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Influence of Abscisic Acid on K^+ Absorption by Leaf Tissues of *Solanum tuberosum* L. (cv. Bintje). Relation with Calcium Fluxes and with Calmodulin

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Abstract. Previous results with potato leaf tissues revealed that a treatment with ABA (10^{-4} M) induced an increase of K^+ uptake (Suleiman *et al.*, 1990a).

In this study, we investigate the relationship between increased K^+ uptake, Ca^{2+} fluxes and calmodulin by treating potato leaf discs with Ca^{2+} channel blockers (La^{3+}, verapamil and nifedipine) and with calmodulin inhibitors (chlorpromazine, W_7: N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide, and compound 4880).

We found: a) all these inhibitors decreased K^+ uptake in the same ratio in tissues treated or not with ABA; b) a synergistic effect between ABA and the Ca^{2+} ionophore A 23187 on K^+ uptake.

The results suggest that the hormone may act on K^+ uptake like a Ca^{2+} agonist, in accord with HUDDART's hypothesis.

In a previous paper we reported that treatment of foliar tissue of *Solanum tuberosum* with abscisic acid (ABA) stimulated K^+ uptake (Suleiman *et al.* 1990b). By using metabolic inhibitors, it appeared that stimulation was due both to an increase in active and passive components.

Elliott (1986) has suggested that in some circumstances hormonal action could involve calmodulin (CaM) and a participation of second messengers especially Ca^{2+}.

Recently, we proposed (Suleiman *et al.*, 1988) a scheme (Fig. 1) based upon data from Elliott (1986), Rincon and Hanson (1986) and Roux *et al.* (1986) to explain the positive effect of ABA on K^+ uptake.

According to this hypothesis, ABA acts like a Ca^{2+} agonist (Huddart *et al.* 1986); a change in cytosolic free $[Ca^{2+}]$ will activate diverse protein-kinases, $(Mg^{2+} + Ca^{2+})$

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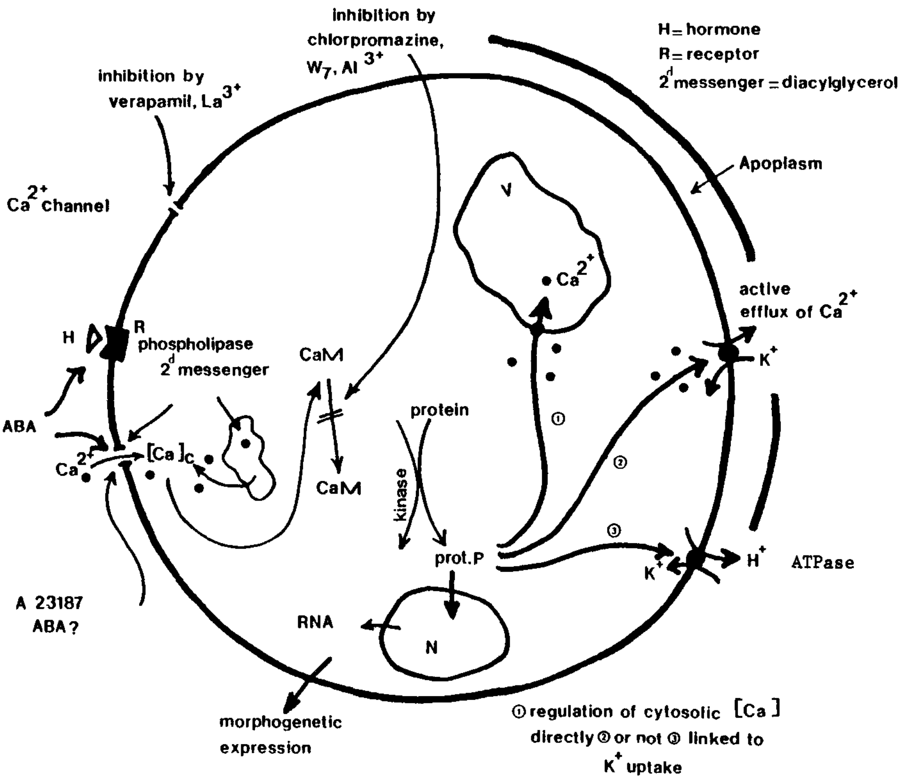

Fig. 1. Scheme to illustrate the effect of ABA on K^+ uptake through an action on Ca^{2+} fluxes and calmodulin (CaM). V : vacuole ; N : nucleus. Adapted from Roux 1986, Elliott 1986, Hanson *et al.* 1986 Suleiman *et al.* 1988.

ATPases (Roux and Slocum 1982) whose initial function would be to regulate endogenous [Ca^{2+}] either by an active efflux to the apoplast or by a sequestration into intracellular compartments.

This hypothesis also takes into account the fact that Ca^{2+} fluxes may be, directly or not, associated with a K^+ balancing flux to maintain electroneutrality (Hanson *et al.* 1986).

The aim of this work was to assemble arguments in favour of this hypothesis. Most of the data showing such a relationship between hormones and calmodulin (CaM) are mainly based upon the use of chemicals which are specific Ca^{2+} channel blockers, selective inhibitors of CaM-regulated functions or are Ca^{2+} ionophores which increase Ca^{2+} fluxes.

Abbreviations: ABA, abscisic acid ; CaM : calmodulin ; DCCD : N,N'-dicyclohexylcarbodiimide ; MES : morpholinopropane sulfonic acid ; W_7 : N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide ; TEA : tetraethylammonium chloride.

The data seem to support the initial hypothesis and might explain the effect of ABA on K^+ uptake in relation to CaM and Ca^{2+} fluxes.

MATERIAL AND METHODS

Plant Material

Potato tubers (*Solanum tuberosum* L. cv. Bintje) from plants grown in the field were provided by the INDRA station of Ploudaniel (Finistère, France). After harvest (in September) tubers were stored at 4 °C in a ventilated room. Antisprouting agents were not applied.

Leaves were obtained from plants grown in vermiculite in growth chambers, irrigated with a Coïc solution (Coïc *et al.* 1961). A photoperiod of 16 h was provided and the irradiance was 25 W m^{-2} (Sylvania Grolux lamps). The average day and night temperatures were 22 and 18 °C.

To measure the effect of abscisic acid (ABA) on cell permeability, discs 10 mm in diameter cut from the leaf blades outside the main vein were used. Incubation with ABA (10^{-4} M) was always carried out for 24 h in the dark at 24 °C. After washing, the discs were used for uptake experiments: K^+ uptake by leaf discs is not induced by any wounding effect (Suleiman *et al.* 1990a).

Uptake

^{86}Rb as a marker for K^+ and ^{45}Ca (IRE, Fleurs, Belgique) were used. The incubation medium contained: ^{86}Rb ($1.85\ 10^5$ Bq l^{-1}) or ^{45}Ca ($3.7\ 10^5$ Bq l^{-1}), 1 mM MES, 1 mM KCl or 0.5 mM $CaCl_2$ at pH 6.0. The uptake was performed at 23 °C. After the appropriate period, discs were collected and washed for 5 min with cold 1 mM KCl or cold distilled water (2 °C). The amount of radioactivity taken up by the discs was measured with a Geiger-Müller counter (Philips PW 4003).

Number of Experiments

Each value is the average of 12 discs and each experiment was performed three or more times.

Inhibitors

Three kinds of inhibitors were used in order to demonstrate the relationship between K^+ uptake, Ca^{2+} uptake and calmodulin involvement.

Calcium channel blockers. Calcium channel blockers have been used in plants to block calcium mediated processes. Ca^{2+} channels can be blocked either by some inorganic ions such as La^{3+} or by several classes of drugs including verapamil or nifedipine (Poovaiah 1987). Lanthanides are thought to remain outside cells and block the entry of Ca^{2+} by occupying Ca^{2+} binding sites on the plasma membrane (Mikkelsen 1976).

Calmodulin Inhibitors. Involvement of CaM is suggested by the use of antagonists which inhibit its action. Many of them are neuroleptic drugs or local anaesthesic including phenothiazines (chlorpromazine) or naphthalene sulfonamide derivates. W_7: N: N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide hydrochloride) and chlorpromazine bind at two high affinity sites on CaM in the presence of saturating Ca^{2+} levels.

Compound 48/80 is a mixture of oligomers resulting from a condensation of N-methyl-p-methoxyphenetylamine with formaldehyde. This chemical, which is quite soluble in water, is the most hydrophylic CaM inhibitor available. By binding to CaM, it will affect the CaM-dependent Ca^{2+} transport ATPase (De Silva *et al.* 1985).

Calcium Ionophore. A 23187 is a low molecular weight lipophilic antibiotic, which translocates Ca^{2+} from an aqueous phase, across a membrane, into another aqueous compartment. The effect of A 23187 is due to mobilization of Ca^{2+} from the apoplast followed by transport into the cytoplasm. This ionophore does not lyse membrane (see De Silva *et al.* 1985; Cheverry *et al.* 1988; and references).

All these compounds were purchased from Sigma: inhibitors were present during absorption period (except for A 23183 present during a pretreatment and absorption phases).

RESULTS

1) Action of Ca^{2+} channel blockers

Lanthanum, verapamil and nifedipine were used. Preliminary experiments were done to check that verapamil (1 mM) and nifedipine (100 µM) produced an

TABLE 1

Effect of Ca^{2+} channel blockers : verapamil (1 mM), nifedipine (100 µM) and lanthanum (200 µM) on ^{86}Rb (KCl, 1 mM) uptake. Measurements after a 24 h treatment with or without ABA (10^{-4} M). Results expressed in nmol h^{-1} $disc^{-1}$ ± SE and in percent of controls (C and ABA)

	0	Verapamil	Nifedipine	Lanthanum
C	8.41 ± 0.25 (100 %)	3.21 ± 0.24 (38 %)	4.80 ± 0.27 (57 %)	3.67 ± 0.14 (44 %)
ABA	11.94 ± 0.16 (100 %)	4.54 ± 0.44 (38 %)	7.88 ± 0.32 (66 %)	5.25 ± 0.18 (44 %)

inhibition of $^{45}CaCl_2$ (0.5 mM) uptake; after a 3 h absorption, the inhibition was about 68 %.

Table 1 shows that treatment of the tissue with ABA increased the rate of K uptake by 42 %, in accord with previous results (Suleiman *et al.* 1990b) and that all channel blockers used descreased K^+ uptake; the inhibition was in the same ratio for control and ABA treated discs (except for nifedipine).

2) Effect of calmodulin inhibitors

Chlorpromazine, W_7 and the highly specific inhibitor of CaM-dependent enzyme activities (compound 48/80 (Gietzen *et al.* 1983) were assayed. These 3 inhibitors produced identical inhibitions for control and ABA treated discs which were respectively 79–76 % for W_7, 70–66 % for chlorpromazine and 34–38 % for 48/80 (Table 2).

3) Action of a Ca^{2+} ionophore: A 23187

When A 23187 (14 μM) was added to the uptake medium, it had no effect on K^+

TABLE 2

Action of calmodulin inhibitors : chlorpromazine (200 μM), W_7 (100 μM) and compound 48/80 (50 mg l^{-1}) on ^{86}Rb (KCl, 1 mM) uptake. Other conditions as stated in Table 1

	0	Chlorpromazine	W_7	48/80
C	8.80 ± 0.35 (100 %)	2.64 ± 0.25 (30 %)	1.85 ± 0.35 (21 %)	5.80 ± 0.38 (66 %)
ABA	12.31 ± 0.49 (100 %)	4.18 ± 0.23 (34 %)	2.95 ± 0.26 (24 %)	7.63 ± 0.29 (62 %)

TABLE 3

Effect of the ionophore A 23187 (14 μM) on ^{86}Rb (KCl, 1mM) uptake. Pretreatment : discs pretreated for 3 h in A 23187. The ionophore was present in the uptake media. Results expressed in nmol h^{-1} $disc^{-1}$ ± SE and in percent of the control (C)

	0	A 23187	Pret. A 23187
C	9.13 ± 0.29 (100 %)	9.40 ± 0.38 (103 %)	10.32 ± 0.37 (113 %)
ABA	12.32 ± 0.45 (135 %)	14.1 ± 0.41 (154 %)	17.42 ± 0.42 (191 %)

uptake by control tissues (Table 3). By contrast, when tissues were pretreated with ABA, a synergistic effect was observed between this ionophore and ABA (+ 54 % of stimulation versus 35 % for ABA alone).

The lack of effect for the control led us to conduct additional experiments. In a first assay, conditions were identical to the former experiment but $CaCl_2$ (0.5 mM) was added to the uptake medium. Indeed, it could be supposed that the lack of effect of A23187 was due to an insufficient calcium supply, though the apoplastic reserve of Ca^{2+} (10^{-3} M) (Poovaiah and Reddy 1987) is adequate to satisfy cell needs. Results were identical to those of the previous experiment.

In a second set of experiments tissues were pretreated (3h) with A 23187 and this ionophore was also present during the uptake period. Table 3 shows that A 23187 slightly stimulated K^+ uptake by control discs (+ 13 %) and amplified the stimulation induced by ABA (91 % versus 35 %).

This result was rather unexpected but not contrary to our hypothesis and led us to check the effect of A 23187 (and of ABA) on calcium ($CaCl_2$, 0.5 mM) uptake. As shown in Table 4, when used separately, A 23187 and ABA did not modify Ca^{2+} absorption but added together produced a clear stimulation of the uptake.

TABLE 4

Action of the ionophore A 23187 (40 μM), present in the uptake medium, on ^{45}Ca ($CaCl_2$ 0.5 mM) absorption by discs pretreated or not with ABA (10^{-4} M). Results expressed in nmol $(3\ h)^{-1}$ $disc^{-1}$ ± SE and in percent of the control (C)

	0	A 23187
C	15.34 ± 0.46 (100 %)	17.05 ± 0.75 (111 %)
ABA	15.79 ± 0.55 (103 %)	19.73 ± 0.69 (129 %)

DISCUSSION

In a previous paper, we reported that pretreatment of potato leaf tissue with ABA induced a stimulation of the absorption of K^+ (Suleiman *et al.* 1990b). The objectives of the present work were twofold. First to determine the relationship between K^+ uptake and fluxes of Ca^{2+} and, second to determine whether ABA-stimulated K^+ uptake is linked to Ca^{2+} fluxes, and to implication of CaM.

Up to now only a few reports have shown a positive effect of ABA on ion or on sugar uptake (Van Steveninck 1975, Hartung *et al.* 1980, Saftner and Wyse 1984).

The mechanism of action at the cellular level remains obscure. It has been suggested that ABA could improve the membrane integrity and act on a H^+ pump coupled to K^+ transport (Van Steveninck and Van Steveninck 1983). De Silva *et al.* (1985, 1986), provided new suggections concerning the mode of action of ABA on stomata. According to these authors, closure of stomata that is said to result from an active efflux of K^+ (Mac Robbie 1982), would result from increased Ca^{2+} entry. Then, Ca^{2+} would act as a secondary messenger binding to the cytosolic protein calmodulin. Guard cells will react to this transitory variation of $[Ca^{2+}]$, the free $[Ca^{2+}]$ in the cytosol being held rather low. Then, many diverse physiological processes will be triggered by enzymes which are stimulated by the binding of Ca to CaM (Roux and Slocum 1982, Poovaiah and Reddy 1987, Owen 1988).

Concerning stomata, ABA could act like a Ca^{2+} ionophore. This idea is supported by the use of specific Ca^{2+} blockers, CaM inhibitors, Ca^{2+} chelators (EGTA) or Ca^{2+} ionophores. This hypothesis has been extended to different mammalian and plant systems (Huddart *et el.* 1986). The hypothesis of a relationship between K^+ and Ca^{2+} was retained in order to explain the stimulation of K^+ uptake by ABA, by using the same chemicals (see Fig. 1).

Our experiments deal with whole tissues and not only stomata.

Every step of this hypothesis was verified. Indeed the various Ca^{2+} channel blockers (La^{3+}, verapamil, nifedipine) decreased K^+ uptake whether tissues were treated with ABA or not. It should be noted that the inhibition occurred in the same ratio for ABA treated discs as for the controls, although a smaller effect was observed with nifedipine (Table 1).

The coupling between K^+ entry and Ca^{2+} fluxes was also confirmed when A 23187 was used as Ca^{2+} ionophore. Although A 23187 has only a small effect when added to the uptake medium for K^+ and Ca^{2+} (Tables 3 and 4) it was seen that A 23187 and ABA produced a synergistic effect for K^+ and Ca^{2+} uptake. This confirms the hypothesis of Huddart *et al.* (1986) that ABA may function as a Ca^{2+} agonist.

The inhibitory effect on K^+ uptake of drugs commonly used to control the binding of Ca to CaM and CaM-dependent processes raised the possibility of a relationship between CaM and K^+ uptake (Table 2). These results are in agreement with Jacoby and Rudich's data (1987) concerning Na^+, K^+ and Cl^- uptake by roots.

A direct Ca^{2+}/K^+ exchange has not been shown because of difficulties in determining free $[Ca^{2+}]$ in the cytosol. Such a coupling has been found by Hanson *et al.* (1986) in experiments where cold shocks produce a leakage of K^+ and a stimulation of Ca^{2+} uptake consecutive to channel opening. Such an opening might be regulated by a binding of ABA to a membrane protein as suggested by Satter and Moran (1988).

The molecular basis for the ABA-Ca^{2+} synergism remains unknown (De Silva *et al.* 1985). The mode of action of ABA with regard to increased K^+ uptake is also unknown (Table 1). Does the observed stimulation result from an action on membrane ATPases as indicated in our scheme (Fig. 1) or does it occur through a change in opening or number of K^+ channels? Nothing allows us to favour one of these possibilities except the fact that ATPases seem to be implicated in K^+ uptake. Indeed, DCCD and orthovanadate, specific plasmalemma ATPase inhibitors, were shown to reduce K^+ uptake by leaf tissue (Suleiman *et al.* 1990b). However, the involvement of K^+ channels cannot be ruled out. The data of Table 5 show that tetraethylammonium chloride (TEA), a K^+ channel blocker (Okhawa *et al.* 1986) inhibited K^+ uptake. This raises the possibility that hormones (here ABA) could act on the configuration and number of K^+ channels as proposed by Satter and Moran (1988).

TABLE 5

TEA sensitivity of ^{86}Rb (KCl, 1 mM) uptake into leaf tissue pretreated or not with ABA (10^{-4} M). Other conditions as in table 1

	TEA, mM			
	0	1	2.5	5
C	7.68 ± 0.39 (100 %)	4.59 ± 0.11 (60 %)	3.53 ± 0.12 (46 %)	3.43 ± 0.11 (45 %)
ABA	11.10 ± 0.57 (100 %)	5.79 ± 0.13 (52 %)	4.80 ± 0.13 (43 %)	4.55 ± 0.12 (41 %)

The stimulation of K^+ uptake by ABA may be explained according to the proposed scheme (Fig. 1). Work in progress indicates that ABA acts on cellular metabolism; indeed, in the same experimental conditions, ABA increased the oxygen consumption and the ATP content: this could explain the involvement of ATPase in the entry of K^+.

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This book, edited within the series of monographs "Quantum Electronics – Principles and Applications" (ed. by P. L. Liao and P. Kelley), is a new revised and extended edition of the equally titled publication from 1982. The book appears at the time when the field of nonlinear laser spectroscopy has sufficiently matured and thus a danger of rapid obsolescence of this book is small. The book offers comprehensive overview of recently developed methods and techniques of nonlinear laser spectroscopy. It belongs to those publications that facilitate the understanding of both the general theoretical principles and practical applications. Theoretical explantation of nonlinear events continues by the description of measuring methods, possible application and examples of measurement. The presentation of the nonlinear Spectroscopy techniques is unified at a level accessible to graduate students and spectroscopists unfamiliar with nonlinear optics. However, some familiarity with laser physics and quantum mechanics are necessary before reading this book.

The book reviewed consists of seven chapters. The first chapter shortly explains principles of tunable lasers and summarizes nonlinear phenomena. The second chapter gives a general theoretical basement for following more special explanations. Chapter 3 describes experimental methods of saturation spectroscopy in gases and condensed phase and explains principles of modulation transfer, dynamic grating and multiphoton and double resonance saturation techniques. Chapter 4 deals with coherent Raman spectroscopy, the powerful method for the characterization of structure and dynamic features of molecules. It describes techniques of measurement of Raman spectra in Stokes and anti-Stokes regions and principles of the Raman-induced Kerr effect spectroscopy, stimulated Raman gain and loss spectroscopy and wave mixing techniques. The next two chapters are concerned with multiphoton absorption and ionization techniques and optical coherent transients. The last chapter dealing with nonlinear sources describes the principles of *n*-order sum and harmonic generation, XUV sources, Raman shifting and infrared sources.

The book is well edited and printed on good paper. The text is accompanied by many figures or schemes (118) and tables (8). The clear explanation of the thema, included problem sets with expected answers and abundant references make this book suitable to be the starting literature for anyone who intends to study the field of nonlinear spectroscopy. It will also be a valuable source of recently developed methods and techniques to researchers working in the area of photosynthesis, particularly in primary photochemistry or plant biochemistry.

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