Transient outward K^+ currents across the plasma membrane of laticifer from *Hevea brasiliensis*

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Abstract Non-inactivating outward rectifying K^+ channel currents have been identified in a variety of plant cell types and species. The present study of laticifer protoplasts from *Hevea brasiliensis*, cells which are specialized for stress response, has revealed, through a switch-clamp method, an outward rectifying current displaying rapid inactivation. The inactivation depended on the external K^+ concentration and on the voltage. This current inactivation appeared clearly different from all those previously described in plant cells and it shared homology with current kinetics of animal *Shaker* family channels. These results, given the recent cloning of plant K^+ channel β-subunits, shed new light on possible plant K^+ channel regulation.

Key words: Current inactivation; K^+ channel; Laticifer; *Hevea brasiliensis*

1. Introduction

Outward rectifying K^+ channel currents have been identified in a variety of cell types and plant species [1,2] including laticifer isolated from the ligneous part of young *Hevea* trees [3]. The most common properties of these outward rectifying channel currents are time- and voltage-dependent activation and in addition their lack of inactivation during continuous depolarization for many seconds [2]. Two outward rectifying channels were cloned from *Arabidopsis thaliana*: (i) the SKOR channel, a *Shaker*-like channel located in the stellar cells [4], (ii) the KCO1, a 'two pore' K^+ channel located in various tissues [5]. Recently, in addition to these outward rectifying channel currents, an instantaneous outward rectifying current [6] and an inactivating outward rectifying K^+ current [7,8] have been described in guard cells. In the present study, we describe a new transient outward rectifying K^+ current resembling that of animal A-type K^+ channels, with a possible transition between inactivating and non-inactivating states.

2. Materials and methods

2.1. Preparation of laticifer protoplasts

Young *Hevea brasiliensis* plants (clone RRIM 600, 2–3 years old) were grown in a greenhouse (>25°C, RH >80%). Protoplasts (Fig. 1) were prepared as previously described [9]: the cell walls were digested in a pH 5.5 standard solution (KCl, NaCl, $MgCl_2$, and $CaCl_2$, 100 μM each) containing 1% cellulysin and 0.2% macerase (both from Calbiochem). The average time for hydrolysis was 15 h at 28°C under constant agitation (60 osc/min). The protoplasts were washed three times in 5 ml of enzyme-free standard solution which was also used as bathing medium (BM) during all the experiments.

2.2. Electrophysiological measurements

All experiments were performed in a chamber (0.5 ml) perfused with BM, or BM containing different K^+ concentrations. Impalements were performed with borosilicate capillary glass (Clark GC 150F) micropipettes (resistance: 50–80 MΩ when filled with KCl 600 mM). As previously described [3] laticifer protoplasts were voltage-clamped using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA, USA) which allows the switch single electrode voltage clamp [10]. In this technique, both current passing and voltage recording use the same microelectrode. Interactions between the two tasks are prevented by multiplexing (commutation rate was adjusted to 3–5 kHz), which ensures that the voltage recorded by the microelectrode tip is sampled and saved only after the current-induced voltage drop across the microelectrode has decayed to a negligible value. Voltage and current were digitized with a personal computer fitted with a Labmaster TL-1 acquisition board. The electrometer was driven using pClamp software (pCLAMP5.5, Axon Instruments). Experiments were performed at 23 ± 2°C.

3. Results

Laticifers are hardly accessible to current electrophysiological techniques, since they are buried in the phloem tissue. The development of a procedure for isolating protoplasts from laticifer cells of *H. brasiliensis* has allowed direct access to their plasma membrane [9]. The single electrode voltage-clamp technique, a priori not appropriate to clamp a network of cells, could be used because in laticifers 30–50% of the cytosolic volume is filled with rubber particles [3]. Therefore, in laticifer protoplasts small cytosolic enclaves are isolated (Fig. 1). They are equivalent to reduced cellular volumes and so could be clamped. Recordings are impossible in the granulous part of the laticifer protoplast as the pipettes are plugged by rubber. The disadvantages inherent in this specialized cells allowed a narrow range of experimentation.

At low external KCl (0.1 mM), the mean polarization of laticifer protoplasts is around −34 mV [9]. Depending on protoplasts, two types of outward rectifying K^+ current were recorded at membrane potentials positive to +10 mV, one showing a full activation (Fig. 2A) as previously characterized [3] and the other showing a large inactivation for strong depolarization (Fig. 2B).

Fig. 3A shows that varying $[K]_o$ from 0.1 to 100 mM reduced the peak and the steady-state currents exhibited at +150 mV. The time to reach the peak current depends both on $[K]_o$ (Fig. 3B) and on voltage (Fig. 3C). The inactivation of this outward current could be fitted by a single exponential. The inactivation half-time was dependent on the voltage and also on $[K]_o$ (Table 1). The increase in $[K]_o$ reduced the percentage

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Abbreviations: BM, bathing medium; KOIC, K^+ outward inactivating current; $[K]_i$, cytosolic K^+ concentration; $[K]_o$, external K^+ concentration; V_h, holding potential; V_m, resting membrane potential

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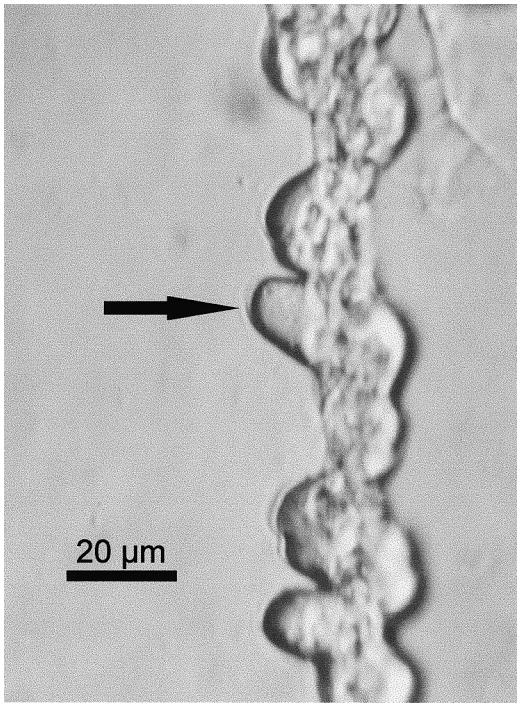

Fig. 1. Protoplast of laticifer vessel from *H. brasiliensis* isolated by enzymatic hydrolysis of the cell wall. The bulge (about 215 μm^2) indicated by the arrow corresponds to a cytosolic enclave, the rest of the laticifer appearing granulous.

of inactivation (Table 1) with a complete disappearance of the current inactivation in 100 mM $[K]_o$ (Fig. 3A). The resting membrane potential (V_m) and the activation potential of the outward current were shifted respectively from −10 mV to +18 mV and from +10 to +50 mV with increasing $[K]_o$, in the same direction as the K^+ equilibrium potential (not shown).

Coming back from 100 to 0.1 mM $[K]_o$ induced the hyperpolarization of the laticifer to about −30 mV and suppressed the inactivation of the outward current (compare Fig. 4A and B). The analysis of successive voltage pulses at +150 mV for the current recorded in BM after the increase of $[K]_o$ showed the reduction of the steady-state current due to the restoration of a slight inactivation for the last depolarizing pulses (inset of Fig. 4B). Both components of this current were reduced by the K^+ channel inhibitor Ba^{2+} (Fig. 4C).

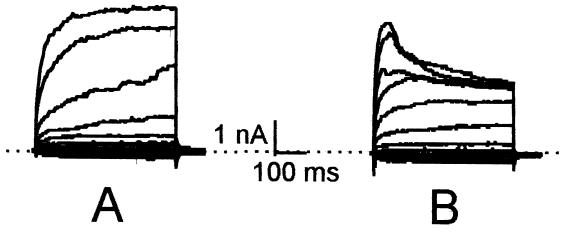

Fig. 2. Two types of profiles for outward rectifying current from different laticifer protoplasts bathed in BM ($[K]_o = 0.1$ mM). The voltage was held at V_m and then stepped in 20 mV increments to voltages from −90 mV to +150 mV. There was a resting phase of 500 ms between each 500 ms voltage pulse. A: Non-inactivating outward K^+ current (out of 16). B: Transient outward K^+ current (out of 3). Currents are shown after leak subtraction (G = 10 nS). Dashed line refers to zero current.

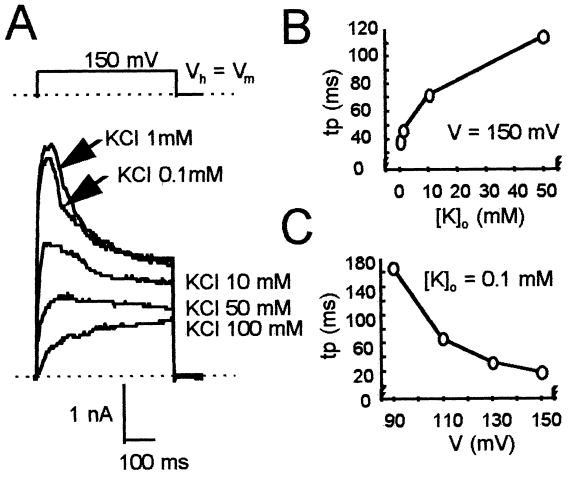

Fig. 3. Transient outward K^+ currents of laticifer protoplasts. A: Current as a function of $[K]_o$ from 0.1 to 100 mM. Currents are presented after leak subtraction. Dashed line refers to zero current. B: Time to reach the peak current (tp) as a function of $[K]_o$. C: Time to reach the peak current (tp) as a function of the imposed voltage.

4. Discussion

The laticifers display original anatomical and physiological characteristics related to their specialization, polyisoprene

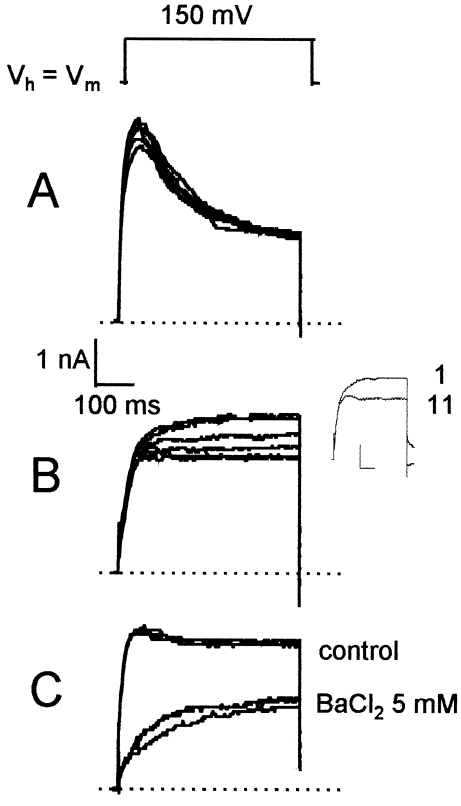

Fig. 4. Outward rectifying K^+ currents activated by 11 successive pulses at +150 mV in the bathing medium, (A) before and (B) after the increase of $[K]_o$ reported in Fig. 2 (only six out of 11 pulses of the protocol are shown). Inset in B: Steps 1 and 11 of the protocol: a slight inactivation reappears on the 11th pulse compared to the first one. C: Inhibition of the current activated by the three latest pulses by 5 mM $BaCl_2$. Currents are shown after leak subtraction. Dashed lines refer to zero current.

Table 1
Inactivation half-time and percentage of inactivation of the transient outward rectifying current from laticifer protoplasts as a function of $[K]_o$ and voltage

		$[K]_o$ (mM)			
		0.1	1	10	50
Inactivation half-time (ms)					
V (mV)	90	140			
	110	91	174		
	130	44	112	120	
	150	36	88	120	140
Inactivation (%)					
V (mV)	90	10			
	110	14	15		
	130	31	29	11	
	150	40	38	19	6

Percentages were calculated from $(I_{peak}-I_{steady\ state})/I_{peak}\times 100$.

(natural rubber) synthesis in response to a wounding stress [11]. Using the switch clamp technique which allowed us to keep the physiological cytosolic medium (composition not controlled), we had previously characterized a non-inactivating outward rectifying K^+ current (Fig. 2A) in laticifer protoplasts [3]. This current shows characteristics similar to those of outward rectifying K^+ channels already described in various plant cells [1,2] and considered by Stoeckel and Takeda [12] to be equivalent to the animal delayed rectifier outward K^+ channels.

About 15% of the laticifer protoplasts also exhibited an outward rectifying K^+ current showing a rapid, voltage-dependent inactivation (<500 ms, Figs. 2B and 4A) therefore named KOIC for $\underline{K}^+$ outward inactivating current. The KOIC differs from I_{AP} currents of guard cells [7] which need a hyperpolarizing pulse to be activated and show an inactivation time longer than 5 s [7,8]. Furthermore, the I_{AP} inactivation is poorly voltage-dependent [7], and the increase of $[K]_o$ decreased the KOIC amplitude in the opposite direction to what is observed for I_{AP}. KOIC also appeared different from the KCO1 current which could inactivate but only slightly and over 10 s [5], and from all the outward rectifying K^+ currents described in plant cells which never inactivate [1,2,4].

The KOIC inactivation (<500 ms) resembles that of some *Shaker*-type currents (30–1000 ms) largely described in animal cells [13] and particularly the one we observed for the Kv1.3 currents of Jurkat T cells (300 ms) [14], Kv1.3 inactivation is also voltage-dependent [15]. The inactivation of KOIC is sensitive to change of $[K]_o$, with a higher inactivation at low $[K]_o$ as observed for the C-type inactivation of *Shaker* channel currents [16]. The KOIC inactivations could also be explained by the 'ball and chain' model [17] which involves a 'ball' with a fixation site on the channel pore. The slowing down of the KOIC inactivation (until it disappears) after the $[K]_o$ increase favoring a K^+ uptake could be linked to an increase of $[K]_i$, as shown for the 'ball and chain' model [17]. However, the modification of the current kinetics is a phenomenon well studied in animal cells. The phosphorylation of the channel subunits could slow down the inactivation [18]. Interestingly, fast inactivation typical of an A-type current can be conferred on non-inactivating *Shaker*-related channels (delayed rectifier current) by association with β-subunits [19]. Recently, plant β-subunits close to the animal β-subunits were cloned [20,21], suggesting that such regulation could exist for plant K^+ channels. Thus, the KOIC regulation could be related to that of *Shaker* family currents. The role of the KOIC for *Hevea* laticifers, specialized for wounding response, could be to repolarize the membrane after a depolarizing wave, a well known phenomenon in plants [22,23]. Indeed this is the role of the A-type and delayed rectifier outward K^+ channels in animal cells [24]. Although these results remain scarce because of difficulties in working with this peculiar model, it has brought new insight into plant K^+ channel regulation.

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