Plant Physiol. (1992) 100, 1962–1967
0032-0889/92/100/1962/06/$01.00/0

Received for publication March 20, 1992
Accepted August 13, 1992

Effects of Deuterium Oxide on Growth, Proton Extrusion, Potassium Influx, and in Vitro Plasma Membrane Activities in Maize Root Segments[1]

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ABSTRACT

Elongation of subapical segments of maize (*Zea mays*) roots was greatly inhibited by ^2H_2O in the incubation medium. Short-term exposure (30 min) to ^2H_2O slightly reduced O_2 uptake and significantly increased ATP levels. ^2H_2O inhibited H^+ extrusion in the presence of both low (0.05 mM) and high (5 mM) external concentrations of K^+ (about 30 and 53%, respectively at 50% [v/v] ^2H_2O). Experiments on plasma membrane vesicles showed that H^+-pumping and ATPase activities were greatly inhibited by ^2H_2O (about 35% at 50% [v/v] ^2H_2O); NADH-ferricyanide reductase and 1,3-β-glucan synthase activities were inhibited to a lesser extent (less than 15%). ATPase activities present in both the tonoplast-enriched and submitochondrial particle preparations were not affected by ^2H_2O. Therefore, the effect of short incubation time and low concentration of ^2H_2O is not due to a general action on overall cell metabolism but involves a specific inhibition of the plasma membrane H^+-ATPase. K^+ uptake was inhibited by ^2H_2O only when K^+ was present at a low (0.05 mM) external concentration where absorption is against its electrochemical potential. The transmembrane electric potential difference (E_m) was slightly hyperpolarized by ^2H_2O at low K^+, but was not affected at the higher K^+ concentrations. These results suggest a relationship between H^+ extrusion and K^+ uptake at low K^+ external concentration.

Incubation in ^2H_2O of animal and plant tissues and organs has been used extensively for proton density-labeling experiments to study synthesis, degradation, and turnover of proteins (8), to investigate the role of water in the structure and function of biosystems (29), and to suppress solvent signal from the water in proton NMR (11). However, a toxic effect of ^2H_2O has been observed on growth of *Escherichia coli* (4), growth and development of plants (2, 6, 28), and seed germination (12). It has been suggested that these effects, obtained at high ^2H_2O concentrations and after long exposure periods, are due to a loss of activity of the deuterated molecules synthesized in the presence of ^2H_2O and to a reduction in protein biosynthesis and increase in protein degradation (6). This increase in the rate of protein breakdown is attributed to an action of ^2H_2O on the biochemical and ultrastructural properties of certain membranes, particularly the tonoplast, probably through a direct or chaotropic effect of ^2H_2O (7). The effect of ^2H_2O on membrane functions has not yet been specifically investigated in plant materials. An effect of ^2H_2O on plant membrane functions might be hypothesized on the basis of the consideration that H^+ translocating activities, which play a central role in the function of plant membranes (27), could also utilize $^2H^+$ even if to a lesser extent.

The experiments described in this paper were aimed at studying the effects of ^2H_2O on H^+ extrusion and related transport activities in subapical maize (*Zea mays*) root segments. Plasma membrane vesicles of high purity from maize roots were used to compare the in vivo effect of ^2H_2O on H^+ transport with the in vitro effect of ^2H_2O on H^+ pumping and ATPase activities and to clarify whether ^2H_2O inhibits specifically the H^+-translocating activities or membrane activities as a whole. Finally, the possibility of using ^2H_2O as a tool to investigate the involvement of H^+ in membrane transport has been analyzed.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* cv Dekalb XL 640 A) seeds were surface-sterilized for 30 min with 1% NaClO and germinated in the dark for 2 d at 26°C on wet filter paper saturated with 0.5 mM $CaSO_4$. Twenty-four hours prior to the experiments, seedlings were transferred to aerated 0.5 mM $CaSO_4$ solution and maintained in the dark at 26°C. After this incubation period, the main roots were about 40 to 50 mm long. Six-millimeter long subapical segments were excised from the main roots between 2 and 8 mm from the tip (23). The root segments were prewashed for 1 h in an aerated solution of 0.5 mM $CaSO_4$. Samples of 15 segments (about 115 mg fresh weight) were then washed twice for 30 min in 10 mL of 0.5 mM $CaSO_4$ in a water bath thermoregulated at 26°C and agitated at 80 oscillations min^{-1}. After this period, about 2.5 h from the excision, recovery from wounding was completed (23).

Measurement of Elongation

Fifteen subapical root segments, preincubated as described above, were put in line over a small groove made in a Plexiglas plate and their total length was measured with calipers. The segments were then transferred into 10 mL of

[1] Research supported by National Research Council of Italy, Special Project RAISA, sub-project 2, paper No. 528.

a medium containing 0.5 mM $CaSO_4$, 0.25 mM K_2SO_4, 0.1 mM Mes-Na, pH 6.0, with or without ^2H_2O. After 3 h of incubation under agitation (80 oscillations min^{-1}) at 26°C, the length of the segments was measured again. Growth of the segments was expressed as percent increase with respect to the initial length. Elongation of intact roots was determined by measuring the roots with calipers before and after 3 h of incubation in aerated medium.

Oxygen Uptake and ATP Levels

Oxygen uptake was measured in a differential respirometer (Gilson IGRP 20) on batches of 15 subapical segments incubated in 10 mL of 0.5 mM $CaSO_4$, 0.25 mM K_2SO_4, and 0.1 mM Mes-Na, pH 6.0, with or without ^2H_2O at 26°C, under agitation, where CO_2 in the flask was absorbed on a filter paper wetted with 0.2 mL of 3 N KOH placed in the central vessel. Every 30 min, the oxygen uptake was measured and the manometers were opened to avoid anaerobiosis.

The ATP levels were determined in the neutralized perchloric acid-soluble fraction of the root segments using the LKB 1243–200 ATP monitoring reagent in a LKB-Wallac 1250 Luminometer.

Measurement of H^+ Extrusion and K^+ Influx

After preincubation, samples (15 subapical segments) were transferred into 10 mL of a solution containing 0.5 mM $CaSO_4$, 0.1 mM Mes-Na, pH 6.0, and 0.05, 0.5, or 5 mM K^+ (as SO_4^{2-}) as required, with or without ^2H_2O, and incubated at 26°C under agitation. The pH values were measured with a Radiometer PHM 84 pH meter after 30 min of incubation. Back titrations of the media of the control and treated samples were undertaken as described by Lado et al. (18) with a Radiometer TTT 80 titrator.

K^+ uptake was measured using $^{86}Rb^+$ as tracer. Fifteen root segments were preincubated as described above, and then incubated with the radiolabeled ion (7.4 kBq $^{86}Rb^+$ μmol^{-1} K^+) in 10 mL of a solution containing 0.5 mM $CaSO_4$, 0.1 mM Mes-Na, pH 6.0, and 0.05, 0.5, or 5 mM K^+ (as SO_4^{2-}) as required, with or without ^2H_2O. At the end of incubation times (15 and 30 min), the segments were washed twice at 4°C for 15 min with the corresponding nonradioactive solutions. The segments were then homogenized in 1 mL of 0.1 M HNO_3 and heated at 100°C for 10 min. Radioactivity was determined by counting, in a Beckman LS 7500 scintillation spectrometer, aliquots of the supernatants dissolved in a scintillation cocktail.

E_m[2]

Measurements of E_m were performed by the conventional microelectrode procedure described by Cocucci et al. (5). Four subapical segments preincubated as described above were set up in a lucite cuvette (total volume 4 mL) and maintained under continuous flow (200 mL h^{-1}) of the thermoregulated (26°C) and aerated medium. Micropipettes filled with 2 M KCl with resistance of 10 to 20 MΩ were used. E_m was recorded with a high-impedance electrometer amplifier (WPI K5–700). The data are the means of at least three measurements of E_m of different cells belonging to the third through fifth layers of different segments in the region between 3 and 5 mm from the root apex. All experiments were run in quadruplicate and repeated at least three times. The data presented are those of an experiment taken as typical for each experimental treatment, where the SE did not exceed ± 4%.

Preparation of Plasma Membrane and Enzymic Activity Measurements

Plasma membranes were prepared essentially as described by Palmgren et al. (24). Seventy grams of maize root segments were homogenized in a mortar with 280 mL of 330 mM sorbitol, 50 mM Mops-BTP (pH 7.5), 5 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 1% BSA. The homogenates were filtered and centrifuged 15 min at 13,000*g* and 30 min at 100,000*g*. This microsomal fraction was resuspended in 9 mL of 330 mM sorbitol, 5 mM K-phosphate (pH 7.8), 5 mM KCl, 1 mM DTT, 0.1 mM EDTA, and added to a phase system with a final weight of 36.0 g and composition consisting of 6.5% (w/w) dextran T500, 6.5% (w/w) PEG 3350, 330 mM sorbitol, 5 mM K-phosphate (pH 7.8), 5 mM KCl, 1 mM DTT, 0.1 mM EDTA (4°C) (24).

The phase systems were processed using the three-step batch procedure described by Larsson et al. (20). The final upper phases, containing plasma membranes, were diluted several times in 330 mM sorbitol, 2 mM Mops-BTP (pH 7.8), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, and the plasma membranes were pelleted and resuspended at 2 mg of protein mL^{-1} in the same medium. The purified right-side-out plasma membrane vesicles were then frozen in liquid N_2 and stored at −80°C. Before use, aliquots of these right-side-out vesicles were thawed in water at 20°C and frozen in liquid N_2 a total of four times to produce a mixture of inside-out and right-side-out vesicles (24).

H^+ pumping into the vesicles was measured by monitoring the decrease in absorbance at 495 nm of the pH probe acridine orange. The assay was performed essentially as described by Palmgren et al. (24): the assay medium consisted of 20 μM acridine orange, 2 mM ATP-BTP, 4 mM $MgCl_2$, 10 mM Mops-BTP (pH 7.0), 140 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mg mL^{-1} of BSA (fatty acid free), 2.5 μg of valinomycin, and 50 to 100 μg of membrane protein in a final volume of 2 mL. ATP concentration was kept constant by including 0.25 mM NADH, 1 mM phospho*enol*pyruvate, 15 μg mL^{-1} of lactate dehydrogenase, and 30 μg mL^{-1} of pyruvate kinase. After 5 min of incubation at 20°C, the reaction was started by $MgCl_2$ addition. The rate of H^+ accumulation was estimated from the initial slope of absorbance quenching of acridine orange. ATPase activity was monitored by following the oxidation of NADH coupled to the hydrolysis of ATP. Decreases in absorbance (495 nm for acridine orange and 340 nm for NADH) were monitored in a Jasco Uvidec 510 double-beam spectrophotometer.

1,3-β-Glucan synthase was measured as incorporation of UDP [^{3}H]glucose into polyglucan according to Kauss et al.

[2] Abbreviations: E_m, transmembrane electric potential difference; BTP, (1,3-bis[tris(hydroxymethyl)methylamino]propane).

(14), as described by Palmgren et al. (24). The assay medium contained 330 mM sucrose, 2 mM DTT, 2 mM spermine, 20 mM cellobiose, 0.2 mM $CaCl_2$, 2 mM UDP-[^{3}H]glucose (18.5 GBq $mmol^{-1}$, Amersham International, Amersham, England), 50 mM Hepes-KOH (pH 7.25), and 1 μg of membrane protein in a final volume of 0.1 mL. After 30 min of incubation at 25°C, the reaction was stopped by immersion in boiling water and 95-μL aliquots were withdrawn and added to cellulose filters, which were dried and washed (14). Radioactivity was measured in a Beckman LS 7500 liquid scintillation spectrometer.

NADH-ferricyanide reductase assay was run at 25°C in 2 mL of 330 mM sucrose, 25 mM Hepes-KOH (pH 7.3), 0.25 mM NADH, 0.2 mM $K_3[Fe(CN)_6]$, and 25 to 50 μg of membrane protein. The reaction was started by the addition of NADH and was measured as the absorbance decrease at 340 nm in a Jasco Uvidec 510 double-beam spectrophotometer. No oxidation of NADH in the absence of ferricyanide was recorded. Correction was made for nonenzymic oxidation of NADH.

RESULTS

Effects of Deuterium Oxide on Elongation, Oxygen Uptake, and ATP Levels in Subapical Segments of Maize Roots

Maize root subapical segments grew in length by about 7% after 3 h of incubation. During this period, the segments took up oxygen at a constant rate (about 450 μL of O_2 h^{-1} g^{-1} fresh weight) and the level of ATP (about 98 nmol g^{-1} fresh weight) did not significantly change (data not shown). The presence of ^2H_2O in the incubation medium inhibited the elongation growth of the segments; this inhibition was detectable in 10% ^2H_2O and increased with corresponding increases in ^2H_2O concentration, reaching more than 80% at 70% ^2H_2O (Table I). Experiments performed using intact roots show that 50% ^2H_2O inhibited root elongation by about 68% (data not shown), a value similar to that reported for subapical segments (Table I).

Table I. *Effects of ^2H_2O on Elongation, O_2 Uptake, and ATP Levels in Subapical Segments of Maize Roots*

Maize root subapical segments were incubated in 0.5 mM $CaSO_4$, 0.25 mM K_2SO_4, 0.1 mM Mes-Na (pH 6.0), and ^2H_2O as indicated. Growth of the segments was determined by measuring the length of 15 segments before and after 3 h of incubation. O_2 uptake was measured in a Warburg apparatus at 30-min incubation periods during the 1st h of treatment with ^2H_2O. ATP levels were measured in the perchloric acid-soluble fraction of root segments after 1 h of incubation in ^2H_2O. The data are the mean values of four experiments run in triplicate. Values followed by the same letter within a column are not significantly different at $P \leq 0.05$ ($n = 12$). Values in parentheses indicate percent change with respect to the controls.

Percent ^2H_2O	Growth in Length	O_2 Uptake	ATP
	% increase	$\mu L\ h^{-1}\ g^{-1}$ fresh wt	$nmol\ g^{-1}$ fresh wt
0	7a	452a	98a
10	5.6b (−19)	446a (−1)	102a (+4)
30	4.1c (−41)	435a (−4)	110b (+12)
50	1.9d (−73)	418a (−8)	111b (+13)
70	1.2e (−83)	391b (−13)	123c (+26)

Table II. *Effects of ^2H_2O on H^+ Extrusion of Subapical Segments of Maize Roots in the Presence of 0.05, 0.5, and 5 mM K^+*

The incubation medium was 0.5 mM $CaSO_4$, 0.1 mM Mes-Na (initial pH 6.0), and K^+ (as SO_4^{2-}) and ^2H_2O at the indicated concentrations. H^+ extrusion was measured by titrating the media after 30-min incubations. The data are the mean values of three experiments run in triplicate. Values followed by same letter within a column are not significantly different at $P \leq 0.05$ ($n = 9$). Values in parentheses indicate percent inhibition with respect to the controls.

Percent ^2H_2O	0.05 mM K^+	0.5 mM K^+	5 mM K^+
	ΔH^+ $\mu mol\ h^{-1}\ g^{-1}$ fresh wt		
0	1.2a	1.4a	1.9a
30	1.1a (−8)	1.1b (−21)	1.3b (−32)
50	0.8b (−33)	0.9b (−36)	0.9c (−53)
70	0.6b (−50)	0.6c (−57)	0.8c (−58)

Table I shows that, in agreement with the results on *Lemna minor* fronds reported by Boudet et al. (2), ^2H_2O inhibited oxygen uptake. This inhibition was very low up to 30% ^2H_2O and increased with ^2H_2O concentration. Oxygen uptake was inhibited from the first 30 min of incubation in ^2H_2O and then proceeded at a constant rate for at least 3 h (data not shown). Table I also shows that ^2H_2O significantly increased the ATP levels and that this increase was higher at higher ^2H_2O concentrations.

Effects of ^2H_2O on H^+ Extrusion, K^+ Influx, and Transmembrane Electric Potential of Subapical Segments of Maize Roots

The effect of ^2H_2O on H^+ extrusion was studied in the presence of three different concentrations of K^+ (Table II) that, in agreement with its well-known effect (16, 22), stimulated H^+ extrusion. Table II shows that H^+ extrusion was inhibited by the presence of ^2H_2O in the incubation medium. This inhibition increased with the increase in ^2H_2O concentration and was larger when H^+ extrusion was stimulated by 0.5 and 5 mM K^+.

The effect of ^2H_2O on K^+ uptake was studied in the presence of three K^+ concentrations. The well-known relationship between influx and concentration of K^+ in the external medium (16) was observed and is reported in Table III: K^+ influx greatly increased when external concentration of K^+ was raised from 0.05 to 0.5 mM, and the increase of K^+ influx was low, from 0.5 to 5 mM. Table III shows that at 0.05 mM K^+ external concentration, the influx of K^+ was already inhibited by 30% ^2H_2O and this inhibition increased with the increase in ^2H_2O concentration. At 0.5 mM K^+ the influx of the cation was only slightly inhibited by ^2H_2O, and at 5 mM K^+ the inhibition was absent or very low.

Figure 1 shows the effect of 50% ^2H_2O on E_m of cortical cells of maize root segments at 0.05, 0.5, and 5 mM external K^+. At 0.05 and 0.5 mM K^+, ^2H_2O induced a slight hyperpolarization. The induced hyperpolarization (about 4–6 mV) was reached after less than 5 min of treatment. E_m rapidly readjusted to the values obtained before treatment after ^2H_2O

Table III. *Effects of ^2H_2O on K^+ ($^{86}Rb^+$) Uptake in Subapical Segments of Maize Root in the Presence of 0.05, 0.5, and 5 mM K^+*

Incubation medium was 0.5 mM $CaSO_4$, 0.1 mM Mes-Na (pH 6.0), K^+ (as SO_4^{2-}), and ^2H_2O at the indicated concentrations. K^+ ($^{86}Rb^+$) uptake was measured after 15- and 30-min incubations. The data are the mean values of four experiments run in triplicate. Values followed by the same letter within a column are not significantly different at $P \leq 0.05$ ($n = 9$). Values in parentheses indicate percent inhibition with respect to the controls.

Percent ^2H_2O	0.05 mM K^+	0.5 mM K^+	5 mM K^+
	K^+ uptake μmol h^{-1} g^{-1} fresh wt		
0	2.5a	4.9a	5.4a
30	2.0b (−20)	4.9a (0)	5.4a (0)
50	1.7c (−32)	4.6a (−6)	5.4a (0)
70	1.6c (−36)	4.2b (−14)	5.0b (−7)

removal. At 5 mM K^+, no changes in E_m were recorded after addition of ^2H_2O.

Effects of ^2H_2O on H^+ Pumping, ATPase, NADH-Ferricyanide Reductase, and 1,3-β-Glucan Synthase Activities in Plasma Membrane Vesicles from Subapical Segments of Maize Roots

The inhibition of H^+ extrusion by ^2H_2O, reported in Table II, might be due to a direct action on the H^+-ATPase at plasma membrane level or to an effect on some other metabolic factor able to diminish H^+ extrusion. To clarify this point, the effect of ^2H_2O on H^+ pumping and ATPase activities has been studied in plasma membrane preparations of high purity, obtained by partitioning in aqueous polymer two-phase system (24). Table IV shows that both H^+ pumping and ATPase activities were greatly inhibited (about 30%) by 50% ^2H_2O. The inhibition of ATPase activity did not depend on an inhibition of the ATP regenerating system by ^2H_2O: in fact, the kinetics of ADP phosphorylation were not significantly affected by ^2H_2O (data not shown). The inhibiting effect on H^+ pumping and ATPase did not appear to be due to a nonspecific action of ^2H_2O on plasma membrane; NADH-ferricyanide reductase and 1,3-β-glucan synthase, two other plasma membrane activities, were slightly (less than 14%) inhibited by ^2H_2O (Table IV). Measurements of the ATPase activities present in the tonoplast-enriched fraction (19) and submitochondrial particles showed that they were not, or were very slightly, inhibited by ^2H_2O (data not shown).

Table IV. *Effects of ^2H_2O on H^+ Pumping, ATPase, NADH-Ferricyanide Reductase, and 1,3-β-Glucan Synthase Activities in Plasma Membrane Vesicles from Subapical Segments of Maize Roots*

H^+ uptake into vesicles was monitored as absorbance decrease at 495 nm of the pH probe acridine orange. ATPase activity, in the presence of an ATP-regenerating system, was coupled to oxidation of NADH and measured as absorbance decrease at 340 nm. NADH-ferricyanide reductase was measured as the decrease in the absorbance at 420 nm. 1,3-β-Glucan synthase activity was measured as incorporation of UDP-[^{3}H]glucose into polyglucan (for details, see "Materials and Methods"). Results are the mean ± SE of three experiments each with three replicates ($n = 9$).

Specific Activities	^2H_2O		Percent Change
	0%	50%	
H^+ pumping (ΔA_{495} min^{-1} mg^{-1})	0.41 ± 0.02	0.27 ± 0.01	−34%
ATPase (ADP release) (μmol min^{-1} mg^{-1})	0.18 ± 0.01	0.12 ± 0.005	−33%
NADH-ferricyanide reductase (μmol ferricyanide min^{-1} mg^{-1})	0.21 ± 0.01	0.18 ± 0.01	−14%
1,3-β-Glucan synthase (μmol glucose min^{-1} mg^{-1})	0.86 ± 0.04	0.77 ± 0.03	−10%

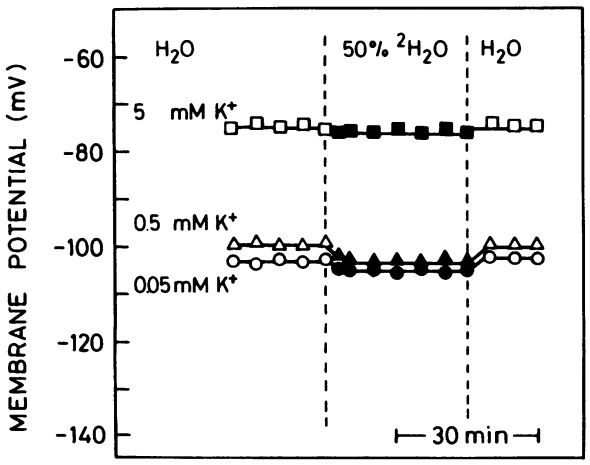

Figure 1. Effects of 50% ^2H_2O on transmembrane electric potential of cortical cells of maize root segments. Incubation medium: 0.5 mM $CaSO_4$, 0.1 mM Mes-Na, pH 6.0, K^+ as indicated (as SO_4^{2-}), with (closed symbols) or without (open symbols) 50% ^2H_2O.

DISCUSSION

The presence of ^2H_2O (30–70%, v/v) in the incubation medium greatly inhibited elongation of subapical segments of maize roots. At the same concentrations of ^2H_2O, O_2 uptake was only slightly inhibited (less than 13%) and the level of ATP significantly increased (up to 26%) (Table I). These results suggest that, under these conditions (^2H_2O concentrations lower than 70% [v/v] and short incubation times), ^2H_2O does not inhibit overall cell metabolism, that the inhibition of growth is not due to a reduction of energy supply, and therefore that deuteration of biomolecules should be low and ^2H_2O should be present in the plant cells mainly as a free exchangeable fraction (13, 29).

The relationship between growth in length and H^+ efflux reported for maize roots (3, 10, 21) suggests that the effect of ^2H_2O on growth might be due to an inhibition of the acidification of the medium resulting from the inhibition of H^+ extrusion. This possibility is also suggested by the consideration that ^2H_2O could inhibit membrane activities through its chaotropic effect (7) or through a direct reduction of ^2H_2O on H^+ translocation. In agreement with these observations, the

H^+ extrusion activity of subapical segments of maize roots was greatly inhibited by the presence of ^2H_2O in the incubation medium (Table II). This inhibition increased with the concentration of ^2H_2O and was more evident when H^+ extrusion was stimulated by the presence of K^+. A similar inhibition by ^2H_2O (about 35%) has been found in vitro on both H^+ pumping and ATPase activities measured in plasma membrane vesicles of high purity (Table IV).

These results indicate that ^2H_2O directly inhibits H^+ translocation at the plasma membrane level. NADH-ferricyanide reductase and 1,3-β-glucan synthase, two plasma membrane activities present in the membrane vesicles, were slightly affected by ^2H_2O (14% or less, Table IV), thus suggesting that the inhibiting effect of ^2H_2O on the H^+-ATPase is not due to a general action of ^2H_2O on plasma membrane activities. Furthermore, the ATPase activities present in both tonoplast-enriched and submitochondrial particle preparations were not inhibited by ^2H_2O (data not shown), suggesting that ^2H_2O does not inhibit all the H^+-ATPase activities but that ^2H_2O specifically inhibits the H^+-ATPase at the plasma membrane level ("P-type" ATPase, 26). This specificity of action might be related to the biochemical differences that characterize the different H^+-ATPase categories. The effect of ^2H_2O on plasma membrane H^+-translocating ATPase could be due to an action on the phosphorylation-dephosphorylation mechanism and/or it could be due to a lower efficiency of H^+-ATPase in translocating $^2H^+$ instead of H^+.

The increase in ATP level observed in the presence of ^2H_2O (Table I) appears to be in agreement with the lack of inhibition of mitochondrial ATPase and with the reduction in the activity of the H^+ pump at the plasma membrane level that has been reported to consume as much as one-quarter to one-half of the cellular ATP (26).

The inhibiting action of ^2H_2O on H^+ extrusion was accompanied, at 0.05 mM external K^+, by a large inhibition of K^+ influx. At higher K^+ concentrations (0.5 and 5 mM), there was slight or no inhibition (Table III). It has been suggested that K^+ absorption is mediated by a high-affinity system, which predominates at low external K^+ concentration and is involved in the active K^+ absorption, and by a low-affinity system, which predominates at high K^+ concentration and is responsible for passive influx (9, 15). The inhibition of K^+ influx at low external K^+ concentration (0.05 mM) indicates that the presence of ^2H_2O inhibits mainly the high-affinity system, which operates against the electrochemical potential of K^+. ^2H_2O slightly hyperpolarized E_m (Fig. 1), suggesting that K^+ influx was inhibited even if the driving force for K^+ influx was not decreased by the presence of ^2H_2O. This suggests that at 0.05 mM external K^+ the absorption is not directly related to its electrochemical potential, in agreement with the hypothesis of an active transport of K^+. The parallel inhibition by ^2H_2O of both H^+ extrusion and K^+ influx together with the slight effect on E_m suggests that the inhibition of K^+ influx might be a consequence of the inhibiting action of ^2H_2O on the H^+-ATPase. This parallel inhibition can suggest a direct coupling between K^+ uptake and H^+ extrusion. It has been suggested that this mechanism should be excluded on the basis of thermodynamic and kinetic considerations (17) and it has been proposed that the high-affinity system for K^+ influx could be mediated either by a K^+-H^+ cotransporter (17, 25) or by a K^+-ATPase (17, 30). The parallel inhibition of H^+ extrusion and K^+ influx seems to be in agreement with the hypothesis of a K^+-H^+ cotransport.

At 0.5 and 5 mM external K^+, when K^+ is almost at equilibrium, the slight effect of ^2H_2O on K^+ influx was accompanied by no significant changes in E_m. The observation that, under these conditions, neither the driving force for K^+ influx nor K^+ influx are affected by ^2H_2O is in agreement with a passive electrophoretic movement of the cation (1, 23).

The fact that the decrease in H^+ extrusion induced at 0.5 and 5 mM K^+ by ^2H_2O was not accompanied by any depolarization of E_m indicates that ^2H_2O did not change the ratio between the influx and efflux of charges through the plasma membrane and might be explained by a decreased reabsorption of H^+ cotransported with other solutes and/or an increase in K^+ efflux. The lack of depolarization could also be explained by the consideration that at these higher external concentrations of K^+, E_m was governed mainly by ion gradients and that the reduction in H^+ extrusion did not influence E_m.

In conclusion, ^2H_2O appears to inhibit H^+ extrusion in maize root subapical segments through a direct effect on the plasma membrane H^+-ATPase; the decrease in root elongation might be a consequence of this inhibition. The effect of ^2H_2O on K^+ influx and E_m appears to depend on its action on the H^+-ATPase at the plasma membrane level, and supports the hypothesis of the possibility of a H^+-K^+ cotransport at low external K^+ and of a passive movement of K^+ driven by E_m at high external K^+.

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