RAPID STIMULATION OF K^+-H^+ EXCHANGE BY A PLANT GROWTH HORMONE

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Summary: The growth-promoting phytotoxin fusicoccin[1] stimulates both [$^{86}Rb^+$]-K^+ uptake and H^+-excretion from oat coleoptiles by at least 5-fold after a lag of less than 90 seconds. Both processes are affected similarly by metabolic inhibitors and external pH. FC appears to activate a K^+-H^+ exchange which is only partly specific for K^+, and which can transport more H^+ than K^+. The natural plant growth hormone indoleacetic acid[1] also stimulates K^+-uptake, but only after a long lag, and to a maximum of 30%, suggesting that IAA does not affect directly the K^+-H^+ exchange process, and that the two hormones induce H^+-excretion, and thus cell elongation, by different mechanisms.

INTRODUCTION

The natural plant growth hormone, indoleacetic acid (IAA) and the phytotoxin fusicoccin (FC) induce rapid cell elongation in oat coleoptile (1-3) and pea stem tissues (3-5). Both hormones also cause these tissues to excrete protons (2, 5-8) which are believed to induce cell elongation by lowering the pH of the wall solution (5, 9-11) and thus activating some cell wall degrading enzyme (12).

Two mechanisms have been suggested to explain the action of these hormones. The hormones may activate a plasma membrane-associated ATPase, resulting in the electrogenic pumping of protons across this membrane (11, 13). This idea is supported by the hyperpolarization of the membrane potential induced by IAA (14, 15) or FC (12, 15, 16). Alternatively these hormones might activate an electroneutral K^+-H^+ antiport (17, 18). The fact that IAA and FC can stimulate equal amounts of H^+-excretion and K^+ uptake over periods of 3-24 hours is consistent with this hypothesis (18-21). To distinguish between these two mechanisms information is needed concerning the amounts of hormone-induced H^+-excretion

Abbreviations: FC (fusicoccin); IAA (indoleacetic acid); CCCP (carbonyl cyanide m-chlorophenylhydrazone); CHI (cycloheximide); DNP (dinitrophenol).

and K^+ uptake, and the lags between addition of the hormones and the start of the responses.

FC and IAA stimulate rapid H^+-excretion from oat coleoptile tissues after lags averaging 1 and 12 minutes respectively (22). The first objective of this study was to determine whether these hormones induced K^+-uptake after similar lags and to similar extents, as required by the K^+-H^+ antiport mechanism. The second objective was to measure the kinetics of FC-induced K^+-uptake to see if it is consistent with the hyperpolarization mechanism.

MATERIALS AND METHODS

The experimental material consisted of deleafed 10-mm sections cut from 25-32 mm coleoptiles of Avena sativa L., cv. Victory (1). In order to compare these results with those of the earlier H^+-excretion studies (2, 6-8) the sections were peeled by removal of the epidermal layer with fine forceps, and were then incubated one hour in water. Unless otherwise indicated, sections were incubated in 1 mM MES-tris buffer, pH 6.0, containing 1 mM K^+ (Cl^- or SO_4^{2+}), $\pm$ 1 mM Ca^{2+} and with [$^{86}Rb^+$]Cl (0.3-3 µc/ml, 36 µMoles/mC) as tracer for the K^+. Optimal levels of IAA and FC (both 10 µM) were added as indicated. After the desired incubation period the sections were quickly rinsed with water, the liquid in the leaf cavity was removed with a stream of air and the sections were given a 3 or 10 minute chase in 4 ml of cold 10 mM KCl. The sections were placed in scintillation vials with a naphthalene-dioxane counting mixture and the radioactivity was measured. H^+-excretion was determined as described earlier (6,8). All experiments were repeated a minimum of three times.

RESULTS AND DISCUSSION

The effect of FC and IAA on [$^{86}Rb^+$]-K^+ uptake is shown in Fig. 1. IAA enhances K^+ uptake by a maximum of 30%, and only after a lag that normally exceeds 30 minutes. Since the effect of IAA on K^+-uptake is considerably less and slower than its effect on proton excretion (8,22), it seems unlikely that IAA is directly activating a K^+-H^+ antiport. The slight stimulation of K^+-uptake may, instead, be in response to the IAA-induced hyperpolarization of the membrane potential (14, 15).

FC, on the other hand, stimulates K^+-uptake by over 5-fold, and within 2 hours the sections have taken up over 25% of the [$^{86}Rb^+$] from the incubation medium. The stimulation of uptake is detectable within 90 seconds after addition of the FC (Fig. 2). The effects of FC on both K^+-uptake (Fig. 2) and

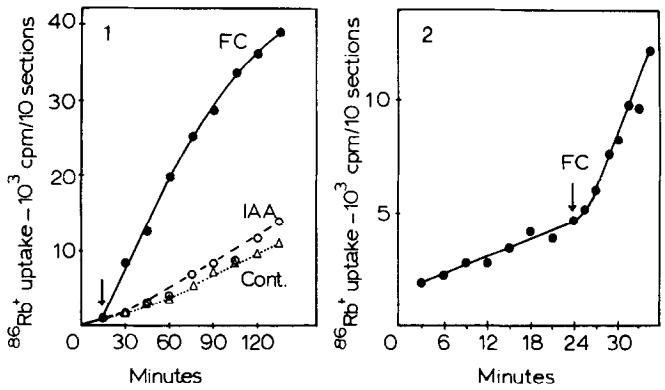

Fig. 1. Effect of FC and IAA on [^{86}Rb]-K^+ uptake into oat coleoptiles. Peeled sections incubated in 5 ml of MES-tris buffer, pH 6.0 + 1 mM $CaSO_4$ and 0.5 mM K_2SO_4, + 2.1 μc[^{86}Rb]Cl. At arrow 10 μM IAA or FC added, and groups of 10 sections were removed every 15 minutes, given a 10 minutes chase in cold 10 mM KCl, and counted.

Fig. 2. Measurement of the lag prior to the start of FC-induced [$^{86}Rb^+$]-K^+ uptake. Conditions same as in Fig. 1 except the solutions contained 1 mM KCl instead of K_2SO_4 + $CaSO_4$. FC (10 μM) added at arrow.

Table 1: Effect of inhibitors on FC-induced [$^{86}Rb^+$]-K^+ uptake and H^+ excretion.

Inhibitor	[$^{86}Rb^+$]uptake, +FC cpm/hr/10 sections	FC-induced pH drop
None	20,638	-0.36 units
CCCP, 10 μM	2,132	-0.01
DNP, 0.1 mM	1,821	-0.01
Mannitol, 0.4 M	14,233	-0.16

Sections pretreated 1 hr in water, then incubated in lots of 10 in 2 ml MES-tris (1 mM, pH 6.0) + 1 mM KCl and inhibitors as noted. After 15 minutes 0.3 μc [^{86}Rb]Cl added, and where indicated 10 μM FC. Sections harvested one hour later, given a 10 min. chase in cold 10 mM KCl and counted. The pH of the solution was 6.0 at the start, and was measured after removal of the sections.

H^+-excretion (22) of Avena coleoptiles are so similar in regard to amount and speed of response that it must be concluded that the two processes are coupled.

The kinetics of [$^{86}Rb^+$] uptake shown in Figure 2 are more consistent with a K^+-H^+ exchange mechanism than a hyperpolarization-driven K^+-uptake. If the hyperpolarization is the driving force the rate of [$^{86}Rb^+$] uptake should

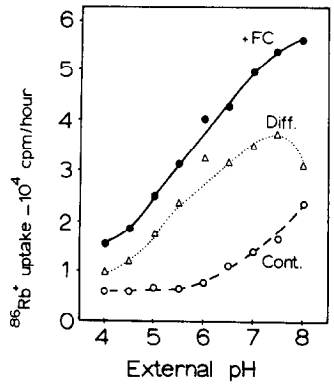

Fig. 3. Effect of external pH on $[^{86}Rb^+]$-K^+ uptake. Groups of 10 sections incubated 1 hr in 2 ml of 1 mM MES-tris, pH 4.0-8.0, containing 0.5 mM K_2SO_4, 0.4 μc $[^{86}Rb]Cl$ and ± 10 μM FC. Sections then given a 3 minute chase in cold 10 mM KCl and counted. FC (—●—); control (— -o-); difference between FC and control (···Δ···).

be proportional to the amount of hyperpolarization, with the result that the maximum rate of K^+ uptake should not occur until the time of maximum hyperpolarization. $[^{86}Rb^+]$ uptake reaches a maximum rate within 3 minutes after addition of FC, while the FC-induced hyperpolarization in barley roots (13) and squash cotyledons (21) reaches a maximum only after 12-25 minutes. The time of maximum K^+-uptake agrees well with the time needed for a maximum rate of FC-induced H^+-excretion to be reached (22), however, as expected with a K^+-H^+ exchange process.

If FC is activating a K^+-H^+ antiport in coleoptiles, factors which modify the rate of proton excretion should exert a similar effect on K^+-uptake. It is shown in Table 1 that the metabolic inhibitors CCCP and DNP completely block both the FC-induced K^+-uptake and H^+-excretion, and similar results were obtained with KCN and NaN_3 (data not shown). Isotonic mannitol causes a partial inhibition of both processes. H^+-excretion is sensitive to the external pH, with both the rate of excretion and the amount of FC-stimulation increasing from pH 4 to 7 (8). K^+-uptake is also affected by the external pH, with the magnitude of the FC-effect increasing from pH 4 to about 7.5 (Fig. 3). Modification of the rate of FC-induced H^+-excretion, then, results in a comparable modification of K^+-uptake.

Table 2: Ability of ions to compete with FC-induced [$^{86}Rb^+$] uptake into coleoptile tissues.

Competing Ion	[$^{86}Rb^+$] uptake, cpm/hr/10 sections	% Inhibition
None	31,710	....
KCl, 1 mM	14,553	54%
NaCl, 1 mM	17,647	44
LiCl, 1 mM	36,660	-16
$MgCl_2$, 1 mM	20,771	34
$CaCl_2$, 1 mM	30,838	3

Groups of 10 sections pretreated 1 hr in water, then incubated 1 hr in 2 ml of 1 mM MES-tris, pH 6.0, containing 0.1 mM KCl, 0.6 μc [^{86}Rb]Cl, 10 μM FC, and competing ions as indicated. Sections were given a 10 minute chase in 10 mM KCl and counted.

The uptake process is not specific for K^+-Rb^+. Na^+ competes nearly as efficiently as K^+ for [$^{86}Rb^+$] uptake, and Mg^+ is only slightly less effective (Table 2). Ca^{2+}, on the other hand, is without effect, and Li^+, if anything, enhances Rb^+ uptake.

If the K^+-H^+ exchange is electroneutral the ratio of K^+ uptake to H^+-excretion should be 1:1. Marrè et al. (20) obtained a ratio of 0.9 for FC-induced K^+-H^+ exchange over a three-hour period with pea stem sections. In the presence of 1 mM KCl we have obtained apparent uptake rates for K^+ of up to 0.30 μMoles/hr/10 sections as compared with H^+-excretion rates of 0.29 μMoles/hr/10 sections, giving a ratio of 1.0. Thus an electroneutral K^+-H^+ exchange can occur. But when the external solution contains only 0.01 mM K^+ the rate of uptake of external K^+ is reduced by over 95% while the H^+-excretion rate is reduced by less than 25% (24). Under conditions of low exernal K^+, then, the system can transport more H^+ than K^+, and will not be electroneutral.

These data would seem to fit a model similar to that proposed by Poole (23). A K^+-H^+ carrier transports protons across the plasma membrane using the energy of ATP hydrolysis to drive this step. The rate of transport will depend on

the ability of the carrier to recycle to the inside of the plasma membrane in a non-protonated form. The first step is dissociation of protons from the carrier on the external side, and this will be facilitated by a high external pH. The dissociated carrier can accept a monovalent cation, preferably K^+, and return rapidly to the inside of the plasma membrane, therefore completing an electroneutral K^+-H^+ exchange. Or it can return more slowly without an accompanying cation, generating a membrane potential. As predicted by this model the rate of H^+-excretion is enhanced by the presence of K^+ in the external medium (20, 24). The role of FC would presumably be to stimulate the H^+-excretion part of this exchange, with the result that K^+-uptake would also be stimulated. Auxins, on the other hand, also cause cells to excrete protons and enlarge, but must do so by another mechanism.

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