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Close coupling between extrusion of H^+ and uptake of K^+ by barley roots

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Abstract. Extrusion of H^+ by intact barley (*Hordeum vulgare* L.) roots was automatically titrated. Simultaneously, uptake of K^+ into the roots, transport of K^+ through the roots, and (as a residual term) accumulation of K^+ within the root tissue were determined. When no monovalent cation was present in the medium the steady rate of H^+ release was close to zero. Addition of K^+ stimulated H^+ extrusion within less than 1 min. The stimulation of H^+ release was apparently limited only by the movement of K^+ through the apoplast of the roots. The steady rate of H^+ extrusion depended on the availability of external K^+ and saturated at a K^+ concentration of about 100 $\mu mol \cdot dm^{-3}$. Half-maximum rates of net K^+ uptake and H^+ extrusion were reached at a K^+ concentration of about 10 $\mu mol \cdot dm^{-3}$. With (slowly absorbable) sulfate as the only anion present, the stoichiometry between H^+ release and net K^+ uptake was one. In conclusion, the uptake of K^+ across the plasmalemma of the cells of the root cortex is electrically coupled to H^+ extrusion.

Key words: *Hordeum* (roots) – Plasmalemma – Potassium (uptake, accumulation, transport) – Proton extrusion – Root (K^+ uptake, H^+ extrusion).

Introduction

Experiments with membrane vesicles (for a review, see Sze 1985) and root protoplasts (Lin 1985) indicate that the uptake of cations and anions is driven by the electrical potential difference ($\Delta\psi$) in combination with the proton concentration gradient (ΔpH) across the plasmalemma. If this were true, continuing ion uptake would require continuing H^+ expulsion in order to maintain the pH gradient. We investigated whether uptake of K^+ by the cells of the root cortex indeed required an electrogenic expulsion of H^+ and whether this kind of mechanism would still function if the availability of K^+ was very low. Glass and Siddiqi (1982) took the position that at an external K^+ concentration below 1 $mmol \cdot dm^{-3}$, evidence was against electric coupling; Cheeseman and Hanson (1979) postulated for corn roots a separate energy-linked, chemical mechanism for a $K^+ - H^+$ antiport.

Attention was paid to the method by which H^+ extrusion from roots was measured. Calculations of H^+ efflux from recorded declines of the pH of the medium surely lead to underestimations because the buffering capacity of the cell walls and of the medium must be taken into account (this was overlooked by Glass and Siddiqi 1982; Mengel and Schubert 1984; Watad et al. 1986). Even back-titration of the solution and the tissue to the original pH (as it was practiced by Pitman 1970; Pitman et al. 1975a, b; Cheeseman and Enkoji 1984; Römheld et al. 1984) may not produce an error-free result if the declining pH of the solution had a feedback effect on H^+ extrusion. The experiments of Glass and Siddiqi (1982) and data reported in the present paper provide evidence that this can happen. We therefore chose to record H^+ extrusion at constant pH by automatic titration of the solutions in which the roots were suspended, in the same manner as H^+ extrusion from epidermal strips was recorded before (Raschke and Humble 1973). Simultaneously with the extrusion of H^+, we recorded the uptake of K^+ by roots. We wish to emphasize, that what we determined were net fluxes of H^+ and K^+, and we shall call these net fluxes "release of H^+" or "extrusion of H^+", and "uptake of K^+". It was possible to estimate unidirectional ion movements from these net fluxes; these unidirectional fluxes we shall call "influx" and "efflux".

In this investigation, K^+ was offered to the roots in combination with sulfate (with the exception of one experiment). Sulfate is taken up by (barley) roots at low rates (Glass and Siddiqi 1982); for most practical purposes, interference by anion uptake did not need to be taken into consideration (except when the pH of the suspension medium was brought to a value below 5).

Material and methods

Roots of 4-d-old "low salt" seedlings of barley (*Hordeum vulgare* L. convar. *distichon* Alef., cv Aura) were used. The caryopses germinated for 24 h on filter paper moistened with 0.5 mmol·dm^{-3} $CaSO_4$. Then they were planted on nylon gauze over aerated 0.5 mmol·dm^{-3} $CaSO_4$ and transferred to a growth chamber where they were illuminated continuously with 300 µmol·m^{-2}·s^{-1} from Osram fluorescent tubes, type 25, at 22° C from 6:00 to 18:00 h and at 18° C from 18:00 to 6:00 h.

Detached roots were mounted in two-chambered Plexiglas vessels (Fig. 1; Jeschke 1972). One of the chambers, A (18 ml), contained the apical ends (25–30 mm) of the roots. The cut ends (2–3 mm) extended into the other chamber, Xyl (9 ml). The partition between the two chambers was sealed with silicone grease. Chamber Xyl was filled with 0.5 mmol·dm^{-3} $CaSO_4$, 0.5 mmol·dm^{-3} $MgSO_4$. Chamber A contained the same solution and, in addition, K_2SO_4 at various concentrations. The solutions in both chambers were stirred vigorously by streams of air. All experiments were run at approx. 22° C.

Both chambers were drained every 30 min and refilled by fresh solutions. The solutions drawn were analysed for K^+ by flame emission photometry (Flammenphotometer 700, "Eppendorf"; Netheler and Hinz, Hamburg, FRG). The decrease in the K^+ concentration in chamber A was a measure for net K^+ uptake across the plasmalemma of the cells of the root cortex. The transport of K^+ along the root in the xylem vessels was estimated from the increase in K^+ concentration in chamber Xyl. The difference between K^+ uptake and K^+ transport represents the amount of K^+ accumulated within the roots.

In the experiment shown in Fig. 5, the decline in K^+ activity in the solution in chamber A was followed with a K^+-sensitive electrode (Type MI-442; Microelectrodes, Inc., Londonderry, N.H., USA); the reference electrode (Type MI-409) dipped into a separate vial containing 3 mol·dm^{-3} KCl. A salt bridge filled with 0.05 mol·dm^{-3} Na_2SO_4 in 4% agar established the electrical connection to chamber A (Fig. 1).

The pH of the solution in chamber A was maintained by automatic titration. The pH-stat consisted of a combination micro pH glass electrode, a Metrohm pH meter 605, an Impulsomat 614, and a Dosimat 655 (Deutsche Metrohm, Filderstadt, FRG). In order to reduce the leakage of K^+ from this combination, the customary 3 mol·dm^{-3} KCl-filling of the reference electrode was replaced by a 0.1 mol·dm^{-3} solution. In addition, only selected electrodes were used which leaked <0.2 µmol K^+ per 30 min into a K^+-free medium. Acid or base was added as 0.5 meq·dm^{-3} H_2SO_4 or $Ca(OH)_2$. The normality of the $Ca(OH)_2$ used was determined before each experiment by titration with 1 meq·dm^{-3} H_2SO_4. Consumption of acid or base, as well as the pH in chamber A, were recorded on a strip-chart recorder. Changes in H^+ flux could be detected that occurred within less than 1 min. All ion fluxes were related to the fresh weight of the root tissue in chamber A.

Unidirectional influx of K^+ (Fig. 6) was estimated from measurements of short-term $^{86}Rb^+$ influx. Apical root sections of 30 mm length were placed, at 10 roots each, in plastic beakers containing solutions of various K^+ concentrations in phosphate buffer (pH 5.8) and in 3 mmol·dm^{-3} $CaCl_2$, 0.5 mmol·dm^{-3} $MgSO_4$. The $^{86}Rb^+$ was added as the chloride salt. These experiments were run at approx. 22° C while the solutions were stirred by a continuous stream of air. After 20 min of exposure to $^{86}Rb^+$, samples of five roots each were removed, rinsed three times for 1 min each with 0.5 mmol·dm^{-3} $CaSO_4$, blotted and weighed. The $^{86}Rb^+$ content of these sections was measured by Čerenkov counting.

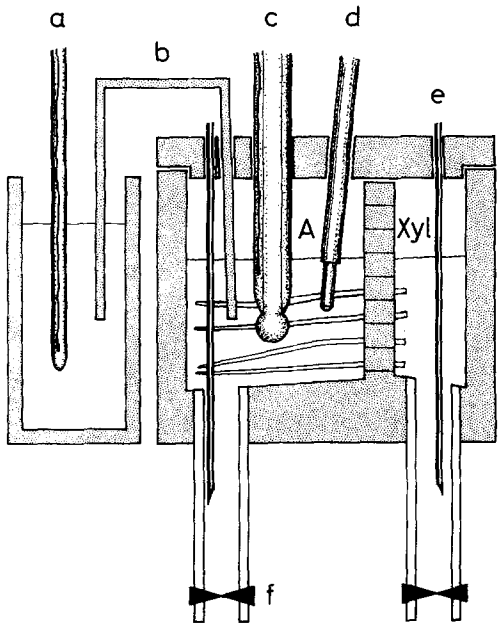

Fig. 1. Apparatus designed for measuring simultaneously H^+ extrusion by automatic titration and net fluxes of K^+ using a K^+-sensitive microelectrode or flame photometry. *a* = micro reference electrode, *b* = salt bridge, *c* = combined micro pH glass electrode, *d* = K^+-sensitive microelectrode, *e* = air, f = drain; *A*: chamber containing the roots; *Xyl:* chamber containing the cut ends of the roots

Results

With only divalent and no monovalent cations present in the medium (pH 5.8, Figs. 2, 3) an apparent release of H^+ by barley roots proceeded slowly at rates <0.5 µmol·(g FW)$^{-1}$·h^{-1}. Addition of K_2SO_4, resulting in a concentration of 0.2 mmol·dm^{-3} K^+, stimulated the rate of H^+ extrusion within less than 1 min (Fig. 2). Within 15 min a new steady rate of H^+ extrusion of about 6.5 µmol·(g FW)$^{-1}$·h^{-1} was established (Figs. 2, 3). The approach to the steady state was exponential: log $(1-v/v_{max})$ was linear with time. The time constant was 0.26·min^{-1} and the half-time 2.7 min. A step-wise increase of the external Ca^{2+} concentration from 0.5 to 1, and further to 2 mmol·dm^{-3}, at an external pH of 5.8, did not

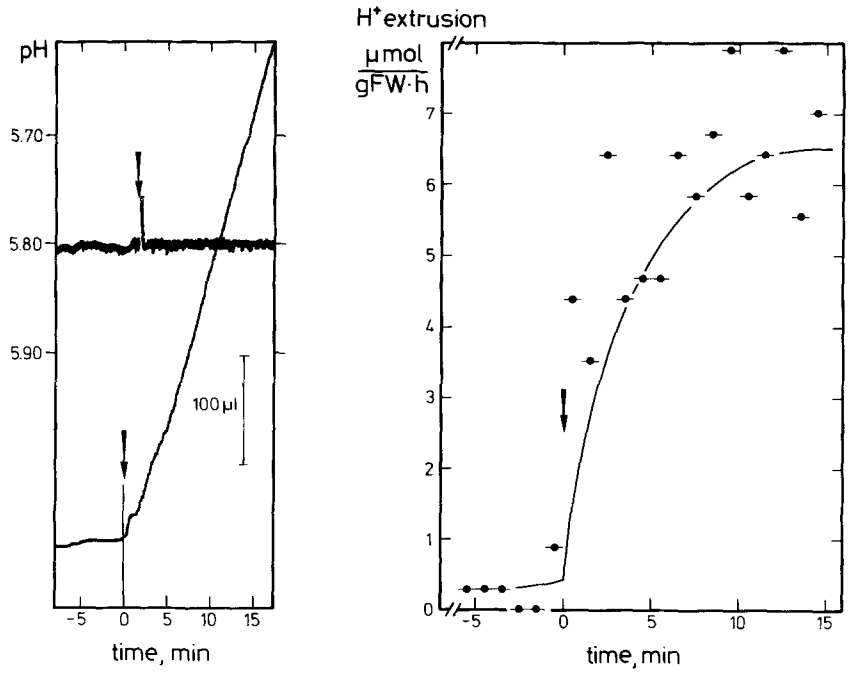

Fig. 2. Rapid stimulation of H^+ extrusion from barley roots after addition (*arrow*) of K_2SO_4 to the medium (final K^+ concentration: 0.2 mmol·dm^{-3}). The medium always contained 0.5 mmol·dm^{-3} $CaSO_4$ and 0.5 mmol·dm^{-3} $MgSO_4$. *Left panel*: titration record of a solution of $Ca(OH)_2$ (0.746 meq·dm^{-3}) added to the medium for maintaining the pH at 5.8; *right panel*: rates of H^+ release calculated from the titration record at intervals of 1 min

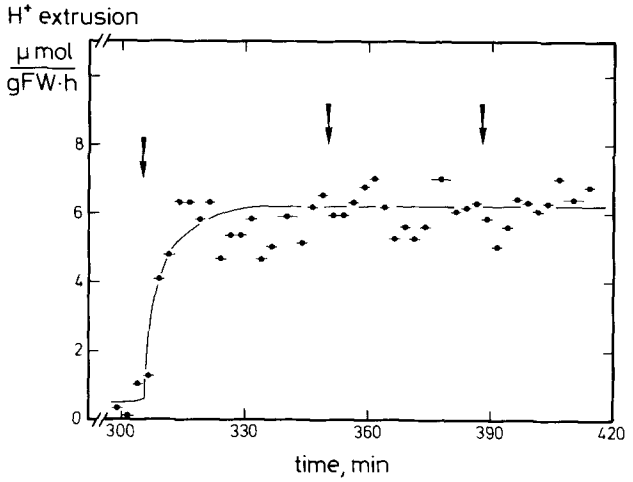

Fig. 3. Effect of an increase of the external Ca^{2+} concentration on the K^+-stimulated H^+ extrusion from barley roots. At the time of the *first arrow* K_2SO_4 was added to give a final K^+ concentration of 0.2 mmol·dm^{-3}; at the times of the *second* and the *third arrows* the external Ca^{2+} concentration was increased from 0.5 to 1, and from 1 to 2 mmol·dm^{-3}. This experiment was performed several times. The same set of roots could be used for repetitions of the experiment, each time with the same result, as long as the roots were rinsed three times with K^+-free solution, before each new cycle started

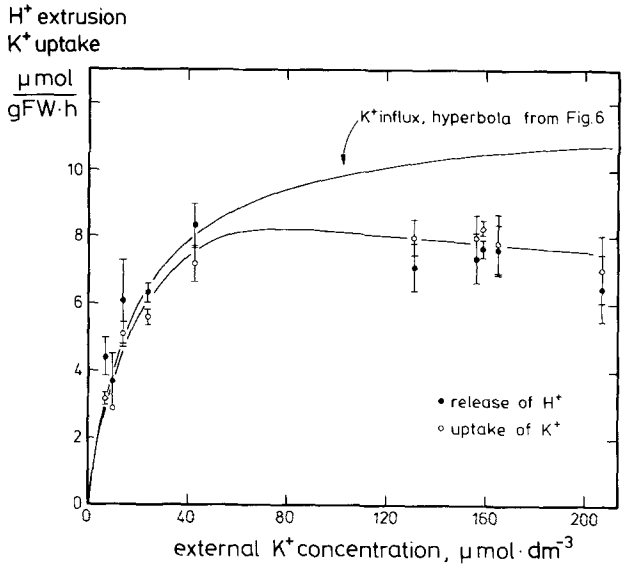

Fig. 4. Rates of net H^+ extrusion and net K^+ uptake by barley roots exposed to solutions of K_2SO_4 at various concentrations. The Michaelis-Menten hyperbola of unidirectional K^+ influx from **Fig. 6** was entered for comparison

cause any further increase in the release of H^+ (Fig. 3), which indicates that the recorded acidification was not caused by ion exchange with H^+ bound to the cell walls.

The dependence of the rate of H^+ extrusion on the external K^+ concentration was determined either by exposing roots to various external K^+ concentrations (Fig. 4) or by using the K^+ depletion of the medium by the roots' activity (Fig. 5). In the depletion experiments, the release of H^+ into the solution did not decline before the K^+ content of the medium had fallen to about 60 µmol·dm^{-3} (Fig. 5, refer also to Fig. 4). Above this concentration, release of H^+ was virtually in-

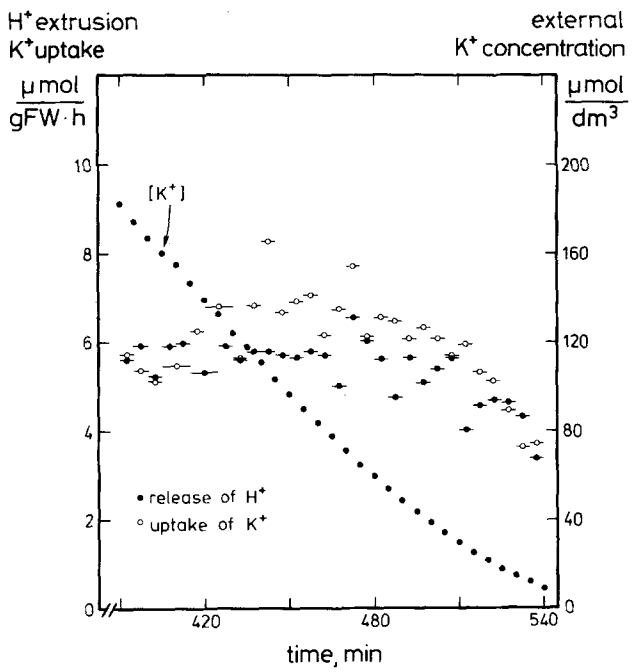

Fig. 5. Effect of a K^+ depletion of the medium on the rates of H^+ extrusion and K^+ uptake of barley roots. Depletion was repeated three times, using the same set of roots. At the beginning of each depletion, the medium was replenished with K^+ to a concentration of 0.2 mmol·dm^{-3}

dependent of the external K^+ concentration (Figs. 4, 5). As a matter of fact, H^+ release even declined slightly at K^+ concentrations above about 100 μmol·dm^{-3} (Fig. 4). Rates of K^+ uptake, as determined from the K^+ depletion of the medium, were nearly equal to the simultaneous rates of H^+ release over the whole concentration range of K^+ covered (Figs. 4, 5). However, a discrepancy emerged when release of H^+ was related to unidirectional influx of K^+ (Fig. 4). The influx had been measured using $^{86}Rb^+$ as a tracer (following Epstein et al. 1963; Fig. 6). The maximum rate of K^+ influx, v_{max}, was 11.8±0.6 μmol·(g FW)$^{-1}$·h^{-1}; the external K^+ concentration for the half-maximum rate of influx, K_m, was 19.9±7.3 μmol·dm^{-3} (Fig. 6). (It is unlikely that, at the low K^+ concentration we used, the presence of 3 mmol·dm^{-3} $CaCl_2$ affected the determination of the kinetic parameters, Epstein 1976.) We recognize in Fig. 4 that a divergence between the rates of unidirectional K^+ influx and net K^+ uptake developed as the external K^+ concentration exceeded 20 μmol·dm^{-3} (Fig. 4), and we note that it was the curve of net uptake of K^+ which coincided with the curve of release of H^+. The difference between influx of K^+ and uptake of K^+ was most probably the result of an efflux of K^+ into the roots' environment. Compartmental analysis showed that barley roots leak some K^+ while they absorb this ion (Behl and Jeschke 1982).

With sulfate as the only anion present, and at an external pH of 5.8, the stoichiometry of the apparent exchange of H^+ for K^+ by the roots was one (Figs. 4, 5, 8, 9). When the external pH was lowered step by step from a value of 5.8 to one of 4.2, and thus the pH gradient across the plasmalemma of the cells of the root cortex was artificially increased, rates of K^+ uptake declined from 8.6 to 3.8 μmol·(g FW)$^{-1}$·h^{-1} (Fig. 7), and also the rates of H^+ release decreased. However, H^+ extrusion declined more strongly than K^+ uptake. Stoi-

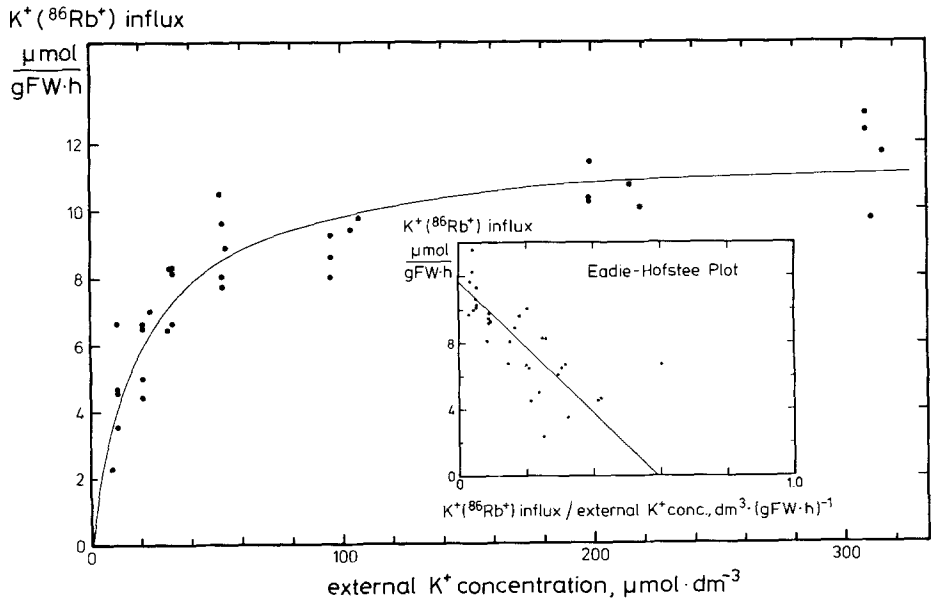

Fig. 6. Effect of various external K^+ concentrations (chloride and sulfate as anions) on rates of unidirectional K^+ influx into barley roots. The influx of K^+ was determined in five separate short-term absorption experiments with $^{86}Rb^+$ as a tracer for K^+ (corrections were applied for differences in specific activity). V_{max} and K_m were derived from the inserted Eadie-Hofstee plot. v_{max}=11.8±0.6 μmol·(g FW)$^{-1}$·h^{-1}; K_m=19.9±7.3 μmol·dm^{-3}

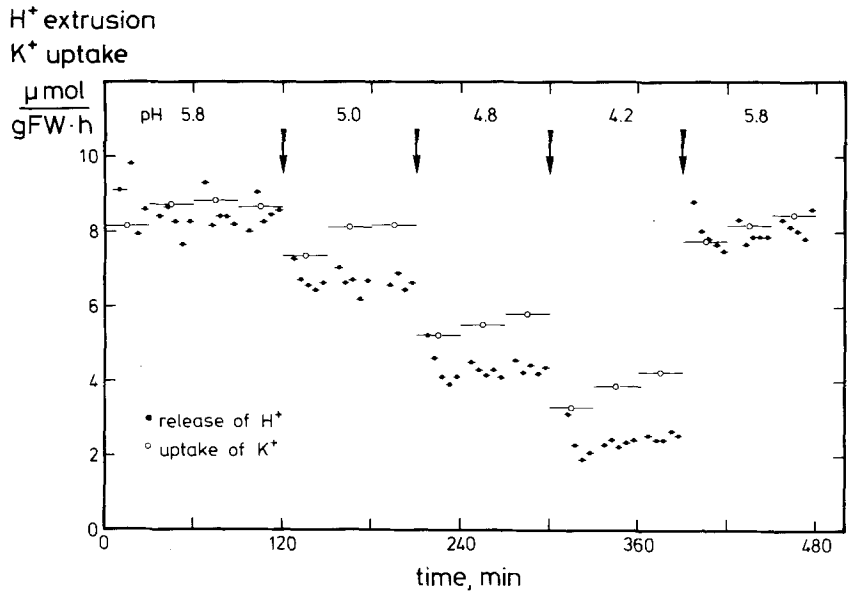

Fig. 7. Effect of the external pH on the rates of H^+ extrusion and K^+ uptake by barley roots exposed to 0.1 mmol·dm^{-3} K_2SO_4. The rates of H^+ extrusion were computed for intervals of 5 min duration, the rates of K^+ uptake for intervals of 30 min duration

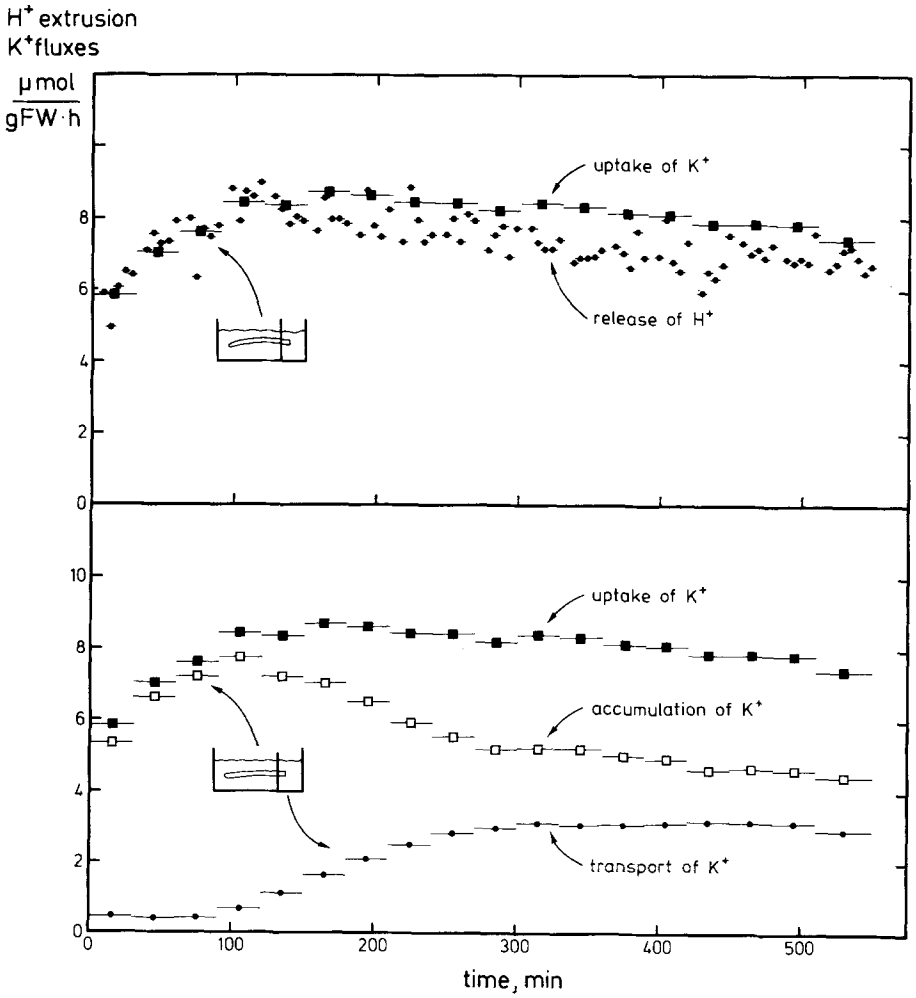

Fig. 8. Time course of the rates of H^+ extrusion, K^+ uptake, K^+ transport, and K^+ accumulation (as a residual term) by barley roots

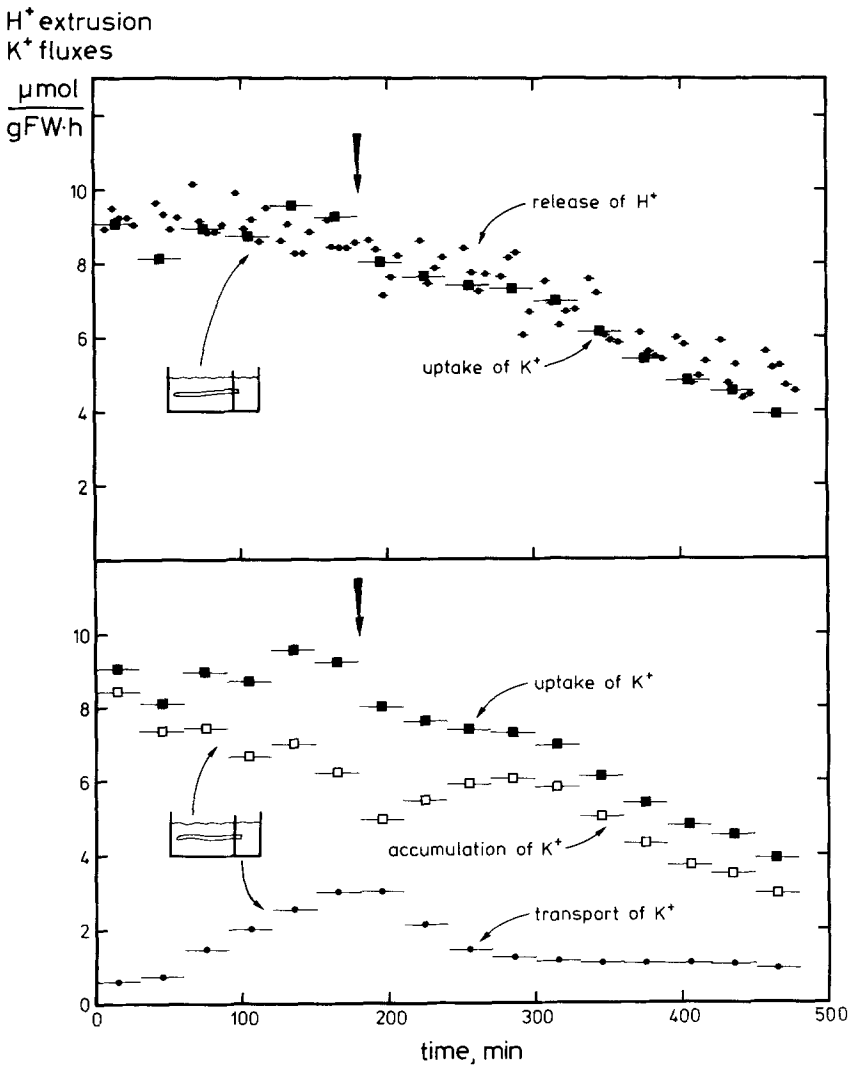

Fig. 9. Effect of 1 mmol·dm^{-3} *p*-fluorophenylalanine on rates of H^+ extrusion, K^+ uptake, K^+ transport, and K^+ accumulation by barley roots. At the time of the *arrow*, *p*-fluorophenylalanine was added to the solution in chamber A (**Fig. 1**)

chiometries between the K^+ and the H^+ exchanges were 1.04 at pH 5.8, 1.23 at 5.4, 1.31 at 4.8, and 1.67 at 4.2 (Fig. 7). When the external pH was raised again from pH 4.2 to 5.8 both, K^+ uptake and H^+ extrusion, increased within less than 5 min. The rate of K^+ uptake was again near 8 μmol·(g FW)$^{-1}$·h^{-1}, and the stoichiometry between uptake of K^+ and H^+ extrusion had returned to the value of one (Fig. 7).

Uptake of K^+ and extrusion of H^+ followed very similar time courses over a span of 9 h (Figs. 8, 9). During the first 2 h of exposure to K^+, the rate of uptake of K^+ equalled the rate of release of H^+. During this initial phase, K^+ was preferentially accumulated in the tissue; rates of K^+ transport into chamber Xyl (Fig.1) were small (Figs. 8, 9). Rates of K^+ transport then increased; concomitantly H^+ extrusion slowed down slightly (by about 1 μmol·(g FW)$^{-1}$·h^{-1}) below the rates of K^+ uptake (Fig. 8). When, however, K^+ transport into the xylem was inhibited by the addition of 1 mmol·dm^{-3} *p*-fluorophenylalanine to the medium, the stoichiometry between K^+ uptake and H^+ extrusion remained at the value of one (Fig. 9), and the amount of K^+ accumulated within the roots equalled K^+ uptake.

Discussion

The results of our experiments with barley roots support the view that uptake of K^+ is driven by an electrogenic extrusion of H^+. A continuous very slow release of H^+ (probably resulting from release of CO_2 or organic acids) into a K^+-free medium accelerated rapidly as soon as K^+ was made available (Fig. 2). The response time was shorter than the temporal resolution of the titrator (<1 min). Sulfate was the only anion present in the medium and could not be measurably absorbed within the time span of the experiment (Glass and Siddiqi

1982). Obviously, the cells of the root cortex of barley do not need to take up anions when they import K^+; like guard cells of *Vicia faba* they extrude H^+ (Raschke and Humble 1973). The stoichiometry between H^+ extrusion and K^+ uptake was one at K^+ concentrations from below 10 up to 200 $\mu mol \cdot dm^{-3}$ in the medium (Figs. 4, 5). (Deviations from a stoichiometry of one will be discussed later.) It is not neccessary to assume separate mechanisms for K^+ uptake at low and high external concentrations of K^+, as Glass and Siddiqi (1982) proposed, nor to assume the operation of an energy-linked $K^+ - H^+$ antiporter (Cheeseman and Hanson 1979). Evidence for a H^+ pump in the plasmalemma of root cells does exist (for a review, see Spanswick 1981), uptake of K^+ into root cells of barley has been reported to dissipate existing electrical potential differences (Pitman et al. 1975b), and it is likely that K^+ channels are present in root cells, in analogy to their operation in guard cells (Schroeder et al. 1984, 1987).

It is unlikely that some of the acidification recorded was the consequence of an export of organic acids. Peterson and Böttger (1986) determined that release of organic acids accounted for not more than 0.2 to 0.3% of the total acidification of the medium by roots of *Zea mays*.

The rapidity of the roots' response to the availability of K^+ may be of great importance to the plant. The plant disposes of a machinery that is ready to absorb K^+ (and probably also other required ions), as soon as water movement and growth bring nutrients in contact with the surface of the root. The rapidity of the response indicates that the installation of H^+ pumps and K^+ channels in the plasmalemma of the root cells does not need induction. Within less than 1 min extrusion of H^+ increased when K^+ was offered in the medium. The lag between K^+ addition and establishment of a steady H^+ release appeared to have been determined by the ion-exchange properties of the apoplast. The half-time of this delay (2.7 min; Fig. 2) was in the same order as that of K^+ exchange between the apoplast of whole barley roots and the external medium (2.2 min; Walker and Pitman 1976).

Because there was only a slowly absorbable anion, sulfate, in the solution the pH in the tissue must have been regulated by a production of organic acids that kept pace with export of H^+ and import of K^+. Malate formation in roots as a consequence of cation uptake has already been determined by Hiatt and Hendricks (1967). Processes occurring in roots during K^+ uptake seem to be similar to those operating in guard cells (Allaway 1973 in conjunction with Raschke and Humble 1973).

The basic model of K^+ uptake and organic-acid production of roots in response to their H^+ extrusion needs refinement. For instance, we know that ion transport across the plasmalemma is not a one-way traffic but rather an exchange process (Walker and Pitman 1976). It was therefore to be expected that net uptake of K^+ into barley roots would not follow the hyperbola of the uptake of $^{86}Rb^+$ as a tracer for K^+ when external concentrations were higher than 40 $\mu mol \cdot dm^{-3}$ in the solution (Fig. 4). The saturation curve of net K^+ uptake fell below the curve for the unidirectional K^+ ($^{86}Rb^+$) influx because K^+ was increasingly released as uptake accelerated. Obviously net uptake of K^+ balanced the release of H^+.

A second addition to the model becomes necessary in order to accommodate the fact, that SO_4^{2-} is not an ion that is completely excluded from import. Some SO_4^{2-} was taken up in symport with H^+ which caused a reduction of the net release of H^+. In our interpretation, such an effect became evident when the pH of the solution was lowered and the increased difference in pH between solution and tissue provided the driving force for an increased uptake of SO_4^{2-} (Fig. 7). The coupling between anion uptake and H^+ release will be the topic of a further publication.

Finally, K^+ transport into the xylem needs to be taken into consideration. Less than 2 h after K^+ was made available, this cation began to appear in the efflux of the xylem (Fig. 8). Transfer of K^+ into the xylem caused K^+ uptake to exceed H^+ release. This imbalance disappeared as soon as transport of K^+ into the xylem was stopped by application of *p*-fluorophenylalanine (Fig. 9). Export of K^+ salts from the root to the shoot may have a feedback effect on the import of inorganic anions and the production of organic anions by the roots. Maintenance of anion balance in turn will affect the stoichiometry between uptake of K^+ and release of H^+.

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