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Deltamethrin rescues run down of K^+ outward rectifying channels in *Vicia faba* guard cells

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ABSTRACT The dominant outward rectifier K^+ currents were examined in protoplasts from *Vicia faba* guard cells. In whole-cell patch-clamp recordings, we generally observed that the conductance of the K^+ inward and the outward rectifier gradually decreases. As a consequence of this run down a new steady state was achieved which was 90 ± 5% lower than that obtained at the beginning of the recording. The run down of the outward rectifier could be greatly reduced by pre-treating protoplasts either with the membrane permeable drug deltamethrin or by perfusing protoplasts with a pipette solution containing 5 μM cyclosporine A. Furthermore, after the rundown the conductance of the outward rectifier could be partially restored upon addition of 5 μM deltamethrin to the bath medium. Since deltamethrin and cyclosporine A are established inhibitors of the calcium sensitive phosphatase calcineurin, the data argue for a participation of this type of phosphatase in the control of the activity of K^+ outward rectifier channel in guard cells. **Acta Biol Szeged 46(3-4):19-20 (2002)**

Pyrethroids are well known neurotoxins causing hypersensitivity, tremors, ataxia and paralysis in both vertebrates and invertebrates. Because of their high insecticidal potency, they are widely used mainly against mosquitoes. In search of their mode of action it was found that the type-II pyrethroid, deltamethrin (dm) prolongs the open time of sodium channels (Narahashi 1992), decreases the open channel probability (P_O) of voltage-gated chloride channels (Forshaw et al. 2000) and inhibits the calcium-calmodulin-dependent protein phosphatase, calcineurin (Enan and Matsumura 1992). The presence of this phosphatase in plants indicates the possibility that dm may also affect physiological processes in these organisms. In the present study, we investigated the effect of dm on stomatal K^+ outward rectifying channels.

Materials and Methods

Plant materials and experimental solutions

Vicia faba L. c.v. Hangdown plants were grown for 2-3 weeks in the greenhouse on soil. Guard cell protoplasts (GCPs) were prepared as described previously (Homann 1998). GCPs were kept in a bath solution consisting of 10 mM potassium glutamate, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM MES/KOH (pH 5.5), and osmolarity was adjusted to 500 mosmol/kg with mannitol. For patch-clamp whole-cell recordings, patch pipettes were filled with a solution containing 150 mM potassium glutamate, 0.838 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM K-ATP, 1 mM EGTA, 10 mM HEPES/KOH (pH 7.5). This gives a concentration of free Ca^{2+} of 200 nM. The osmolarity was adjusted to 520 mosmol/kg with mannitol. After attaining the whole-cell configuration, deltamethrin containing bath solution was added to the external solution. Deltamethrin (Calbiochem-Novabiochem Co., USA) was diluted from a stock (2 mM) dissolved in dimethyl sulfoxide (DMSO). DMSO (max. 0.16% v/v) alone did not affect the potassium currents. All other chemicals were purchased from Sigma (St. Louis, MO).

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Electrophysiological recordings

Potassium currents were recorded using standard patch-clamp whole-cell recordings. Currents were filtered at 3 kHz and sampled under control of pClamp 5 hard/software (Axon Instruments, Foster City, CA, USA). Membrane currents in combination with the electrical parameters of the cells were recorded with the software driven EPC-7 and EPC-9 amplifier (HEKA Lambrecht, Germany).

Results and Discussion

Figure 1 A shows a typical recording of membrane currents across the plasma membrane of a *Vicia faba* guard cell protoplast. Currents were measured in the whole cell configuration upon clamping the membrane from the holding voltage (-80 mV) to a series of test voltages between -80 mV and +100 mV. The resulting currents obtained immediately after breaking into the whole cell configuration reveal the typical features of the slow activating K^+ outward rectifier (I_{Kout}) found in these cells. We found that many protoplasts tested had no or only very small inward rectifiers. Generally, I_{Kout} declined with time after achieving the whole cell configuration. In the present case I_{Kout} decreased between the first I/V scan (t = 0) and a late scan about 10 min after reaching the whole cell configuration (t = 560 s) by 66% (Fig. 1B). In comparable long lasting experiments we found the same type of gradual decline in I_{Kout}. The decline of K^+ current could result from a loss of regulatory molecules and reflect a process generally known as "wash out". To test therefore if the decline in K^+ current is the consequence of a fatal decrease in access conductance, we measured the K^+ current in parallel with the electrical parameters of the cell with a lock-in-amplifier. During the same time neither G_a nor the membrane capacitance, C_m changed significantly.

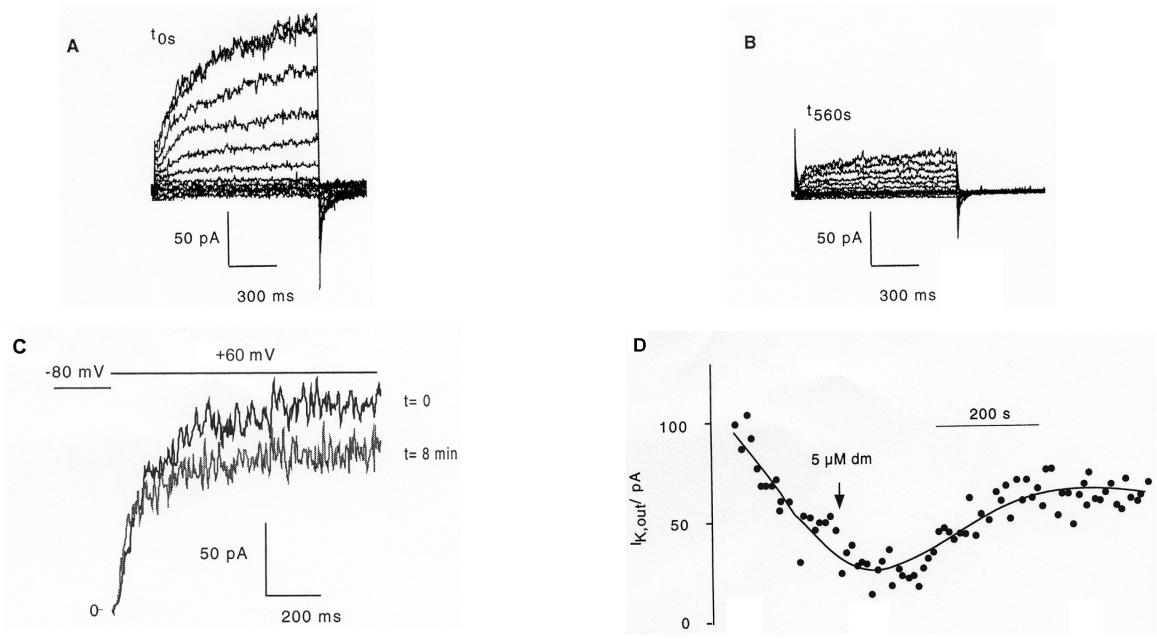

Figure 1. Whole cell recordings in protoplasts from *Vicia faba* guard cells. For further details see text.

A number of studies have already revealed a sensitivity of both guard cell K^+ channels to protein phosphorylation/dephosphorylation. To examine whether these processes are involved in the relaxation, we examined the difference in decline after omitting Mg-ATP from the pipette medium. However, the respective whole cell recordings revealed no difference in the decline of I_{Kout} between recordings with or without 2 mM Mg-ATP in the pipette solution. In further experiments the effect of protein phosphatase inhibitors on the decline process was tested. Therefore we used deltamethrin, a pyrethroid known to inhibit the calcium-calmodulin-dependent protein phosphatase calcineurin in animals (Enan and Matsumura 1992) and plants (Allen and Sanders 1995). Figure 1C shows a typical recording of I_{Kout} in a guard cell protoplast pre-treated for 20 min with 10 µM deltamethrin. Again, upon access to the cell, a positive voltage step from the holding voltage -80 mV to +60 mV reveals the slow activation of I_{Kout}. But unlike in control cells, this deltamethrin pre-treated cell showed only a reduced decline of I_{Kout} over the time of recording. The result that a phosphatase inhibitor hastened the decline of the outward rectifier compared to untreated protoplasts suggests that the activity of a calcineurin type phosphatase is responsible for the relaxation of this current.

Deltamethrin induced recovery illustrated in Figure 1D was also observed in other experiments. The mean deltamethrin induced recovery of I_{Kout} reveals that I_{Kout} recovered to about 72 ± 12% of the control value over a period of 8 min after administration of deltamethrin. In the next step we examined the specificity of I_{Kout} decline for calcineurin-like phosphatase activity. Therefore we used cyclosporine A, a drug that inhibits phosphatase activity by forming a complex with cyclophilin. Subsequent monitoring of I_{Kout} in standard whole cell recordings showed that this operation prevented the decrease of I_{Kout} in long lasting whole cell recordings. Taken together, the fact that two different inhibitors of the 2B type phosphatase prevent relaxation of the K^+-current stresses the involvement of this type of phosphatase in the regulation of I_{Kout}.

Acknowledgments

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