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Pharmacology of K^+ Channels in the Plasmalemma of the Green Alga *Chara corallina*

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Summary. The outer membranes of plant cells contain channels which are highly selective for K^+. However, many of their properties and their similarities to K^+ channels found in animal cells had not previously been established. The channels open when the cells are depolarized in solutions with a high K^+/Ca^{2+} ratio. In this work, the pharmacology of a previously identified plant K^+ channel was examined. This survey showed that the channels have many properties which are similar to those of high-conductance Ca^{2+}-activated K^+ channels (high G $K^+(Ca^{2+})$). K^+ currents in *Chara* were reduced by TEA^+, Na^+, Cs^+, Ba^{2+}, decamethonium and quinine, all inhibitors of, among other things, high G $K^+(Ca^{2+})$ channels. Tetracaine also inhibited K^+ currents in *Chara*, but its effect on most types of K^+ channels in animal tissues is unknown. The currents were not inhibited by 4-aminopyridine (4AP), caffeine, tolbutamide, dendrotoxin, apamin or tubocurarine, which do not inhibit high G $K^+(Ca^{2+})$ channels, but affect other classes of K^+ channels. The channels were "locked open" by 4AP, in a remarkably similar manner to that reported for $K^+(Ca^{2+})$ channels of a molluscan neuron. No evidence for the role of the inositol cycle in channel behavior was found, but its role in K^+ channel control in animal cells is obscure. Potassium conductance was slightly decreased upon reduction of cytoplasmic ATP levels by cyanide + salicylhydroxamic acid (SHAM), consistent with channel control by phosphorylation. The anomalously strong voltage dependence of blockade by some ions (e.g. Cs^+) is consistent with the channels being multi-ion pores. However, the channels also demonstrate some differences from the high G $K^+(Ca^{2+})$ channels found in animal tissues. The venom of the scorpion, *Leiurus quinquestriatus* (LQV), and a protein component, charybdotoxin (CTX), an apparently specific inhibitor of high G $K^+(Ca^{2+})$ channels in various animal tissues, had no effect on the K^+ channels in the *Chara* plasmalemma. Also, pinacidil, an antihypertensive drug which may increase high G $K^+(Ca^{2+})$ channel activity had no effect on the channels in *Chara*. Although the described properties of the *Chara* K^+ channels are most similar to those of high conductance $K^+(Ca^{2+})$ in animal cells, the effects of CTX and pinacidil are notably different; the channels are clearly of a different structure to those found in animal cells, but are possibly related.

Key Words Ca^{2+} · decamethonium · quinine · charybdotoxin · tetracaine · 4-aminopyridine · apamin · dendrotoxin · pinacidil · inositol

Introduction

The giant cells of the green alga *Chara corallina* usually have a large negative resting potential of, say, −200 mV. The cell is excitable, with depolarization probably caused by Ca^{2+} influx and Cl^- efflux (Lunevsky et al., 1983); repolarization is at least partly due to K^+ efflux (Kitasato, 1973). During the action potential, it is likely that the K^+ moves through the same channels which dominate plasmalemma conductance in another condition, when the cell is depolarized in solutions with a high K^+/Ca^{2+} ratio; in these circumstances, the PD stays near the K^+ equilibrium potential ("K state"). For the cell to go into the K state, it must normally have an action potential (Keifer & Lucas, 1982; Beilby, 1985, 1986*a*); action potentials are accompanied by a rise in cytoplasmic Ca^{2+} (Williamson & Ashley, 1982), which suggests a role for Ca^{2+} in the gating of the K^+ channels, although gating may be controlled only by membrane depolarization.

The original aim in this work was to try to distinguish pharmacologically the K^+ channels that dominate plasmalemma conductance during the action potential, when the cell is in K state, and an inward rectifying K^+ channel recently reported in *Nitella* by Sokolik and Yurin (1986). I have been unable to characterize the inward rectifier in the *Chara* plasmalemma, but work is currently in progress investigating the pharmacology of the K^+ channels responsible for repolarization during the action potential. In the present study, the pharmacology of the K^+ channels which are open when the cells of *Chara* are in K state is investigated, and their properties are compared with those of several different channels found in animal cells.

The pharmacological properties of five relatively widespread and well-characterized channels are summarized in the Table. ATP-sensitive channels have been found in various tissues, such as β-

Table 1. Summary of effects of various inhibitors on the K^+ conductance of the plasmalemma of *Chara corallina*, compared with effects on some different types of K^+ channels found in various animal tissues

Inhibitor	*Chara* plasmalemma	High *G* K(Ca)	Low *G* K(Ca)	Classic delayed rectifier	Inward rectifier	ATP-sensitive
TEA^+	+[1,2]	+[3]	−[3]	+[4]	+[4]	+[5]
Cs^+	+	+[3]	?	+[4]	+[4]	?
Ba^{2+}	+	+[3]	?	+[4]	+[4]	+[5]
Quinine	+	+[6]	+[3]	+[7]	?	+[5,8]
4-aminopyridine	−[9]	−[9,10,11]	?	+[11,12]	?	+[13]/−[5]
Charybdotoxin	−	+	?	−	?	−
Decamethonium	+	+	?	−	?	−[5]
Apamin	−	−[14,15]	+[15,16]	?	?	−[5]
Tubocurarine	−	?	+	?	?	?
Dendrotoxin	−	−	−	?	?	?
Tolbutamide	−	−[17]	?	−[18]	−[19]	+[5,17,19,20]
Tetracaine	+	?	?	+[21]	?	+[5]

[1] + indicates inhibition; − no effect; ? not known.
[2] Most references are given here, but if not, they are in the text.
[3] Matthews, 1986.
[4] e.g. Hille, 1984.
[5] Castle & Haylett, 1987.
[6] Lebrun et al., 1983; Guggino et al., 1987.
[7] Fishman & Spector, 1981.
[8] Cook & Hales, 1984; Findlay et al., 1985.
[9] Does not inhibit channels, but slows the rate of PD-dependent closure.
[10] Thompson, 1977.
[11] Hermann & Gorman, 1981.
[12] Gillespie, 1977.
[13] Kakei, Noma & Shibasaki, 1985.
[14] Lebrun et al., 1983; Guggino et al., 1987.
[15] Pennefather et al., 1985.
[16] Hugues et al., 1982.
[17] Trube, Rorsman & Ohno-Shosaku, 1986.
[18] Rorsman & Trube, 1986.
[19] Belles, Hescheler & Trube, 1987.
[20] Arkhammar et al., 1987.
[21] Almers, 1976.

cells and exhausted muscle; conductance is increased by a decrease in cytoplasmic ATP concentrations (Noma, 1983; Cook & Hales, 1984; Arkhammar et al., 1987). The classic delayed rectifier opens upon depolarization and is insensitive to cytoplasmic Ca^{2+} levels; it has a distinctive pharmacology, however, from other channels which open upon depolarization (Table). The inward, or anomalous rectifier opens upon hyperpolarization, opposite to the channel found in the *Chara* plasmalemma. In various animal tissues, two types of Ca^{2+}-activated K^+ channels ($K^+(Ca^{2+})$) have been characterized, which have an increased probability of being open with an increase in cytoplasmic Ca^{2+} levels: one has a high unitary conductance [around 200 pS; high *G* $K^+(Ca^{2+})$] and is rapidly activated upon depolarization (Moczydlowski & Latorre, 1983); the other has a lower unitary conductance (about 30 pS), is slower activating and is less PD sensitive (e.g. Latorre, 1986). There are many more types of K^+ channel, such as stretch-activated channels, and those whose gating is mediated by binding of chemicals or hormones to receptors; in fact, at least 30 types of K^+ channel are known (Yellen, 1987), but for the moment these other types will not be considered.

Each major type of K^+ channel appears to have a unique pharmacology. For example, high *G* $K^+(Ca^{2+})$ channels, which are particularly widespread in animal tissues (Schwarz & Passow, 1983; Matthews, 1986), are inhibited by many chemicals whose specificities are usually unknown and probably very varied. Another set of compounds affect different types of K^+ channels, but not the

$K^+(Ca^{2+})$ channels (*see* Table). This variety of action is exploited to try to characterize one of the K^+ channels known to exist in the plant plasmalemma. Evidence suggests that the channels found in plant cells are most like high G $K^+(Ca^{2+})$ channels that are widespread in animal cells, and that appear to occur even in *Paramecium* (Eckert & Brehm, 1979).

From recent work with different animal tissues, it appears that there may be more than two types of Ca^{2+}-activated K^+ channel (Hermann & Erxleben, 1987; Smart, 1987), but the relationship of these either to each other or to the proposed $K^+(Ca^{2+})$ channels found in plant cells is unclear.

Materials and Methods

Small leaf cells (3 to 5 mm long) of *Chara corallina* were cut and allowed to stand overnight in APW (mM: 1 NaCl, 0.1 KCl, 0.5 $CaCl_2$, 2 HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)/NaOH, pH 6.8). To get into the K state, the cell was depolarized while in an external solution ("5K") containing (in mM): 5 KCl, 0.5 $CaCl_2$, 1 NaCl and 2 HEPES/NaOH, pH 6.8. Solutions were flowed at 3 to 10 ml/min (i.e. 3 to 10 chamber volumes/min), but within an experiment, rates were not altered, to eliminate any possible flow effects. Cells were illuminated by a fiber optics source, at an intensity of approximately 50–100 μmol m^{-2} sec^{-1}.

Cells were exposed to 0.1 mM $LaCl_3$ for about 10 min to reduce irreversibly Ca^{2+} conductance, thus reducing excitability and enabling measurements across a wide voltage span (over about 500 mV). The $LaCl_3$ was thoroughly washed away until curves restabilized (La^{3+} also inhibits, reversibly, the K^+ channels: *unpublished results*). Sharp rectifications were still apparent at around −350 and +150 mV, but the reason(s) for these is unknown. The cells treated with Li^+ and inositol were not "lanthanized," to minimize possible interference with the inositol cycle and its consequent effects on Ca^{2+} metabolism (e.g. Hokin, 1985).

All venoms were dissolved in aqueous stock solutions, and, except for the LQV, were stored frozen; the LQV and CTX were kept at 0–5°C (C. Miller, *personal communication*). Aqueous stocks of quinine and 4-aminopyridine were prepared fresh each morning; 250 mM tolbutamide was prepared each morning in ethanol, and after dilution into aqueous solutions, the pH was readjusted with small amounts of NaOH; controls with equal concentrations of ethanol were always used. Pinacidil stock solution (50 mM) was also dissolved in ethanol, and suitable controls were always used.

Current-voltage (I/V) relations of the plasmalemma of *C. corallina* were used to measure the K^+ and "leak" conductances (Beilby, 1985). The voltage across the plasmalemma was space-clamped by the insertion of a Pt/Ir wire along the axis of the intact cell (*see* Tester, Beilby & Shimmen, 1987); the longitudinal wire was made after the method of Findlay and Hope (1964). An efficient space clamp was essential as the distortion due to the cable properties of the cell tends to linearize the I/V curves (Smith, 1984); this would be especially important when clamping the plasmalemma in the high-conductance K state, when the cable length would be much shorter than that of the plasmalemma normally. The glass potential-measuring electrode, which was filled with 3 M KCl, was inserted into the cytoplasm [salt leakage from the electrode tip (Blatt & Slayman, 1983) should not be significant in the giant *Chara* cells]. The positioning of the electrode could be determined by several methods; if the tip penetrated the plasmalemma only, the cell contents did not gush up into the electrode during insertion, and the cell did not have an action potential during the insertion. Also, the shape of both an action potential and the K-state I/V curve were quite distinctive if the electrode tip penetrated the tonoplast.

I/V curves were obtained with a bipolar staircase of voltage-clamp commands, with 60-msec pulses to various potentials separated by 180 msec at the baseline clamp potential (Beilby, 1985). As described in Beilby and Beilby (1983), the voltage clamp was controlled by a Minc 11 computer and the data logging provided a time resolution of 2 msec. Positive current represents a net outward movement of positive charge.

The rate of channel closure was measured by the decrease in current required to clamp the plasmalemma PD to −150 mV after a PD jump from the resting potential, where the channels are mostly open. At −150 mV, the channel closes slowly, in a time- and PD-sensitive manner. The clamp was held for 10 sec, and current was sampled every 2 msec.

All inorganic chemicals were from BDH, Poole, England; most organic chemicals were from Sigma. Three batches of the venom of the scorpion *Leiurus quinquestriatus* (LQV) were from Sigma, and one batch was from Latoxan, Rosans, France. The partially purified charybdotoxin (CTX) was kindly supplied by Prof. Christopher Miller, and the pinacidil was a gift from Leo Laboratories, Aylesbury, England.

Results and Discussion

When the cell is in K state, the steady-state I/V characteristics are remarkably stable for at least 24 hr (Beilby, 1985). The I/V curves showed two characteristic regions of negative-slope conductance (e.g. Figs. 1 and 4), which are believed to represent the rapid voltage-dependent opening of K^+ channels as the potential moves from around −250 to −150 mV; the second region represents the closure of the same channels as the potential moves more positive to about 0 mV. Between the two regions of negative-slope conductance, membrane conductance is dominated by K^+; beyond are regions with unspecified "leaks." It can be seen (e.g. Fig. 4) that net K^+ movement can occur both into and out of the cytoplasm. The K^+ conductance across the plasmalemma at the resting potential is estimated by subtracting the estimated leak conductance (e.g. the broken line in Fig. 4) from the slope of the curve through the resting (reversal) PD. Most data are presented here without lines drawn, so the raw data can be seen without directing the reader's eye in a possibly biased way. Leak conductance can also be estimated by addition of large amounts of TEA^+, for example, to reduce K^+ current. This assumes, however, that the TEA^+, and its accompanying anion is not affecting other currents.

TEA^+, Na^+, Cs^+ and Ba^{2+} reduced the K^+ con-

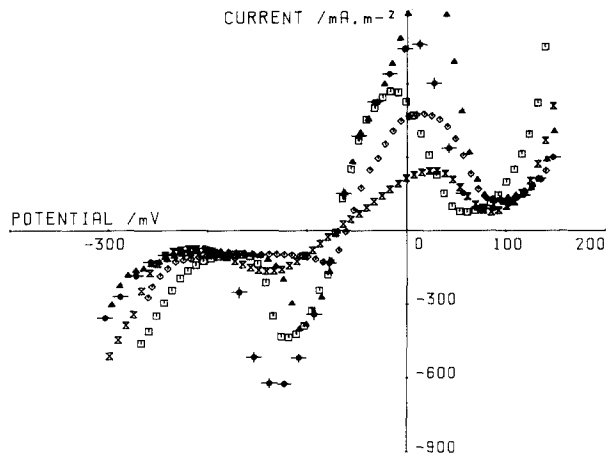

Fig. 1. Effect of TEA^+, Na^+, Cs^+ and Ba^{2+} on K state of *Chara corallina*. *I/V* relations measured in a 5K solution only (○), or after addition of 5 mM TEACl (X), 0.1 mM CsCl (△), 10 mM NaCl (□), or 1 min after addition of 1 mM $BaCl_2$ (◇). Control curve (○) average of six curves run during the experiment; all points were averaged within 15 mV spans (horizontal bars), with standard errors of the mean (vertical bars)

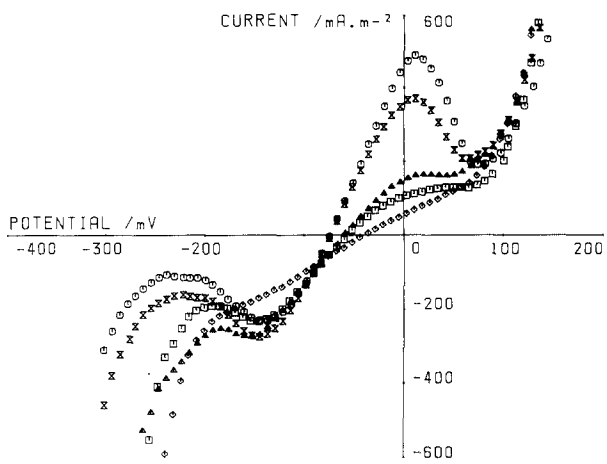

Fig. 2. Inhibition by quinine of *Chara* K^+ current. *I/V* relations in control ("5K") solution (○) and 1 (X), 4 (△) 6 (□) and 8 (◇) min after addition of 1.0 mM quinine. Final curve (◇) with 5 mM TEACl also added. Note increase in leak conductance within 1 min (X), which is not inhibited by TEA (◇)

ductance in the K state (Fig. 1), as well as the K^+ efflux during the action potential.[1] An example of the effect of these ions on the K state is shown in Fig. 1, but details of inhibition by these compounds will be provided in a later paper.[1] Blockade of K^+ current by Cs^+, Na^+ and Ba^{2+} appears to be voltage dependent, and that by TEA^+ voltage independent. Analysis of the voltage dependence of inhibition of the K^+ channel by Cs^+ shows that the channels are multi-ion, single-file pores,[2] as is found with delayed rectifier, inward rectifier and $K^+(Ca^{2+})$ channels (Latorre, 1986; Cecchi et al., 1987). Li^+ also inhibits the channels (*see below*), and H^+ has also been reported to decrease K^+ conductance (Beilby, 1986*b*; Plaks, Sokolik & Yurin, 1987).

Quinine inhibits many types of K^+ channel, including $K^+(Ca^{2+})$ channels (*see* Table); in *Chara*, 0.3 to 1.0 mM quinine reduced K^+ conductance in the K state (Fig. 2). The blockade appeared to be voltage independent. However, the leak conductance also increased upon addition of quinine, an affect which was not inhibitable by 5 mM TEA (Fig. 2). Effects on the leak and the K^+ conductance increased with increasing time (Fig. 2) and concentration, but unless the cell became very leaky, both effects were reversible. Quinine (at 1 mM) has recently been shown to inhibit leaf movements in *Samanea* by blocking K^+ channels (Satter et al., 1988).

A common inhibitor of the delayed rectifier K^+ channel is 4-aminopyridine (4AP), which is believed not to inhibit $K^+(Ca^{2+})$ channels (Table). It did not inhibit K^+ conductance in *Chara*, but when applied at 1 to 3 mM, depolarized the cell by about 2 mV min^{-1}. It caused a large increase in conductance at voltages more negative than −120 mV, and removed the region of negative-slope conductance at negative potentials (Fig. 3*a*). This is the region where the channels close in a voltage- and time-dependent manner after a jump from the resting potential, where the channels are mostly open. As the slope of the line through the resting PD was not greatly altered (where K^+ movement dominates membrane conductance), and the leak conductance did not appear to be increased (Fig. 3*a*, curve measured 3 min after addition of 4AP), it is reasonable to conclude that the effect is due to an increase in the time taken for the channels to close. When the rate of channel closure was measured by recording the decrease in current after a PD jump from the resting PD to −150 mV, 4AP clearly and reversibly reduced the rate of channel closure (Fig. 3*b*). The apparent "locking open" of the channels described here is remarkably similar to the effect of 4AP on $K^+(Ca^{2+})$ channels of molluscan neurons (Hermann & Gorman, 1981). It should be noted that the gating

[1] Tester, M. 1988. Blockade of potassium channels in the plasmalemma of *Chara corallina* by tetraethylammonium, Ba^{2+}, Na^+ and Cs^+. *J. Membrane Biol. (in press)*

[2] Tester, M. 1988. Potassium channels in the plasmalemma of *Chara corallina* are multi-ion pores: Voltage-dependent blockade by Cs^+ and anomalous permeabilities. *J. Membrane Biol. (in press)*

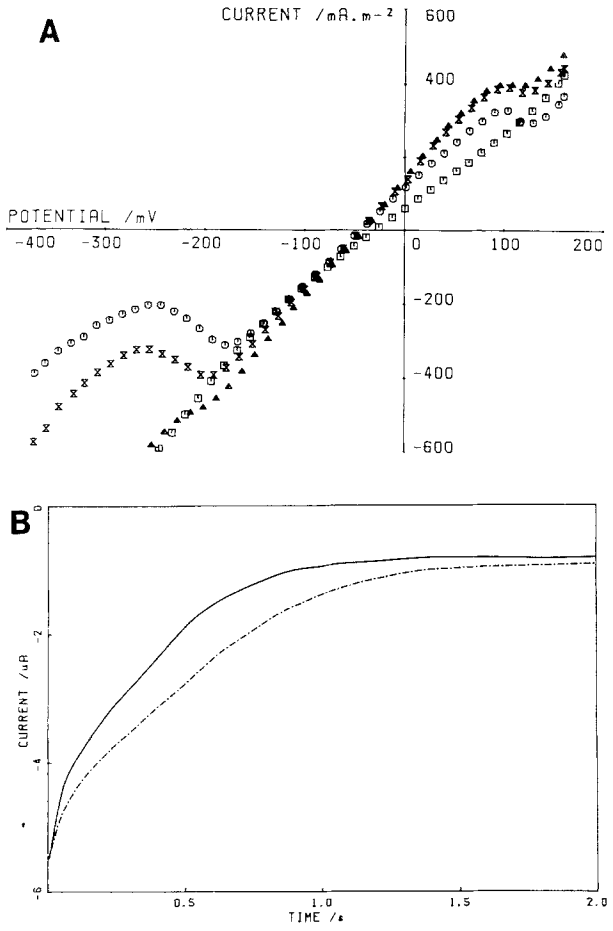

Fig. 3. Effect of 4-aminopyridine on K^+ current. (*a*) *I/V* relations measured in 5K solution only (○), and 3 (X) and 7 (△) min after addition of 3 mM 4AP. Reduction in some current occurs after addition of 5 mM TEACl (□). (*b*) Effect of 4AP on the rate of channel closure. Closure was measured by the decrease in current required to clamp membrane PD after a jump from the resting potential (−72 mV) to −150 mV, where the channel closes in a time- and PD-sensitive manner. Cell in 5K solution only (solid line), and 2 min after addition of 3 mM 4AP (broken line). Clamp was held for 10 sec, but only the first 2 sec presented; current was stable from 2 sec until the end of the clamp. The effect was reversed within 3 min of removal of the 4AP (line not drawn). Cell surface area 1.6×10^{-5} m²

of the channels at positive potentials was not greatly affected. There was also a small increase in membrane *G* at less negative voltages (*see also* Keifer & Lucas, 1982), which was partly reduced by TEA; however, the inhibition by 4AP of the voltage-dependent channel closure was not prevented or reversed by TEA (Fig. 3*a*). Addition of 25 to 50 mM caffeine had little effect on K^+ conductance in *Chara,* contrary to one brief report which suggested caffeine had a similar effect to that of 4AP in animal tissues (Hermann & Gorman, 1980). Recently, 65 and 40 pS K^+ channels in the plasmalemma of guard cell and suspension culture protoplasts, respectively, have been shown to be insensitive to 10 mM 4AP (Schauf & Wilson, 1987*a*,*b*).

The crude venom of the Israeli scorpion, *Leiurus quinquestriatus* (LQV), and a minor protein component called charybdotoxin (CTX) inhibit high conductance $K^+(Ca^{2+})$ channels in numerous animal tissues (Miller et al., 1985; Leneveu & Simonneau, 1986; Smith, Phillips & Miller, 1986; Guggino et al., 1987; Lancaster & Nicoll, 1987). Inhibition by CTX is believed to be specific, not affecting the delayed rectifier (Miller et al., 1985; Leneveu & Simonneau, 1986; Hermann & Erxleben, 1987), ATP-sensitive K^+ channels (Castle & Haylett, 1987) or Na^+, Ca^{2+} or Cl^- currents in a variety of cell types (Hermann & Erxleben, 1987; C. Miller, *personal communication*). When applied to the outside of a *Chara* cell, neither the crude venom nor the partially purified CTX affected the K^+ state. CTX is a small polypeptide, and it would be expected to be able to penetrate the turgid walls of *Chara,* which have been reported permeable to molecules of up to 60 kD (Kiyosawa, 1975). However, the extremely basic nature of CTX (Smith et al., 1986) may impede its movement through the cell wall, so a study of the naked membrane with patch-clamp techniques would be appropriate.

Another inhibitor of high conductance $K^+(Ca^{2+})$ channels is decamethonium (Latorre, 1986; Villarroel, Alvarez & Latorre, 1986), which apparently does not affect the delayed rectifier of squid axons (White & Bezanilla, quoted in Latorre & Miller, 1983). When applied externally to cells of *Chara* at concentrations up to 1.0 mM, decamethonium reversibly reduced the K^+ conductance. The effect was slow, however, increasing over at least 20 min (Fig. 4). Also, the block appeared largely voltage independent in *Chara,* in contrast to the work with animal tissues. This could well be due to a difference in the structure of the channel antechamber; this may be narrower in the plant channel, so the decamethonium cannot traverse a significant amount of the voltage drop across the membrane. This could also account for the lack of inhibition by CTX, which, like decamethonium, is believed to lodge in the channel antechamber (MacKinnon & Miller, 1987).

A frequently used blocker of TEA-insensitive, low-conductance $K^+(Ca^{2+})$ channels is apamin, which affects neither high *G* $K^+(Ca^{2+})$ channels, nor ATP-sensitive channels (Table). In *Chara,* apamin had no effect (at up to 1 μM for up to 50 min) on the plasmalemma K^+ conductance; nor did tubocurarine chloride (at 20 μM for over 90 min), which inhibits the same channels (Cook & Haylett, 1985; Dun, Jiang & Mo, 1986; Bourque & Brown, 1987).

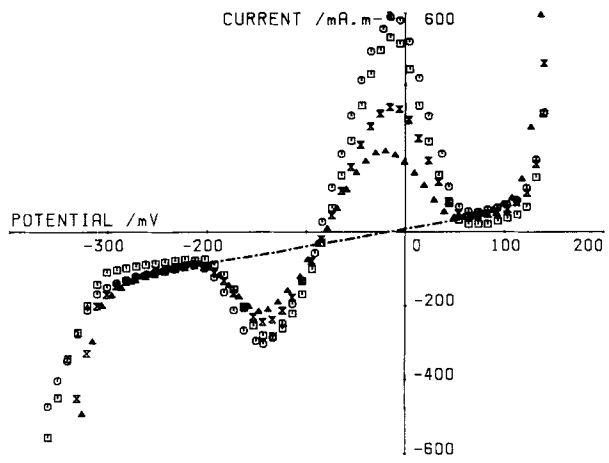

Fig. 4. Inhibition of K^+ current by decamethonium. *I/V* relations measured in 5K solution before (○) and 60 min after (□) treatment, and after 12 (X) and 18 (△) min in 1 mM decamethonium. The broken line represents the assumed leak conductance over the potentials where K^+ movement dominates membrane conductance

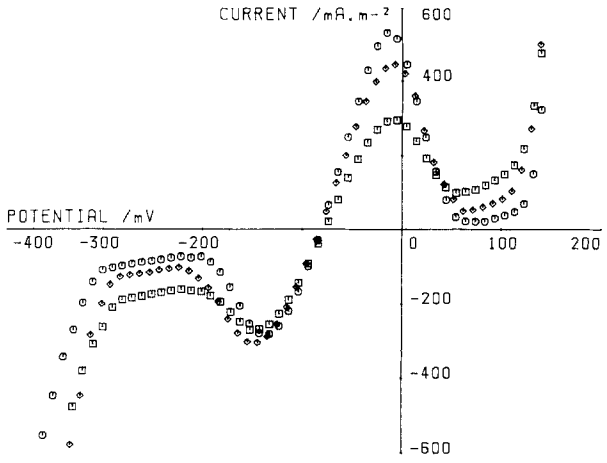

Fig. 5. Effect of tetracaine hydrochloride on K^+ current. *I/V* relations measured in 5K solution only before (○), and after (◇) treatment, and 2 min after addition of 1.0 mM tetracaine hydrochloride (□)

Interestingly, 100 μM tubocurarine has been reported to inhibit the dark-stimulated closure of *Mimosa* pinnae (Applewhite, 1972), a process thought to involve a large and rapid loss of K^+ from particular cells (Allen, 1969; Kumon & Suda, 1984), but the physiological basis of this result is unknown.

A component of the venom of the mambas *Dendroaspis polylepis* and *D. angusticeps,* dendrotoxin (DTX), appears to inhibit a transient K^+ current in some neurones (Benoit & Dubois, 1986; Halliwell et al., 1986), and a slowly inactivating, 4AP-sensitive K^+ current in other reports (Weller et al., 1985; Penner et al., 1986; Stansfield et al., 1986; Schauf, 1987), but apparently not $K^+(Ca^{2+})$ currents (Cook & Haylett, 1985; Halliwell et al., 1986). The concentration applied in this work was approximated after Halliwell et al.'s use of 300 nM toxin (although other workers used only 1–10 nM: Benoit & Dubois, 1986; Penner et al., 1986; Stansfield et al., 1986), using the molecular weight of DTX of 7 kD and assuming 2.5% wt/wt of the whole venom was DTX (Harvey & Karlsson, 1980). When 100 μg/ml of the crude venom of *D. polylepis* was applied to *Chara,* there was no effect on K^+ conductance.

Tolbutamide has been reported to inhibit ATP-sensitive K^+ channels, but not to affect the delayed rectifier, inward rectifier nor the high *G* $K^+(Ca^{2+})$ channels (Table). In *Chara,* K^+ conductance was not affected by up to 3 mM tolbutamide, when left on for up to 30 min.

Tetracaine hydrochloride inhibits ATP-sensitive and delayed rectifier K^+ channels, as well as Na^+ channels (Almers, 1976), but its effects on $K^+(Ca^{2+})$ channels are unknown (Table). In *Chara,* 0.1 to 1.0 mM tetracaine rapidly reduced the K^+ conductance (Fig. 5); however, when left on the cell for several minutes, or in higher concentrations, cells depolarized to around +40 mV and became very conductive. If left on for only 1 or 2 min, the effects were slowly reversible.

Ouabain has been reported to inhibit a Ca^{2+}-stimulated K^+ efflux in red blood cells (Blum & Hoffman, 1971), and reduces K^+ influx in some, but not all plant cells (MacRobbie, 1962; Raven, 1968). When applied to *Chara* at up to 1 mM, there was no effect on the K-state *I/V* relations.

The crude venom of *Androctonus australis* Hector, which includes, among many other components, an insect toxin of unusual structure and unknown action (Darbon et al., 1982), also had little effect on the K state in concentrations up to 30 μg/ml.

The antihypertensive drug, pinacidil, may act by increasing the open probability of high *G* $K^+(Ca^{2+})$ channels (results of Hermsmeyer & Reno, reported in Weston & Abbott, 1987). However, when applied to cells of *Chara,* 100 μM pinacidil had no effect on the K^+ conductance. When cells hyperpolarized in APW are exposed to increased external K^+ concentrations, the potential remains very negative until the cell has an action potential, after which the cell will remain in K state (*unpublished results*; Keifer & Lucas, 1982). Pinacidil was applied to cells still hyperpolarized in 5 mM K^+, but it did not promote depolarization, suggesting that

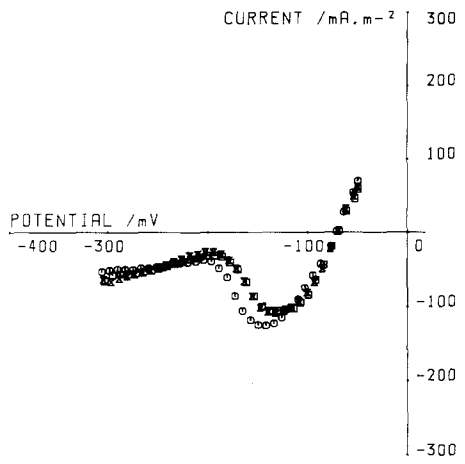

Fig. 6. Effect of Li^+ and inositol on K^+ current. *I/V* relations measured in 5K solution only (○), 12 min after addition of 10 mM LiCl (X), and 5 min after further addition of 100 μM inositol (□)

K^+ channels were not significantly opened by the pinacidil (*results not presented*).

Relatively high concentrations of Li^+ are believed to inhibit quite specifically the recycling of inositol-1-phosphate to inositol in animal cells (e.g. Berridge, 1984; Hokin, 1985), and there is some evidence for a similar cycle in plant cells (Irvine, Letcher & Dawson, 1980; Heim & Wagner, 1986; Morse, Crain & Satter, 1987; Ettlinger & Lehle, 1988). When 10 mM LiCl was applied to cells of *Chara*, the K^+ conductance was inhibited in a voltage-dependent manner (Fig. 6). This inhibition, which is readily reversible, is reminiscent of the voltage-dependent block of the channels by Cs^+ (Fig. 1; *see also* footnotes 1 and 2), and suggests that the blockade of the channels by Li^+ is direct, a perhaps not unexpected result (Sokolik & Yurin, 1986). The effect of Li^+ was maximal within 2 min of application, which, at least from data on animal cells, is likely to be long enough for inositol depletion and substantial inositol-1-phosphate accumulation to occur within the cell (Hokin, 1985), but the Li^+ was left on for 12 min in the example presented to ensure effects in the giant cells of *Chara* could occur. To determine if any of the inhibition was due to a blockade of the inositol cycle, 100 μM inositol was then applied to cells already inhibited by Li^+, to replenish the pool of inositol depleted due to inhibition of inositol-1-phosphate phosphatase. Although the inositol was not shown to enter the cells in this system, it readily penetrates other plant cell membranes (e.g. Ettlinger & Lehle, 1988). However, there was no effect of inositol on the K^+ conductance (Fig. 6), suggesting that the effect of Li^+ on K^+ conductance was a direct one, and is not evidence for the role of inositol in the control of the K^+ channel. Li^+ has been reported to inhibit in a voltage-dependent manner high G $K^+(Ca^{2+})$ channels in animal tissues (Latorre, 1986). However, the role of the inositol cycle in the control of K^+ channel gating in animal tissues is unclear, although a recent paper has demonstrated the increase of a $K^+(Ca^{2+})$ current upon addition of inositol trisphosphate and tetrakisphosphate together (Morris et al., 1987).

The overall plasmalemma conductance in K state is greatly reduced by lowering cellular ATP levels with sodium cyanide + salicylhydroxamic acid (Fig. 7*a*). However, most of this reduction in conductance upon addition of cyanide is due to a decrease in the background "leak" conductance, as can be seen by a large reduction of G with cyanide when the K^+ conductance is reduced by addition of TEA (Fig. 7*b*). When the K^+ conductance only is calculated, by subtracting total currents from currents in TEA (Fig. 7*c*), it can be seen that after 15 min in cyanide, there is a small, but definite decrease in the K^+ current. This reduction in K^+ conductance occurs more slowly than that for the background conductance, the nature of which will be discussed in another paper (Blatt, Tester & Beilby, *in preparation*). Poisoning with CN + SHAM greatly reduces ATP levels in plant cells within seconds (e.g. Blatt, 1987), and the reduction in K^+ conductance over a much slower time course is in accordance with the report of the increased gating of the high conductance $K^+(Ca^{2+})$ channels in animal tissue upon phosphorylation (Kloerke, Petersen & Jorgensen, 1987). The channels clearly do not behave like the recently discovered ATP-sensitive channels in animal tissues (Noma, 1983; Cook & Hales, 1984).

Patch-clamp studies of the plant plasmalemma have found K^+ channels which open upon depolarization with unitary conductances of 20 to 110 pS (Schroeder, Hedrich & Fernandez, 1984; Moran et al., 1986; Azimov, Geletyuk & Berestovsky, 1987; Schroeder, Raschke & Neher, 1987; Bertl & Gradmann, 1987; Schauf & Wilson, 1987*a*,*b*; Satter et al., 1988). All unitary conductances measured for the plasmalemma of plant cells are greater than all measures of unitary conductance for both delayed and inward rectifier channels, which appear to be between 2 and 18 pS, and usually less than 10 pS (e.g. Table in Latorre & Miller, 1983). The plant unitary conductances appear to be nearer the range of unitary conductances reported for high G $K^+(Ca^{2+})$ channels, which although usually between 100 and 260 pS (Latorre, 1986), appear to vary greatly in unitary conductance, even within a preparation, and can be as low as 35 pS (Hermann & Erxleben, 1987).

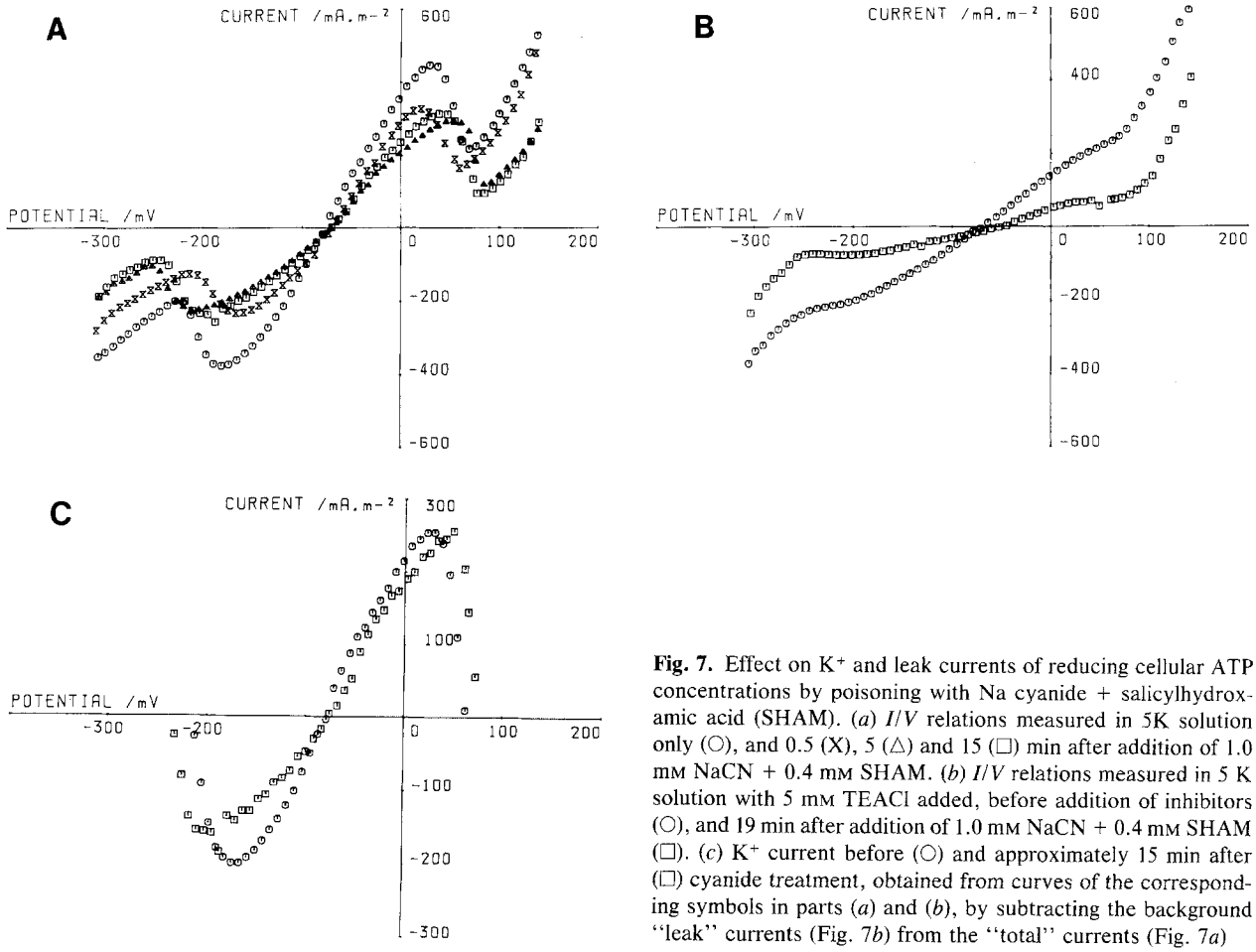

Fig. 7. Effect on K^+ and leak currents of reducing cellular ATP concentrations by poisoning with Na cyanide + salicylhydroxamic acid (SHAM). (*a*) *I/V* relations measured in 5K solution only (○), and 0.5 (X), 5 (△) and 15 (□) min after addition of 1.0 mM NaCN + 0.4 mM SHAM. (*b*) *I/V* relations measured in 5 K solution with 5 mM TEACl added, before addition of inhibitors (○), and 19 min after addition of 1.0 mM NaCN + 0.4 mM SHAM (□). (*c*) K^+ current before (○) and approximately 15 min after (□) cyanide treatment, obtained from curves of the corresponding symbols in parts (*a*) and (*b*), by subtracting the background "leak" currents (Fig. 7*b*) from the "total" currents (Fig. 7*a*)

There is ample scope for patch-clamp studies to demonstrate further analogies, or otherwise, of plant channels with those found in animal cells. Studies using the plant system may, in fact, be superior, as much larger voltage displacements can be made than is usually possible with animal cells. It should be noted that there are almost certainly other K^+ channels in the plant plasmalemma (Sokolik & Yurin, 1986), but the channel described here dominates conductance in the K state of *Chara*.

Increased external Ca^{2+} decreases the K^+ conductance (e.g. Beilby, 1986*a*), which may seem contradictory to Ca^{2+} activation of K^+ channels. However, the site of activation by Ca^{2+} is on the cytoplasmic side of the membrane, opposite to that where the inhibition by Ca^{2+} occurs in *Chara*. The inhibition could be due to the large effects of Ca^{2+} on the surface charge density of the plasmalemma (Sokolik & Yurin, 1986), which is known to affect channel gating by local distortion of electric fields (Frankenhaeuser & Hodgkin, 1957; Hille, 1984); external Ca^{2+} may also directly affect channel gating, as occupation of K^+ channels by external Ca^{2+} may be obligatory for channel closure (Armstrong & Matteson, 1986; Armstrong & Lopez-Barneo, 1987). It is quite likely that these external effects would be greater than changes of internal Ca^{2+} levels by external Ca^{2+} concentration changes. A recent paper measuring cytoplasmic Ca^{2+} concentrations with aequorin showed very little effect on steady-state light emission of washing the cell in 1 mM EGTA compared with keeping the cell in 3.9 mM CaCl (there were only large effects on transient changes in cytoplasmic Ca^{2+} levels: Okazaki et al., 1987). Upon replacement of 10 mM external Ca^{2+} with Mg^{2+}, Brownlee and Wood (1986) measured changes in cytoplasmic Ca^{2+} of about 1 μM in the rhizoid tip of *Fucus* zygotes; this is where the cytoplasmic Ca^{2+} was unnaturally high (at about 2 μM), however, and it is possible that in a normal resting cell, cytoplasmic Ca^{2+} levels are not as sensitive to changes in external Ca^{2+} concentrations. In any case, the relative change in internal Ca^{2+} compared to the changes in external concentrations is small.

Conclusions

Potassium currents in *Chara* were reduced by TEA, Na^+, Cs^+, Ba^{2+}, decamethonium, quinine and tetracaine. The currents were not inhibited by 4-aminopyridine (4AP), caffeine, tolbutamide, dendrotoxin, apamin or tubocurarine, which do not inhibit high *G* $K^+(Ca^{2+})$ channels, but affect other classes of K^+ channels. Charybdotoxin (CTX), an apparently specific inhibitor of high *G* $K^+(Ca^{2+})$ channels in various animal tissues, had no effect on the K^+ conductance in the *Chara* plasmalemma; and pinacidil, a possible opener of high *G* $K^+(Ca^{2+})$ channels, had no effect on the K^+ conductance. There was no evidence for the role of inositol in the control of channel function. Potassium conductance was slightly sensitive to reduction of cytoplasmic ATP levels by cyanide + salicylhydroxamic acid (SHAM). From analysis presented in another paper[2] the channels appear to be multi-ion pores.

Although the pharmacological and other properties of the *Chara* K^+ channels are most similar to those of high-conductance $K^+(Ca^{2+})$ in animal cells, the effects of CTX and pinacidil are notably different. 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* $K^+(Ca^{2+})$ channels found in animals.

Further analogies to channels in animal tissues may be found as more polypeptide toxins are characterized to be highly specific inhibitors of various K^+ channels (*see* recent work by Carbone et al., 1982, 1987; Lucero & Pappone, 1987). The possibility that these toxins could be used to help isolate the proteins could clearly lead to great advances in the study of these channel proteins.

Recent results with perfused cells of the charophyte *Lamprothamnium* suggest the existence of Ca^{2+}-sensitizing soluble components in the cytoplasm, which may work as mediators to activate K^+ channels (Okazaki & Tazawa, 1987). These components may include calmodulin, which has been implicated in the control of $K^+(Ca^{2+})$ channels in animal tissues (Lackington & Orrega, 1981; Kloerke et al., 1987; Okada et al., 1987). Further evidence for the possible role of Ca^{2+} on the control of K^+ channels in *Chara* has recently been found, using a wide variety of Ca^{2+} channel blockers, and this will be described in a subsequent paper.

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