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Inactivation of KAT1 channel of guard cells at sub-millimolar concentrations of external potassium

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ABSTRACT In the present study we investigated the effect of depleting K^+_o on the activity of a plant K^+ channel, the inward rectifier KAT1 from *Arabidopsis thaliana*. Most voltage sensitive K^+ channels are able to sense the concentration of extracellular K^+ (K^+_o) and inactivate if K^+_o decreases to sublillimolar values. The channel was expressed in mammalian HEK293 cells and measured with patch clamp in the whole cell configuration. The effect of K^+_o depletion on channel activity was monitored from the tail currents before during and after washing K^+_o from the medium. The data show that a depletion of K^+_o results in a decrease in channel conductance. This high sensitivity of the channel to K^+_o could serve as a safety mechanism, which inactivates the channel at low K^+_o and prevents in this way leakage of K^+ from the cells via this type of channel.

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KEY WORDS

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HEK293
potassium affinity

Under conditions of low extracellular K^+ (K^+_o), which is the prevailing situation in most soils, K^+_o may only be present at submillimolar concentrations. In the case of these very low K^+_o the equilibrium voltage for K^+ is very negative. Simultaneous recordings of the electrical voltage and K^+-concentration gradient across the plasma membrane of *Arabidopsis thaliana* root cells have shown that the resting voltage can at submillimolar K^+_o be more negative than the K^+ equilibrium voltage (Maathuis and Sanders 1993). Without inactivation of the potassium inward rectifier, cells would in this situation inevitably loose K^+ through this type of channel. It is therefore reasonable to speculate that cells have developed mechanisms to down regulate K^+ channel activity at very low external potassium availability; this may help the plant to prevent unwanted K^+-leakage.

Materials and Methods

For functional expression KAT1 was transfected with 23 µg/ml DNA (pCB6-KAT1) into modified human HEK293 cells. HEK293 cells were transiently transfected using a standard calcium phosphate protocol.

Experiments were performed on cells incubated after transfection at 37°C in 5% CO_2 for 2-3 days. On the day before the experiment, cells were dispersed by trypsin, plated at a low density on 35 mm culture dishes and allowed to settle over night. Dishes were then placed on the stage of an inverted microscope and single cells patch-clamped in the whole-cell configuration according to standard methods using an EPC-9 patch clamp amplifier (HEKA, Lambrecht, Germany). Data acquisition and analysis were performed using PULSE software (HEKA).

K^+ concentrations in bath solution samples were determined as described previously (Bérczi et al. 1982) using a Hitachi Z-8200 atomic absorption spectrophotometer.

Results and Discussion

To examine the dependency of KAT1 activity on extracellular potassium the channel protein was expressed in mammalian HEK293 cells. These cells are suitable for heterologous expression of an inward rectifier, because they exhibit at voltages negative than about 0 mV only a very low endogenous conductance (Figs. 1A, C). With this low background conductance the expression of recombinant KAT1 is easily detectable. Figure 1B shows the current response of a HEK293 cell transfected with kat1 DNA. When clamped from the holding voltage of -10 mV to a series of test voltages between +60 mV and -140 mV, the cell exhibits a large inward current. This inward current exhibits the typical steady state I/V relation and kinetic features of KAT1 (Figs. 1B,C).

To examine the effect of K^+_o on KAT1 conductance the bath medium with 20 mM K^+ was exchanged for a nominally K^+ free solution. Before, during and after removal of K^+_o (Fig. 2A) we determined KAT1 activity by analysis of the respective activation curves. Therefore cells were clamped as in Figure 1 from the holding voltage -10 mV to a series of test voltages in order to fully activate KAT1. From these voltages the membrane was stepped back to the common test volt-

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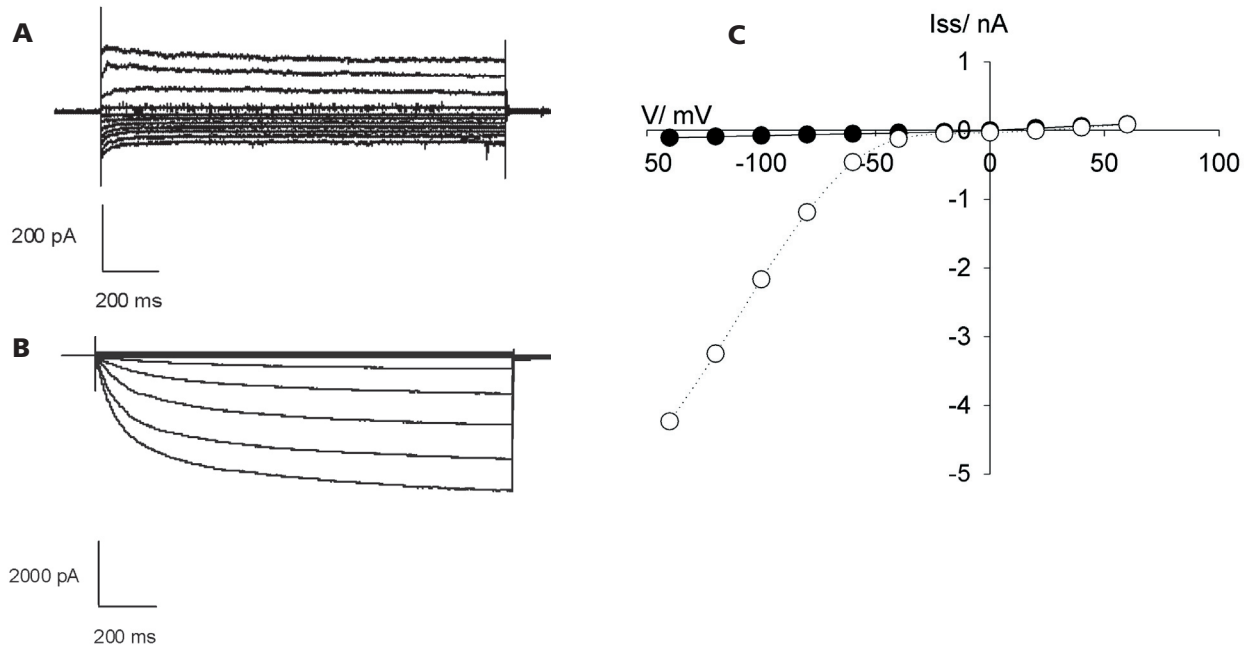

Figure 1. Current/voltage relations of HEK293 cells after a mock transfection (A) or transfection with KAT1 DNA (B). Current responses of cells in control bath medium with 20 mM KCl to standard voltage protocol (top: -10 mV holding voltage, test voltages between +60 and -140 mV in steps of 20 mV, -10 mV post voltage) were recorded in whole cell configuration. Steady state I_{ss}/V relations of currents collected at the end of the test puls as function of clamp voltage re shown for boh cellsin.

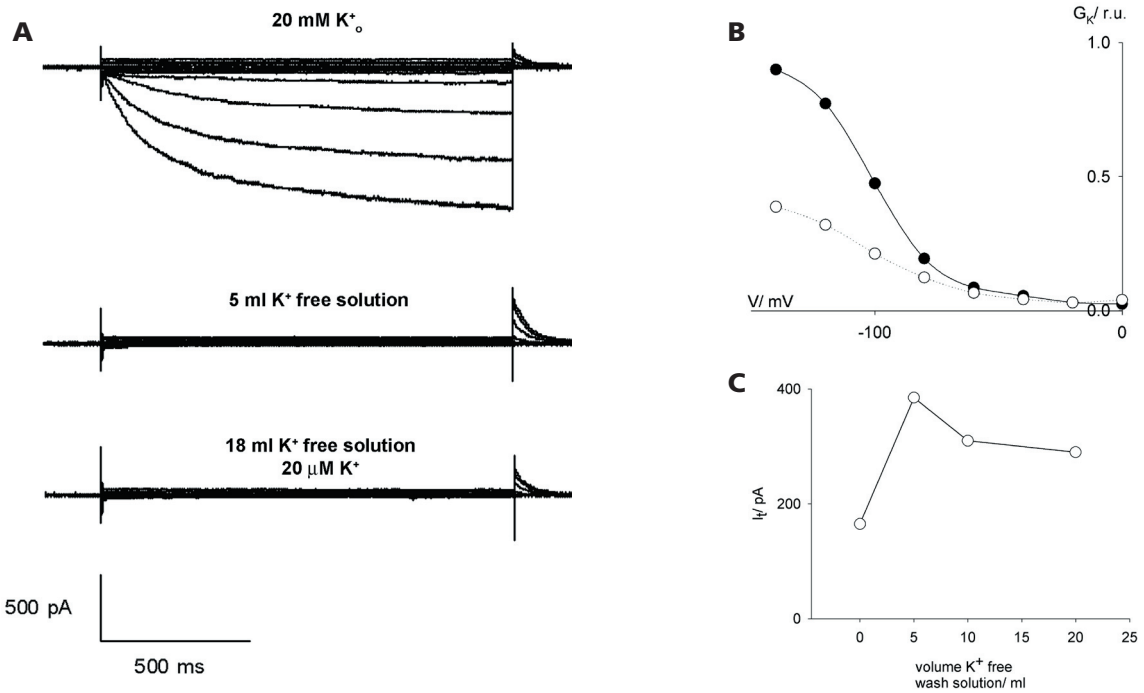

Figure 2. Removal of external K^+_o decreases KAT1 conductance. (A) Current responses of HEK293 cells expressing KAT1 to standard voltage protocol (see Fig. 1) in control medium (top, 20 mM K^+) and at different times after washing with K^+ free medium. The last I/V scan was recorded with an external concentration of 20 µM. (B) G_K/V relation obtained from tail currents in 20 mM (solid symbol) and 20 µM K^+_o (open symbol). (C) Development of tail current amplitude during removal of K^+ from bath medium.

age at –10 mV and the amplitude of the tail currents plotted against the conditioning voltage (Fig. 2B). For a quantitative comparison of the activation curves in different K^+_o the plot was expressed as cord conductance (G_K) according to the equation $G_K=I_t/(V-V_r)$, where I_t is the amplitude of the tail current, V the test voltage at which I_t is collected and V_r the reversal voltage of KAT1.

Washing the cells with a bath medium with nominally K^+ free solution resulted initially in an increase in the tail current amplitude (Fig. 2C). This increase was expected simply because of an increased thermodynamic driving force for K^+. Further dilution of K^+_o, however, resulted in a progressive decrease in tail currents (Fig. 2C). This decrease, which was observed in all experiments during removal of K^+ from the bath medium, clearly demonstrates a reduction in channel activity.

The present data now reveal that also KAT1, a typical Shaker like channel is sensitive to K^+_o.

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