. 4. Concentration-response relationships for SV channel inhibitors: charybdotoxin (*); quinacrine (▼); 9-amino-acridine (♦); (+)-tubocurarine (●); quinine (▲); TEA (■). Charybdotoxin data were derived from outside-out patches containing many channels (holding potential −80 mV); all other experiments were performed in the whole-vacuolar mode with an applied voltage of −100 mV. Means from five experiments each.

tubocurarine, also blocks other ion channels. Smart (1987) reports that calcium-dependent potassium channels of large conductance in the sympathetic nerve system of the rat are inhibited by 25 to 100 μM (+)-tubocurarine. On these channels, (+)-tubocurarine causes a "flickering" block, as is shown for the SV channel (Fig. 1).

Quinine and its stereo-isomer quinidine block various potassium channels (Bleich, Schlatter & Greger, 1987; Guggino et al., 1987; Gögelein, Greger & Schlatter, 1987; Glavinovic & Trifaro, 1988), but also cation and chloride channels in general (Gögelein & Capek, 1990; Gray & Argent, 1990). The mechanism of action is always similar. In low concentrations quinine induces a "flickering" block changing to a "fast" block, if larger doses are applied. The effective concentration varies from 10 μM (Smart, 1987) to 500 μM or more (Gray & Argent, 1990). Thus, the concentration for half-maximum inhibition of the SV channel observed in this study (35 μM) lies in the lower range of the effective doses documented in the literature.

The induction of a "substate" by quinine (Fig. 5) has not been reported so far. Nevertheless, subconductance levels are common for potassium channels. The quoted number varies from two (Barrett, Magleby & Pallotta, 1982), three (Benham & Bolton, 1983; Sakmann & Trube, 1984; Matsuda, 1988), five (Weik, Lönnendonker & Neumcke, 1989), six (Stockbridge, French & Paulman, 1991) to 15 (Geletyuk & Kazachenko, 1989)! Recently, substates have also been detected in potassium channels of plant membranes (Tyerman, Terry & Findlay, 1992). They mostly appear spontaneously, but may be elicited by pharmacologically active compounds as, for instance, by (+)-tubocurarine (Takeda & Trautmann, 1984), deltamethrin (Chinn & Narahashi, 1986), ryanodine (Rousseau, Smith & Meissner, 1987), or by cholesterol, lectin and calmidazolium (Ma & Coronado, 1988). Since most of these substances are lipophilic—as is the membrane-permeant quinine (Schlichter, 1992)—the induction of additional conductance levels may be due to interactions of these compounds with the lipid bilayer surrounding the SV channel protein, rather than the channel protein itself. Our observation that quinine may act upon addition of either side of the membrane is consistent with this mechanism. However, the quinine-induced substate has been observed only when the agent was added to the vacuolar side of the tonoplast.

In fact, quinine was the only tested blocker which was effective if added to either the cytosolic or vacuolar face of the tonoplast. Thus, inhibition by quinine seems incompatible with any of the "classical" models of channel inhibition (Hille, 1984).

Few reports deal with the action of the pH-sensitive fluorescence dye 9-amino-acridine on ion channels. Cook and Haylett (1985) found the reduction of a calcium-dependent potassium conductance in hepatocytes of guinea pigs, whereby the concentration for half-maxiumum inhibition was 73 μM. This compound also inhibits sodium channels in neuroblastoma cells (Yamamoto & Yeh, 1984), where it causes a "flickering," voltage- dependent block, with a EC_{50} of 21 μM. An inhibitory potential for different ion channels is also reported for quinacrine. This dye reduces a calcium-activated potassium conductance in guinea pig hepatocytes (Cook & Haylett, 1985), the calcium-induced calcium efflux from sar-

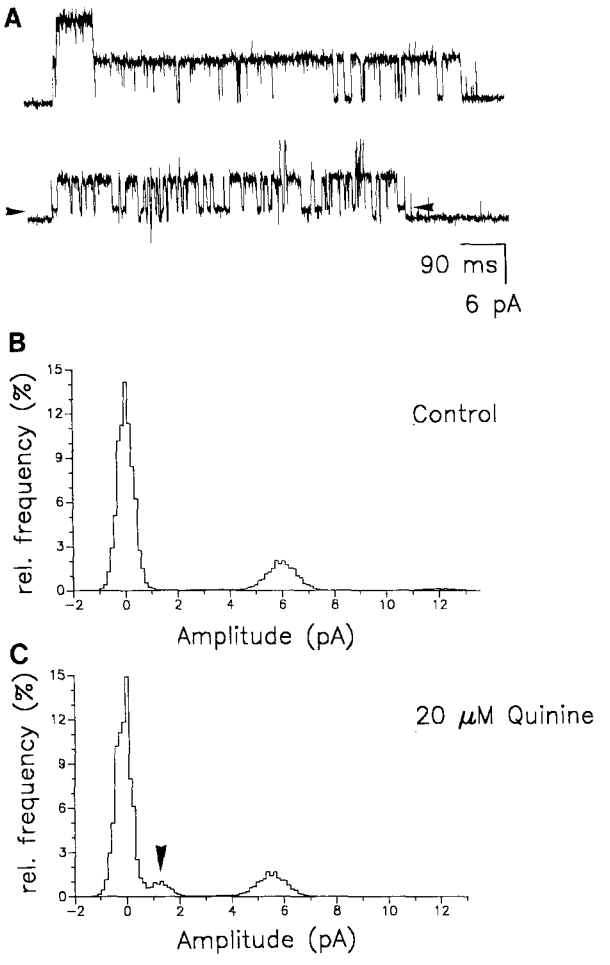

Fig. 5. Effect of 20 μM quinine added to the vacuolar side of single SV channels (*B*), compared to the control (*A*). Arrowheads denote an additional conductance state visible both in the recording (*B*) and the amplitude histogram (*C*), reflecting approx. 20% of the full conductance. Inside-out configuration, holding potential was −80 mV.

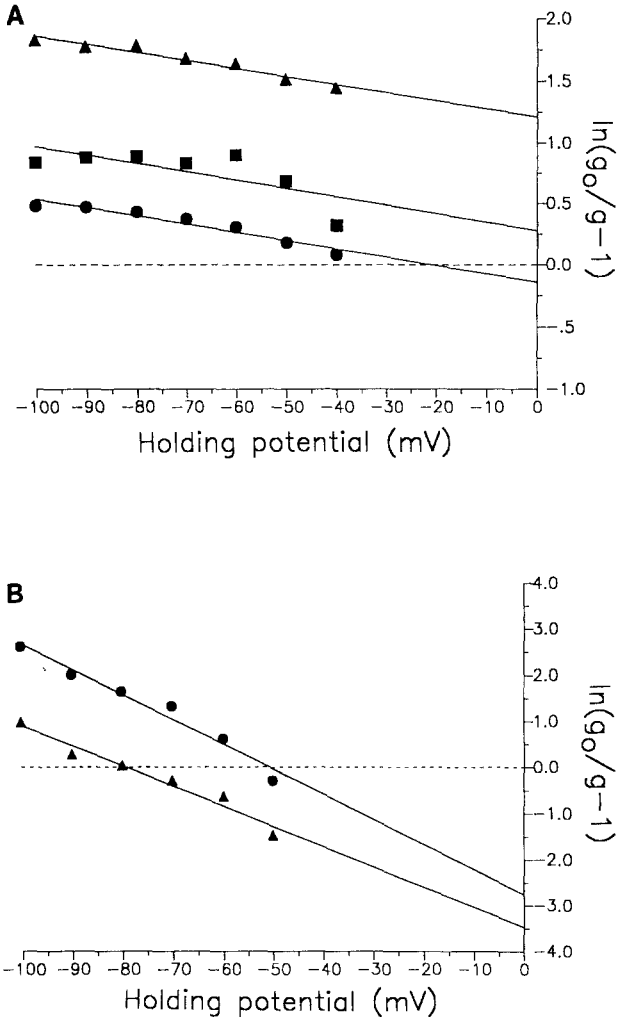

Fig. 6. Voltage dependence of the inhibition of vacuolar current in the presence of (*A*) 9-amino-acridine (● 20 μM; ■ 40 μM; ▲ 80 μM), and (*B*) quinacrine (▲ 0.5 μM; ● 1 μM), respectively. Since the open probability of the SV channel at negative voltages is very low, no data below −40 mV are available. For evaluation of the graph *see text*.

coplasmic reticulum (Fernandez-Belda, Soler & Gomez-Fernandez, 1989), and blocks the acetylcholine-gated cation channel of the neuromuscular junction (Adams & Feltz, 1980*a*,*b*; Tsai et al. 1979).

TEA typically inhibits the delayed rectifier of the squid axon from the axoplasmic side, but has also been reported as an effective inhibitor of Maxi-K channels. On the other hand, its effect on Maxi-K channels depends on the side of application: added to the extracellular side, its EC_{50} is about 0.15–0.4 mM, the effect being hardly voltage dependent. Added to the cytosolic side, however, TEA causes a voltage-dependent inhibition, but at higher concentrations, EC_{50} being 20–30 mM (Vergara, Modczydlowski & Latorre, 1984; Benham, et al., 1985; Hedrich & Kurkdjian, 1988; Villaroel et al., 1988). The SV channel of *C. rubrum* is inhibited only by cytosolic application and high concentrations of TEA, but the effect does not depend on the transtonoplast voltage (Fig. 4). The observed "fast" block, with an apparent reduction of single channel conductance (Fig. 3), is typical for the action of TEA on potassium channels.

All the substances considered so far are known to block—among others—calcium-dependent potassium channels of large conductance. So it seemed promising to test the scorpion toxin charybdotoxin, supposed to be a specific inhibitor of this channel category (Dreyer, 1990). Charybdotoxin obviously inhibits the SV channel of the *Chenopodium* tonoplast with high efficacy (Fig. 4), suggesting a close similarity of the presumably distinct toxin binding site to that of the Maxi-K channel. Charyb-

Table. Synopsis of pharmacological properties of the SV channel in tonoplast of *Chenopodium rubrum* suspension cells

Agent	EC_{50} at −100 mV	Kd_{0mV}	δ	Inhibited channel state
(+)-Tubocurarine	6 μM	60 μM	0.31	Open
9-Amino-acridine	7 μM	25 μM	0.175	Open
Quinacrine	0.3 μM	15 μM	0.675	Open
TEA	20 mM	20 mM	≈0	Open
Charybdotoxin	20 nM	?	?	Closed
Quinine	35 μM	?	?	?

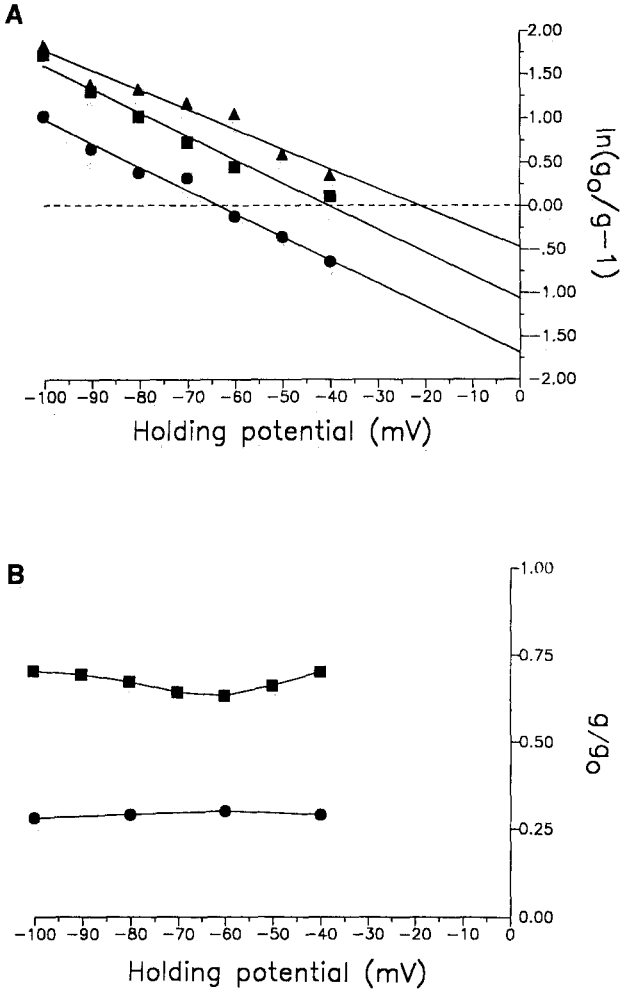

Fig. 7. (*A*) Voltage dependence of SV channels in the presence of 10 μM (●), 20 μM (■), and 50 μM (▲) (+)-tubocurarine. (*B*) Data for 50 mM (●) and 20 mM (■) TEA, respectively.

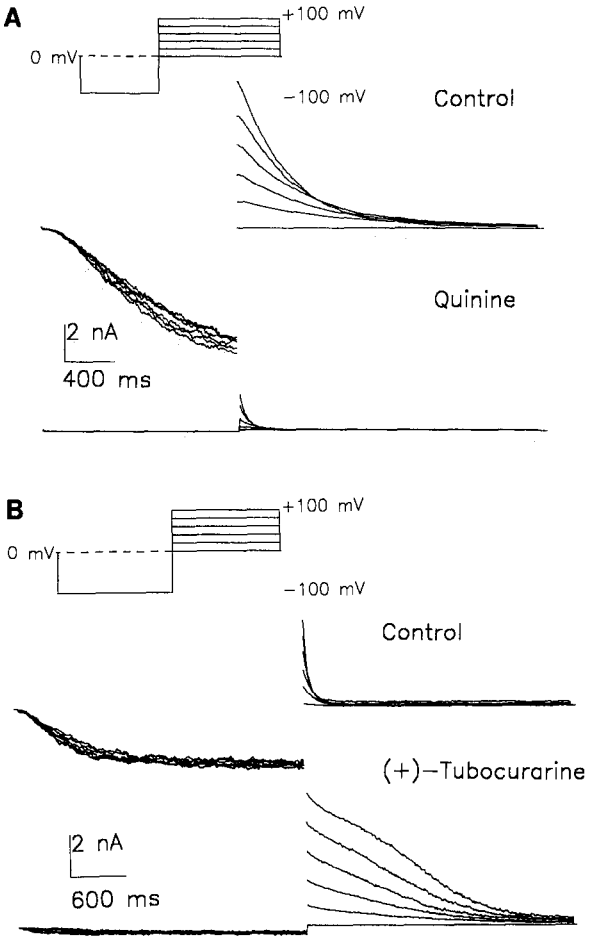

Fig. 8. Effect of 35 μM quinine (*A*) and 50 μM (+)-tubocurarine (*B*) on deactivation currents. Quinine reduces the "tail" amplitudes, but allows the decay of current to be fitted by a single exponential; (+)-tubocurarine raises the "tail" amplitudes, and the currents cannot be approximated by a single exponential. Note the variable time course of the deactivation currents in the controls.

dotoxin furthermore causes a "slow" block in the SV channel (Fig. 3 *E,F*) as is known from Maxi-K channels (Latorre, 1986; Lucchesi et al., 1989).

An intriguing test to classify the SV channel with respect to known potassium channels could be provided by the well-known bee toxin apamine, which inhibits calcium-dependent potassium channels of low conductance, since so far no channel has been reported to be blocked by both charybdotoxin and apamine (Cook, 1988; Moczydlowski, Lucchesi & Ravindran, 1988; Castle, Haylett & Jen-

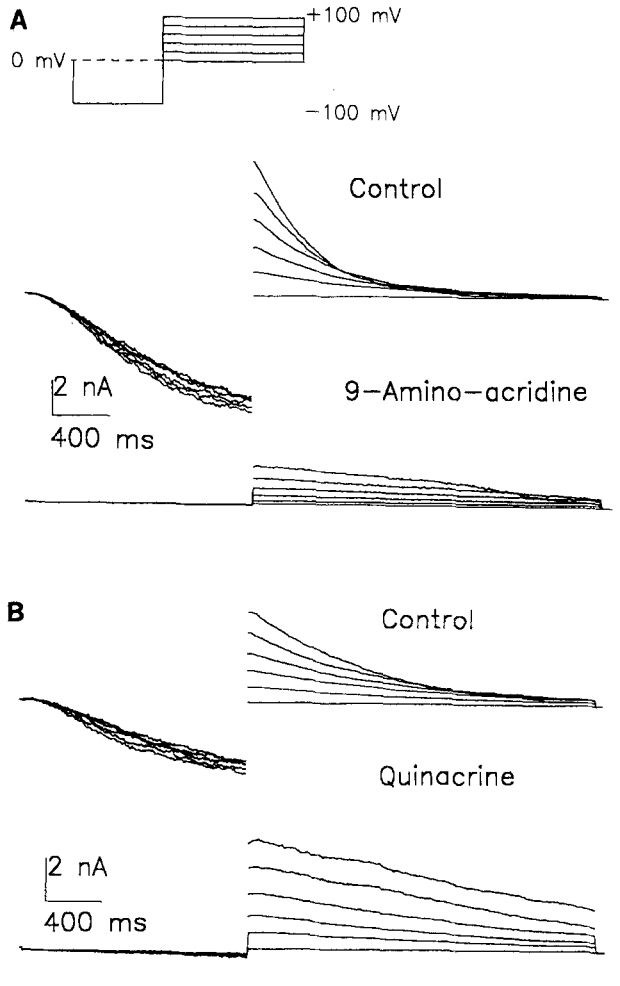

Fig. 9. SV channel deactivation currents by 70 μM 9-amino-acridine (*A*), and 3 μM quinacrine (*B*), respectively; 9-amino-acridine diminishes the initial amplitude, whereas quinacrine slightly enlarges the "tail" amplitude.

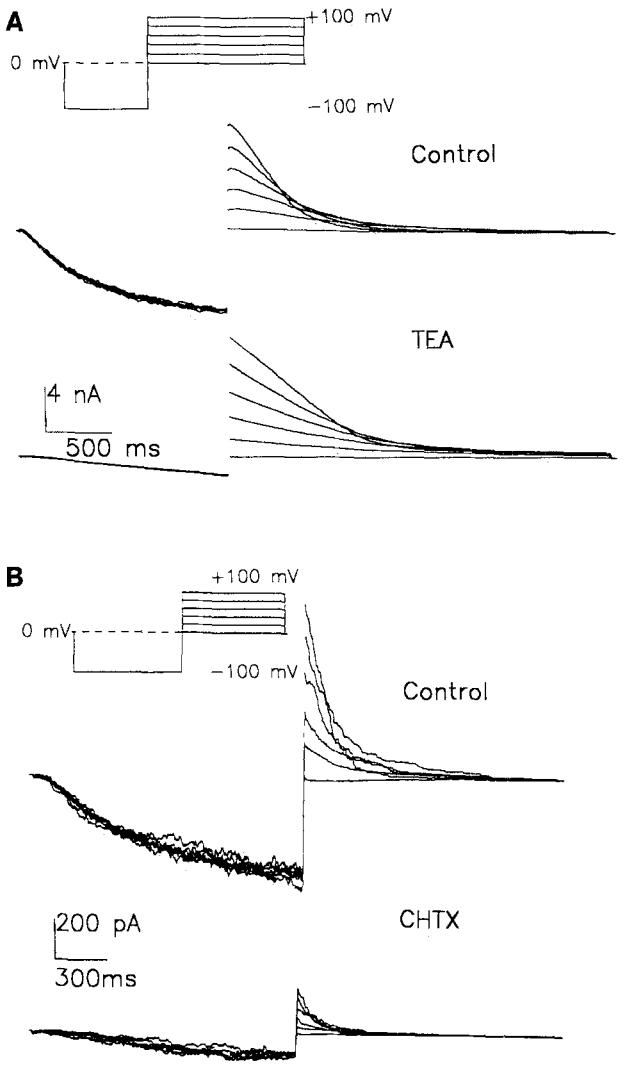

Fig. 10. SV channel deactivation by (*A*) 50 mM TEA and (*B*) 200 nM charybdotoxin; in *B* both time courses can be described by a single exponential with identical time constants.

kinson, 1989). Apamine turned out to be ineffective on the SV channel in *C. rubrum* at concentrations as high as 1 μM (*data not shown*). This failure supports our tentative notion to classify the SV channel with the Maxi-K channel of animal membranes.

Deactivation Currents

All blockers of the SV channel activity presented in this study alter the course of the deactivation currents. The analysis of these tails gives a first estimate about the mechanism of inhibition, i.e., whether a certain compound binds preferentially to the open or the closed channel protein. This analysis is based on the following assumptions. An ion channel toggles between two states, closed and open,

$$C \leftrightarrow O$$

The SV channel is strictly rectifying, so that a voltage jump from negative to positive potential forces the channel to close. This behavior can be described by a first-order kinetic (Matsuda, 1988). Deactivation currents in the control experiments thus follow a single exponential function, where the time constant of the decay depends on the applied positive voltage. If an inhibitor is applied, which binds to the open channel, the following model is applicable:

$$C \leftrightarrow O \leftrightarrow OB$$

Thus, the channel must be activated prior to the binding of the blocking compound. On the other hand, the blocked channel has to pass the opened

state before it can close. So, the deactivation current gains an additional kinetic component and cannot be approximated by a single exponential. In addition, the initial amplitude of the current is altered. At a holding potential of −100 mV the SV channel of *C. rubrum* has an open probability of about 30% (*our unpublished observation*). In the presence of saturating inhibitor concentrations, almost every channel protein is blocked. Since under deactivating conditions the channels must pass through the open state, the initial amplitude of the tails can be larger than in the control. Additionally, this amplitude is dependent on the velocity of the steps $OB \leftrightarrow O$, and $O \leftrightarrow C$, respectively. If the transition of BO to O is slow compared to the step from O to C, then the initial amplitude of the tail currents is smaller than in the control, but the current is very slowly decreasing. This describes the effect of 9-amino-acridine and quinacrine, respectively.

In the opposite case, the initial amplitude is increased, but the decay of the current is not as prolonged. This is valid for the influence of (+)-tubocurarine and TEA on the tails. Since the SV channel probably has more than two kinetic states, and the binding-unbinding reactions of the inhibitors are influenced by both voltage and concentration, a detailed quantitative analysis is rather complicated.

If the closed channel is blocked, the following scheme is valid:

$$C \leftrightarrow CB \leftrightarrow O$$

In this case, channel blockage does not affect the time course of the deactivation currents, but the initial amplitude of the tails must always be smaller than in the control. The inhibition of the SV channel by charybdotoxin perfectly matches this model. Since this compound binds to the closed Maxi-K channel in rat muscles (Anderson et al. 1988), again a fairly similar action of charybdotoxin on both the SV and the Maxi-K channel is inferred.

The used two-state model of channel opening and closing certainly oversimplifies channel performance; our present analysis of inhibitor action still holds true, however, if we assume a more complex channel kinetics. For instance, if a channel exhibits multiple closed states, and a given inhibitor binds preferentially to one of those, the channel's overall closed time is increased.

$$\begin{array}{l} C1 \leftrightarrow C2 \leftrightarrow C3 \leftrightarrow O \\ \quad\quad\updownarrow \\ \quad\quad CB \end{array}$$

Thus, the time-averaged conductance is reduced, but the transition of the open to the closed state is not influenced. Consequently, tail-current amplitudes would be reduced compared to the control; deactivation time constants, however, would not be affected. Furthermore, our approach still holds true, if we assume a channel with multiple open states, one of them binding the inhibitor:

$$\begin{array}{l} C \leftrightarrow O1 \leftrightarrow O2 \\ \quad\quad\updownarrow \\ \quad\quad O1B \end{array}$$

In this case, under our experimental conditions channels are quantitatively "frozen" in the $O1B$ state. Upon deactivation, the inhibitor will dissociate and the channel pass the $O1$ state, resulting in the above outlined consequences.

As has been noted in Fig. 8, tail currents of control experiments may differ substantially. This observation is not restricted to our SV channel data from *Chenopodium*. Ketchum and Poole (1991) present K^+ current data from *Zea mays* protoplasts, where activation kinetics vary from cell to cell. Schroeder (1989) reports similar findings for the outward-rectifying K^+ current in guard cell protoplasts from *Vicia faba*. Quite recently van Duijn et al. (1993) presented similar data on K^+ channels from tobacco protoplasts. Comparable observations also exist for ion channels in the tonoplast of vacuoles other than *Chenopodium*, for instance, by Colombo et al. (1988), Hedrich and Kurkdjian (1988), or Hedrich et al. (1988). Clearly, activation kinetics of plant ion channels may notably vary within a cell population from the the same tissue. By contrast to animal ion channels, plant ion channels might not so critically depend on a rather precise time course of channel activation (and deactivation), because they commonly serve physiological functions of a comparably extended time scale.

Altogether, as summarized in the Table, we provide information concerning affinity and topography of presumed binding sites for established cation, mainly K^+ channel blockers of the SV channel along its transmembrane voltage profile in the *Chenopodium* vacuolar membrane. We tentatively infer a certain structural similarity of the SV channel with the Maxi-K channel in animal systems. In a concurrent study, Laver (1992) has worked out δ values for the apparent cation binding site topography along the transmembrane voltage profile of the large conductance vacuolar K^+ channel in *Chara australis*. He also noticed certain similarities of this vacuolar channel with animal Maxi-K channels. Our pharmacological data provide independent evidence for this notion.

On the other hand, Tester (1988), who had stud-

ied the action of a broad set of channel blockers on plasmalemma K^+ conductance in intact cells of *Chara corallina,* was surprised to find no effect by charybdotoxin, and concluded that "the *Chara* channels are clearly of a different structure to those found in animal cells, but they may well have some structural (and evolutionary?) relationship to the high *G* K^+ (Ca^{2+}) found in animals."

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