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Stimulation of potassium uptake by abscisic acid in potato tuber tissues. Relation with H^+ and Ca^{2+} fluxes

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Abstract

Previous results with potato tuber discs showed that a treatment with abscisic acid stimulated K^+ uptake. In this investigation, we determine the relationship between increased K^+ uptake and H^+extrusion, and Ca^{2+} fluxes by treating tissues with specific Ca^{2+} channel blocker (La^{3+}), calmodulin (CaM) inhibitors (chlorpromazine and W_7), and with Ca^{2+} ionophore (A23187). K^+ uptake increased with increasing external pH whether tissues were treated with ABA or not. Treatment of tissues with La^{3+} inhibited K^+ uptake, whereas CaM inhibitors have no effect. By contrast ABA and A23187 produced a synergistic effect, suggesting that ABA may act in part, on K^+ uptake, like a Ca^{2+} agonist, in accord with Huddart's hypothesis.

Additional key words: calmodulin, H^+-ATPase, lanthanum, *Solanum tuberosum* L.

Introduction

It is well known that abscisic acid (ABA) plays an important role in the control of fluxes of nutrients (ions or sugars) (*e.g.* Van Steveninck 1972, Hartung *et al.* 1980, Saftner and Wyse 1984, Suleiman *et al.* 1991, Penot *et al.* 1991, Feray *et al.* 1992, Penot *et al.* 1993). However, the mode of ABA action at the cellular level remains unclear. It has been postulated that ABA could improve the membrane integrity and act on a H^+-pump coupled to K^+ transport (Van Steveninck and Van Steveninck 1983). It was suggested that ABA acts like a Ca^{2+} agonist (Elliott 1986, Huddard *et al.* 1986, Penot *et al.* 1991). It may change cytosolic Ca^{2+} concentration (by

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Abbreviations: ABA - abscisic acid, CaM - calmodulin, DCCD - N,N'-dicyclohexyl-carbodiimide, FAP - furfurylaminopurine, FC - fusicoccin, f.m. - fresh mass, IAA - indole-3-acetic acid, W_7 - N-(6-aminohexyl)-5-chloro-1-naphthalene-sulphonamide.
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opening of Ca^{2+}channel) and this may activate various enzymes (Evans *et al.* 1991, Roberts and Harmon 1992, for review see Chanson 1993).

The aim of this work was to examine the role of pH in the action of ABA on K^+ uptake and to determine whether ABA-stimulated K^+ uptake is linked to H^+ and Ca^{2+} fluxes.

Materials and methods

Potato tubers (*Solanum tuberosum* L. cv. Spunta) were prepared and treated with ABA as described previously (Reddahi *et al.* 1999). The absorption of ^{86}Rb was used as a marker of potassium uptake (for detail see Reddahi *et al.* 1999). The incubation medium contained 100 mM mannitol, 5 mM buffer MES (pH 6.0), 0.5 mM $CaSO_4$, 1 mM KCl and ^{86}Rb (4.6 kBq cm^{-3}).

For the effect of pH, the buffers used were 5 mM sodium citrate (pH 4), MES (pH 6) and HEPES (pH 8). The tissues were incubated for 3 h. H^+ fluxes were measured in discs of tuber tissues (6-mm diameter, 15 discs, fresh mass about 0.7 g) preincubated at 22 ± 2 °C in 50 cm^3 of the reference medium containing 100 mM mannitol, 0.5 mM $CaSO_4$, and 50 mg dm^{-3} chloramphenicol (initial pH 6.0 to 6.4) and shaken from time to time. After 1.5 h the discs were transfered to 20 cm^3 of the same medium and the pH of the constantly shaked solution (200 cycles min^{-1}) was monitored with the *PHM 61* laboratory pH-meter (*Radiometer*, Copenhagen, Denmark) for various times of ageing. The effectors tested were added to the reference medium and the pH was adjusted.

Results and discussion

Influence of external pH on potassium uptake: The increase of external pH stimulated K^+ uptake for both control and ABA-treated tissues (Table 1). This result could be explained by direct facilitating H^+ efflux and K^+ influx or by an increase in

Table 1. Effect of external pH on ^{86}Rb uptake [nmol g^{-1}(f.m.)] by potato tuber discs incubated for 3 h in the presence of 1 mM KCl. Measurements after 24 h pretreatment with or without 100 μM ABA. The buffers (5 mM) used were sodium citrate (pH 4), MES (pH 6) and HEPES (pH 8). Means ± SE, n = 3; percentage of control in brackets.

	pH 4	pH 6	pH 8
Control	59.1 ± 4.1	64.6 ± 3.8	88.9 ± 4.8
ABA 100 μM	79.0 ± 4.0 (133.7 %)	91.9 ± 4.6 (142.3 %)	123.1 ± 12.1 (138.5 %)

plasmalemma H^+/K^+ ATPase activity (Leonard and Hodges 1973). According to Lin (1979) changes in K^+ uptake with pH are balanced by the change in H^+ efflux, therefore no net charge is accumulated and consequently membrane electrical

potential is not altered; this further supports the involvement of an exchanging mechanism for K^+ uptake. Furthermore ABA stimulated uptake at all used pH values (Table 1).

Relation with calcium flux and with calmodulin: After 24 h of ageing, a 3 h treatment of tissues with lanthanum (calcium channel blocker) inhibited K^+ uptake, whether tissues were treated with ABA or not, whereas W_7 or chlorpromazine (selective inhibitors of calmoduline-regulated functions) showed no effect (Table 2). This insensitivity to calmodulin inhibitors provides further evidence that a Ca^{2+}-ATPase, which is stimulated by calmodulin (Williams *et al.* 1990, Evans *et al.* 1991, Askerlund and Evans 1992, Rasi-Caldogno *et al.* 1992, Chanson 1993, Hwang *et al.* 1997) does not contribute directly to the K^+ uptake.

Table 2. Effect of specific inhibitors on ^{86}Rb uptake [nmol g^{-1}(f.m.)] by potato tuber discs. The tissues were pretreated for 24 h or not with ABA (100 μM) and incubated for 3 h. The inhibitors were added to the uptake medium. Means ± SE, n = 3; percentage of uptake without inhibitors in brackets.

Inhibitor	Control	100 μM ABA
0	82.4 ± 2.0 (100 %)	111.6 ± 4.3 (100 %)
200 mM lanthanum	73.2 ± 4.2 (88.8 %)	80.5 ± 4.2 (72.2 %)
200 mM chlorpromazine	81.3 ± 4.8 (98.7 %)	106.4 ± 6.6 (95.3 %)
100 mM W_7	77.2 ± 4.2 (93.7 %)	119.3 ± 9.4 (106.9 %)
40 mM A23187	107.2 ± 9.3 (130.1 %)	134.5 ± 15.5 (120.6 %)

Concerning A23187, this Ca^{2+} ionophore which was shown to activate the Ca^{2+}-ATPase and to completely release accumulated Ca^{2+} (Askerlund and Evans 1992) stimulated K^+ uptake by 30 % in control discs. This stimulation reached 63 % when the tissues were pretreated for 24 h with ABA (Table 2). These results suggest a coupling between K^+ entry and Ca^{2+} fluxes and plaid in favour of the hypothesis that ABA may functions as a Ca^{2+} agonist (Huddart *et al.* 1986).

Protons fluxes measurements: During a 24 h ageing period, the pH increased irregularly from 6.0 (initial pH value) to about 7.5 ± 0.4 (Figs. 1, 2, Table 3). Moreover, the tissues adjust the pH of their incubation medium to this value independently of the initial pH value (result not shown). Such alkalinisation has been shown in other material (*e.g.* Rubinstein *et al.* 1992).

According to Van Steveninck (1978) the tissues acquire the ability to acidify their incubation medium if the ageing is pursued a long time (> 40 h). In our material, the pH remains constant after 24 h of ageing, and not any acidification was observed even if ageing is pursued a long time (Fig. 1, Table 3). Moreover, except its sensitivity to low temperature, the alkanisation observed does not change for any other treatment during this period (Fig. 1, Table 3). Lin and Hanson (1974) found that net H^+ efflux decreased during ageing and attributed this result to the action of

antiport carriers. In the present case the alkalinisation observed, linked likely to ageing process, seem to be independent of the H^+ driving force as shown by its insensitivity to inhibitors (Table 3), however, the absence of the KCN effect may be explained by the development in the course of ageing of the cyanide-insensitive respiration (Solomos 1977).

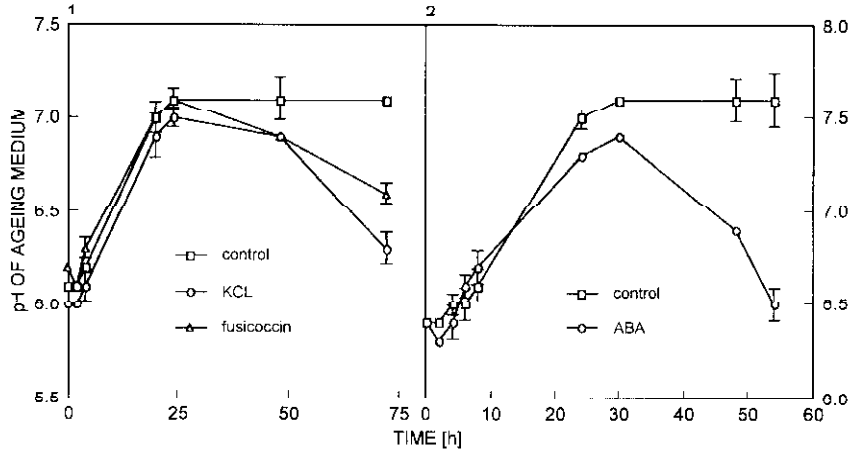

Fig. 1. Changes in pH of the medium in the course of potato discs ageing: Influence of 10 mM KCl and 10 μM fusicoccin (FC).

Fig. 2. Influence of 20 μM ABA on the changes in pH of ageing medium.

Table 3. Action of low temperature (4 °C), inhibitors (KCN and DCCD) and hormones (IAA and FAP) on the changes in pH of ageing medium.

Treatment	Duration of ageing [h]			
	0	24	30	48
0	6.4	7.5 ± 0.06	7.6 ± 0.03	7.6 ± 0.11
4 °C	6.0	6.1 ± 0.09	-	-
1 mM KCN	6.4	8.0 ± 0.11	8.0 ± 0.09	7.9 ± 0.03
100 μM DCCD	6.4	6.9 ± 0.17	7.2 ± 0.09	7.6 ± 0.23
10 μM IAA	6.0	7.3 ± 0.14	-	-
44 μM FAP	6.0	7.5 ± 0.20	-	-

Nevertheless, the capacity of the tissues to acidify their incubation medium was restored after 24 h of ageing by the presence of K^+ in the medium (Fig. 1), as shown in other isolated plant parts (Cocucci *et al.* 1983, Yan *et al.* 1992). This corresponds likely to the establishment of a H^+-pump, thereby it was not the H^+-pumping that limits the net H^+ release. It is possible that the alkalinisation observed is due to the reentry of H^+ back into the cells, this reentry is likely important for the regulation of the intracellular pH, as suggested in maize root by Zimmermann *et al.* (1983)

The dependence of active H^+ extrusion on K^+ uptake appeared evident when H^+ extrusion and K^+ uptake are stimulated by either FC, which is known to stimulates

H^+ extrusion and monovalents cations uptake and hyperpolarizes the plasma membrane (Marrè 1977, Bellando *et al.* 1979) or by ABA. Indeed, FC and ABA promoted after 24 h a net H^+ release by tissue (Figs. 1, 2) even in the abscence of K^+ and increased K^+ uptake, especially by aged tissues (Tables 1, 2). Such a positive effect of ABA on H^+ extrusion was obtained in broad bean by Yan *et al.* (1992).

These results together suggested a regulation of H^+ extrusion by a coupling with the K^+ influx. However, the difference in the responses of K^+ and H^+ fluxes to the ageing, ABA, FC and other effectors suggests that different pathways are involved in the transport of these two ions. The coupling between K^+ influx and H^+ efflux would be rather electric than chemical. The effect of K^+ on H^+ extrusion seem to be correlated with the K^+ uptake rate and depolarization of the plasma membrane (Bellando *et al.* 1979, Cocucci *et al.* 1983, Marrè *et al.* 1974). Conversely the stimulation of the H^+-pump by FC or ABA would hyperpolarize the plasma membrane and thus increase the driving force for K^+ uptake. A similar observation was reported by Pitman *et al.* (1975), Marrè *et al.* (1975), Bellando *et al.* (1979), and Cho and Komor (1980).

This indirect coupling can be explained by the K^+ concentration retained in our experimental conditions. Indeed it has been shown that beyond 10^{-4} M a passive entry of K^+ contributes to its total uptake (Cheesemann *et al.* 1980). The responses of K^+ uptake to metabolic inhibitors is a additional argument in favour of the existence of a passive influx of K^+.

In the conclusion, the hypothesis of a coupling between K^+ and Ca^{2+} and H^+ could be retained in order to explain the stimulation of K^+ uptake by ABA, however, detailed explanation of the mechanisms of ABA-Ca^{2+} synergism requires further studies.

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