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Pharmacology of the Ca^{2+}-dependent K^+ channel in corn protoplasts

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We investigated the sensitivity of the Ca^{2+}-dependent K^+ current, $I_{K(Ca)}$, present in corn protoplasts, to different K^+ channel blockers. $I_{K(Ca)}$ was inhibited by external Cs^+ (10 mM), Ba^{2+} (10 mM), and quinine (0.5 mM): reagents which block many types of outward-rectifying K^+channels. In contrast 4-aminopyridine (5 mM), an inhibitor of delayed rectifier or inactivating K^+ currents, had no effect. Neither of the peptide toxins, apamin or charybdotoxin, specific for Ca^{2+}-dependent K^+ channels in animal cells, inhibited currents when used in the nanomolar concentration range. However, higher levels of charybdotoxin (10 μM) caused marked reduction of $I_{K(Ca)}$.

K^+ channel, Ca^{2+}-dependent; *Zea mays*; Patch-clamp; Inhibitor

1. INTRODUCTION

At the plasma membrane of plant cells there are several types of K^+ channels [1] which have been classified primarily according to their activation, or inactivation, in response to changes in membrane potential. One current which is prominent in many protoplast preparations is the voltage- and time-dependent K^+ current (I_{K^+out}) elicited by membrane depolarization [1]. Although I_{K^+out} recorded in different types of cells exhibits similar activation kinetics and has a comparable activation range [2–7], the degree of homology between the K^+ channel proteins in protoplasts derived from different species and/or tissues is unclear. The range in unitary conductance, 3 to 65 pS [3–5,8–10] recorded for plant plasmalemma K^+ channels, and the observation that multiple regulatory mechanisms (e.g. cytosolic Ca^{2+} [10,11] or abscisic acid [8,12]) can modulate K^+ currents in some protoplasts, suggest that more than one type of K^+ channel may be responsible for I_{K^+out} in these diverse cell systems.

Pharmacological analysis of I_{K^+out} in different protoplast preparations would provide a more detailed categorization of the K^+ channels in plant membranes. In this report we have characterized the effects of various K^+ channel blockers on the time- and voltage-dependent K^+ current observed during depolarization of corn protoplasts. The spectrum of inhibitors which attenuate this current, Cs^+, Ba^{2+}, quinine, charybdotoxin (CTX)[1] and tetraethylammonium ions (TEA^+, shown previously [6]), also inhibit the high conductance, Ca^{2+}-dependent K^+ channel found in many animal cells [13,14]. These data corroborate our previous findings that I_{K^+out} in corn suspension cells is a Ca^{2+}-dependent K^+ current, designated $I_{K(Ca)}$[11], and lend further insight into the structural similarities that exist between channel proteins in eukaryotes from divergent evolutionary paths.

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Abbreviations: CTX, charybdotoxin; TEA^+, tetraethylammonium ion; EGTA, ethylene glycol-bis(β-aminoethyl ether); Tris, tris-(hydroxymethyl) aminomethane; BTP-ATP, bis-tris-propane-adenosine 5′-triphosphate; Mes, 4-morpholineethansulfonic acid; 4-AP, 4-aminopyridine

2. MATERIALS AND METHODS

Corn (*Zea mays*) cell cultures, protoplast preparation and whole-cell recording were performed as described by Ketchum and Poole, 1990 [11]. Currents stored on VCR tape were digitized with a Lab Master analog-to-digital converter (Scientific Solutions, supplied by Axon Instruments, Burlingame, CA) and analyzed using the pClamp software program (Axon Instruments). Intracellular medium (pipette filling solution) contained, in millimolar (mM), 100 K-gluconate, 2 $MgCl_2$, 4 EGTA-Tris, 5 BTP-ATP, 10 Hepes titrated to pH 7.0 with Tris. Extracellular medium consisted of 1 K-gluconate, 2 $MgCl_2$, 1 $CaCl_2$, 8 hemicalcium gluconate, 10 Mes, pH titrated to 6.2 with Tris. In some experiments 100 μM $LaCl_3$ was added to the chamber to promote sealing of the protoplast membrane to the glass pipette. This concentration of La^{3+} has no effect on $I_{K(Ca)}$ [11]. Inhibitors to be tested were added to the extracellular medium. CTX was the generous gift of Dr C. Miller (Brandeis University, Waltham, MA). The mitochondrial signal peptide of pre-ornithine carbamyltransferase, amino acids 1 to 27, was chemically synthesized corresponding to the sequence published by Nguyen et al. [15]. This peptide, which contains 6 positive residues and one partial (histidine) positive charge, was kindly provided by Drs S.K. Randall and G.C. Shore (Dept of Biochemistry, McGill Univ., Montreal, Canada). All other inhibitors were obtained from Sigma (St Louis, MO). In experiments where CTX or apamin were tested, protoplasts were first washed (centrifug-

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ed and resuspended in fresh extracellular medium), 3 times to remove dithiothreitol; this reductant is present during the enzymatic isolation and storage of protoplasts prior to recording.

3. RESULTS

The addition of extracellular CsCl caused marked inhibition of $I_{K(Ca)}$ (Figs 1 and 2A). At 1 mM the time- and voltage-dependent K^+ current was slightly attenuated. When the Cs^+ concentration was increased to 10 mM $I_{K(Ca)}$ was abolished at test potentials between -60 and 0 mV (Figs 1C and 2A). At more positive voltages a portion of the current recovered during the test step. These observations are consistent with the theory that cesium ions can enter the pore from the external surface to block K^+ conduction and are repulsed from the channel with strong depolarization [14].

Inhibition of $I_{K(Ca)}$ was also observed following the addition of $BaCl_2$ (Fig. 2B). A progressive decrease in current was noted as the Ba^{2+} concentration was raised from 1 to 10 mM. The response to $BaCl_2$ was accompanied by a hyperpolarization of the resting membrane potential and a correlative decrease in the time-independent current (not illustrated). These changes are similar to the alterations of potential and conductance which have been observed in corn protoplasts following the addition of TEA^+ [6]. Comparable changes in resting potential after exposure to K^+ channel blockers were noted with both *Nitella flexilis* and *Chara corallina* [16,17].

We tested the sensitivity of $I_{K(Ca)}$ to the organic reagents quinine and 4-aminopyridine (4-AP). Quinine, like Cs^+ and Ba^{2+}, inhibits a variety of K^+ channels

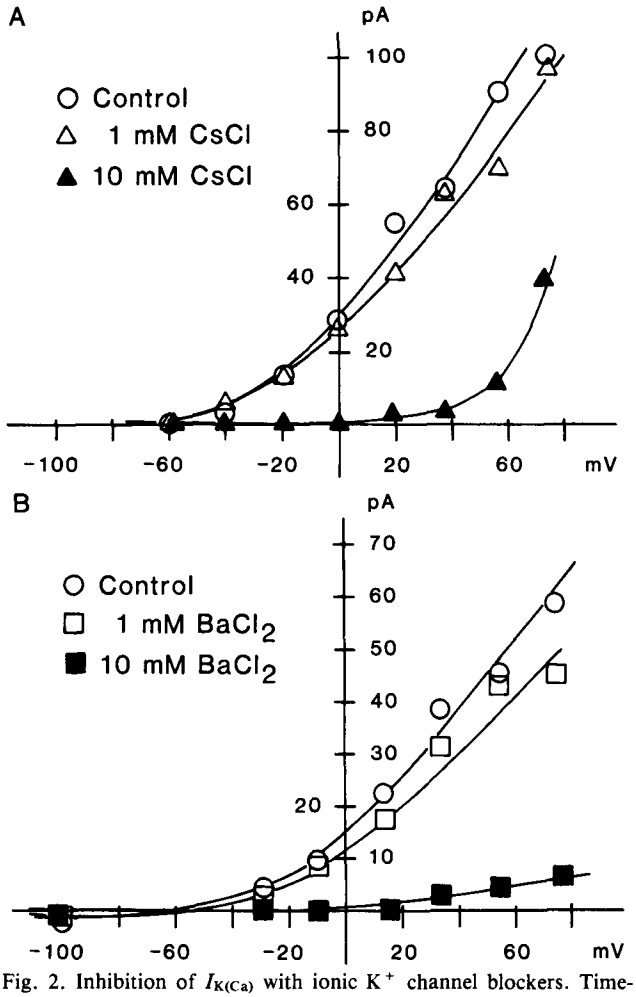

Fig. 2. Inhibition of $I_{K(Ca)}$ with ionic K^+ channel blockers. Time-dependent current expressed as a function of the membrane potential. (A) CsCl inhibition; (○) control, (△) 1 mM CsCl, (▲) 10 mM CsCl. (B) Inhibition by external $BaCl_2$; (○) control, (□) 1 mM $BaCl_2$, (■) 10 mM $BaCl_2$.

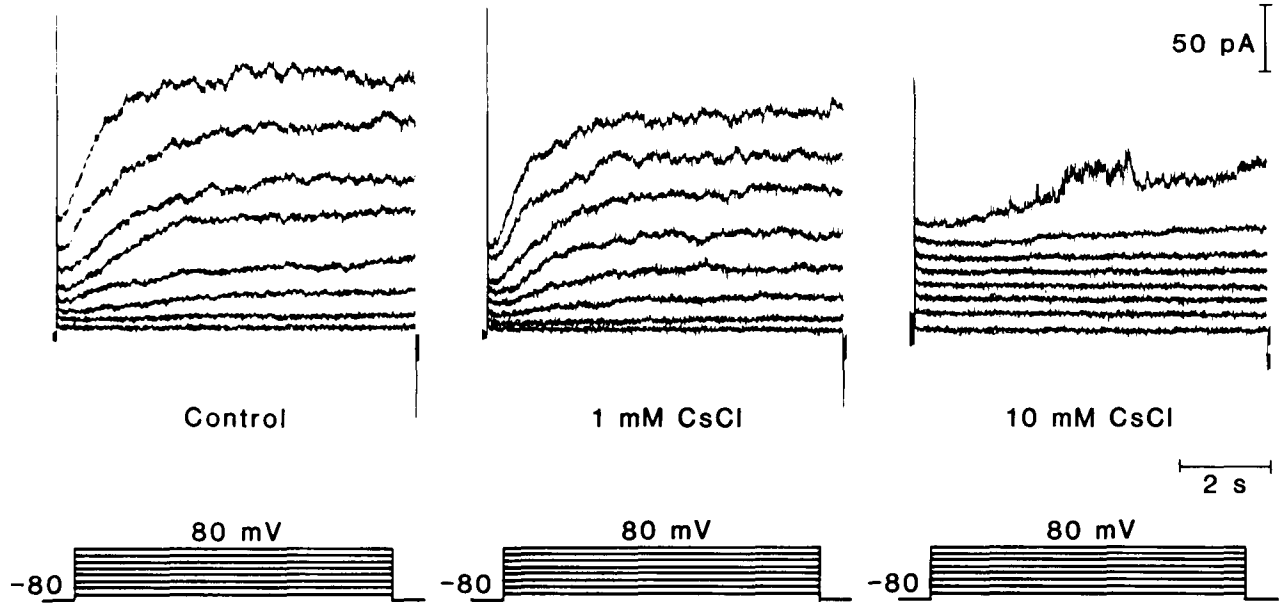

Fig. 1. Cs^+ blockage of $I_{K(Ca)}$. Whole-cell currents recorded at test potentials between −60 and +80 mV, 20 mV intervals, from a holding potential of −80 mV.

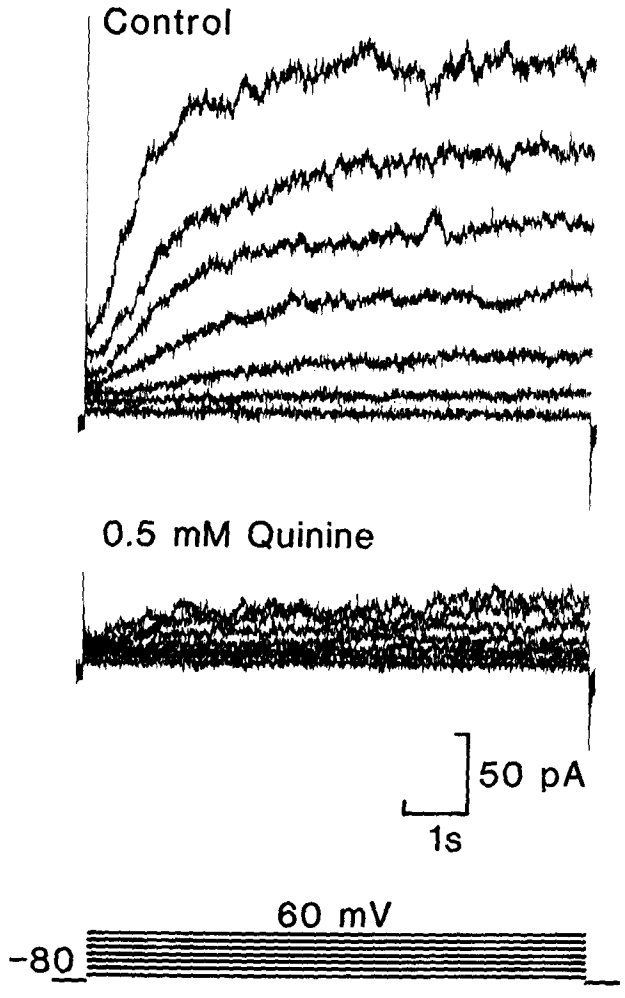

Fig. 3. Reduction of $I_{K(Ca)}$ by quinine. The holding potential was −80 mV and step potentials ranged from −60 to +60 mV.

[18] and was a potent inhibitor of the K^+ conductance in corn (Fig. 3). In contrast 4-AP (5 mM), which blocks either delayed rectifier or inactivating K^+ currents but not Ca^{2+}-dependent K^+ currents [18], caused no change in $I_{K(Ca)}$ (data not shown).

Finally, we assessed the effect of two peptide toxins, apamin and CTX on $I_{K(Ca)}$. These peptides are inhibitors of low and high conductance Ca^{2+}-dependent K^+ channels, respectively [13]. Neither compound influenced K^+ efflux when used in the low nanomolar concentration range. However, micromolar levels of CTX caused marked reduction of $I_{K(Ca)}$ (Fig. 4) which was not observed with comparable levels of apamin (not illustrated). A control peptide, the first 27 amino acids of the mitochondrial signal sequence to preornithine carbamyltransferase, which has a charge density similar to CTX also did not significantly inhibit the Ca^{2+}-dependent K^+ current when applied at 10 μM (data not shown). It should be noted that inhibition in the order of 20% or less would not be detected in our system due to run-down of the control current.

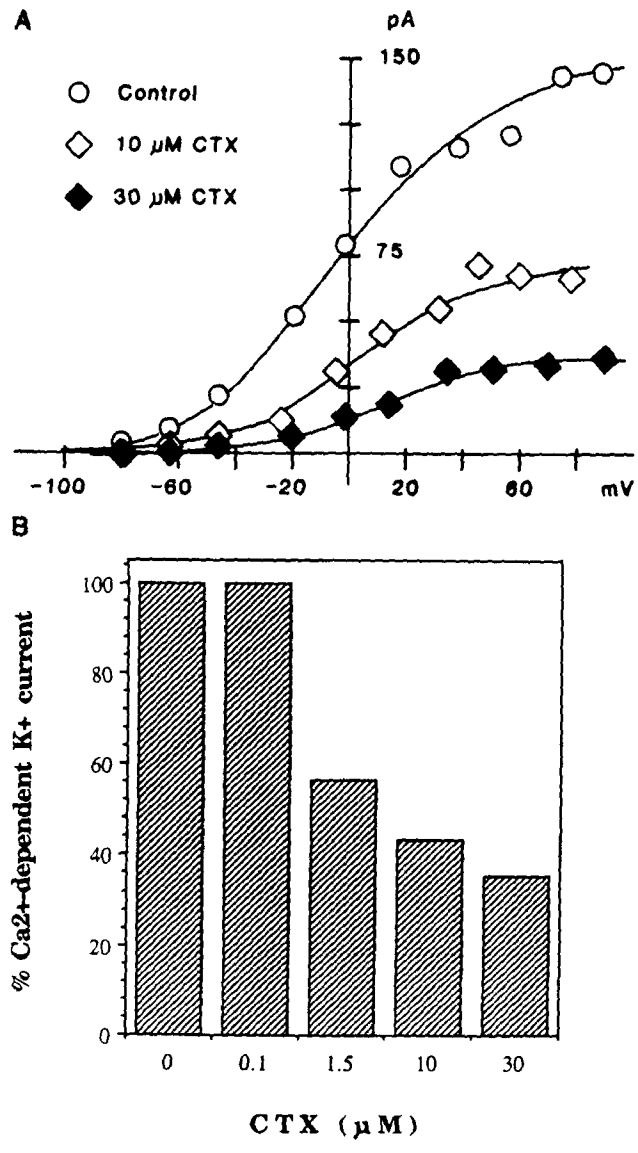

Fig. 4. CTX inhibition of the Ca^{2+}-dependent K^+ current. $I_{K(Ca)}$ observed as a function of the membrane potential. (A) (○) control and CTX at (◇) 10 and (◆) 30 μM. (B) % Ca^{2+}-dependent K^+ current recorded at +60 mV compared with the external CTX concentration. Values are the average of 2–4 cells.

4. DISCUSSION

The structural similarity that exists between different voltage-dependent K^+ channels is reflected in the proficiency with which certain reagents (e.g. TEA^+, Cs^+, Ba^{2+} and quinine [13,18]) inhibit the activity of all classes of K^+ currents. These 'broad-spectrum' inhibitors have been used both to identify potential new K^+ channels and to confirm the classification of currents which demonstrate K^+ selectivity. However in plant tissues, the effectiveness of these compounds has not been well documented. The Ca^{2+}-dependent K^+ channel found in corn suspension cells is inhibited by all 4 reagents ([6], and data presented). These results sup-

Table I

Inhibitors of outward-rectifying K^+ channels in plant cells

Inhibitor	*Zea mays* +/− conc (mM)	*Vicia faba* +/− conc (mM)	*Asclepias tuberosa* [4] +/− conc (mM)	*Samanea saman* [5] +/− conc (mM)	*Dionaea muscipula* [3] +/− conc (mM)	*Chara corallina* [19] +/− conc (mM)
$TEA^{+,a}$	+ 9 [6]	+ 10 [8]	+ 10	+ 6	+ 20	+ 5
Cs^+	+ 10	+ 100 [8][b]	+ 20			+ 3
Ba^{2+}	+ 10	+ 10 [9]			+ 10	+ 1
Quinine	+ 0.5			+ 1		+ 1
4-AP	− 5	− 10 [8]	− 10			− 3[c]
CTX	+ 0.01					− ?
Apamin	− 0.02					− 0.001

(+) Inhibition; (−) no effect; In most cases, IC_{50} has not been determined. The concs. given are those tested.
[a] TEA^+ also inhibits K^+ channels in *Hydrodictyon africanum* [2], *Nitella flexilis* [16], and *Mougeotia* [10]. [b] Internal inhibitor concentration. [c] Enhances current.

port the premise that specific motifs have been conserved in all channel proteins that maintain K^+ selectivity.

In other plant tissues broad-spectrum reagents have inhibited K^+ conductance, although there are dissimilarities in the experimental conditions and concentrations required (Table I). For example, external application of Cs^+ (20 mM) blocked time- and voltage-dependent outward K^+ currents in *Asclepias tuberosa* [4], while inclusion of Cs^+ (100 mM) in the pipette filling solution abolished the activity of the outward-rectifying K^+ current (i.e. I_{K^+out}) in guard cells of *Vicia faba* [8]. In both *N. flexilis* and *C. corallina* Cs^+ (0.01 to 5.0 mM) addition to the extracellular medium inhibited inward K^+ flux but enhanced outward K^+ movement [16,19].

The pharmacology of the K^+ channel in corn suspension cells resembles that of the high conductance, Ca^{2+}-dependent K^+ channel found in animals [13,18]. As well, there are similarities between the channel in corn and the K^+ channel that dominates the 'K-state' in *Chara corallina* [20]; which is also thought to be a Ca^{2+}-dependent K^+ channel. It is however clear that the 'hallmark' reagent of this class, CTX, has a much lower affinity for the K^+ channel in corn in contrast to its strong inhibitory character in animal cells [21]. Nevertheless, when compared to all other reagents tested in plant systems this is the most potent K^+ channel blocker identified to date (Table I). CTX inhibition is not adequately explained as a non-specific interaction with the channel protein since other polycationic reagents, namely the mitochondrial signal peptide and apamin, or La^{3+} [11], do not elicit the same response. Minor changes in the sequence of the peptide toxins (e.g. comparisons between CTX and noxiustoxin [22] or mono-iodination of CTX [23]) have been shown to dramatically affect the efficacy of toxin inhibition. In the latter example the blocking affinity decreased from 1.3 nM to 15 μM. One might speculate that equally minor changes in the vicinity of the CTX binding site could account for the decrease in toxin binding affinity observed here.

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