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Effect of Inorganic Cations and Metabolic Inhibitors on Putrescine Transport in Roots of Intact Maize Seedlings

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ABSTRACT

The specificity and regulation of putrescine transport was investigated in roots of intact maize (*Zea mays* L.) seedlings. In concentration-dependent transport studies, the kinetics for putrescine uptake could be resolved into a single saturable component that was noncompetitively inhibited by increasing concentrations of Ca^{2+} (50 micromolar to 5 millimolar). Similarly, other polyvalent cations, including Mg^{2+} (1.8 millimolar) and La^{3+} (200 micromolar), almost completely abolished the saturable component for putrescine uptake. This suggests that putrescine does not share a common transport system with other divalent or polyvalent inorganic cations. Further characterization of the putrescine transport system indicated that 0.3 millimolar *N*-ethylmaleimide had no effect on putrescine uptake, and 2 millimolar *p*-chloromercuribenzene sulfonic acid only partially inhibited transport of the diamine (39% inhibition). Metabolic inhibitors, including carbonylcyanide-*m*-chlorphenylhydrazone (20 micromolar) and KCN (0.5 millimolar), also partially inhibited the saturable component for putrescine uptake (V_{max} reduced 48–60%). Increasing the time of exposure to carbonylcyanide-*m*-chlorphenylhydrazone from 30 minutes to 2 hours did not significantly increase the inhibition of putrescine uptake. Electrophysiological evidence indicates that the inhibitory effect on putrescine uptake by these inhibitors is correlated to a depolarization of the membrane potential, suggesting that the driving force for putrescine uptake is the transmembrane electrical potential across the plasmalemma.

Polyamines are widely recognized to have multifunctional effects at the cellular level in both plants and animals (for reviews, see refs. 22 and 24). In plants, stress-induced putrescine accumulation has been associated with a number of additional effects that appear to be important in the adaptation of plants to adverse conditions (24). Although early reports (10) suggested that putrescine acted as a "second messenger," incapable of cell to cell transport, more recently, Friedman *et al.* (9) demonstrated that putrescine levels increased in the xylem exudate of salt-stressed sunflower (*Helianthus annuus* L.) plants. A number of other reports have also shown putrescine to be translocated in root and shoot systems (1, 21). Recently, we (7) reported that radiolabeled putrescine loaded into maize roots for 24 h accumulated in the vacuole but was capable of moving back out of the vacuole across the tonoplast and the plasmalemma. Thus, we proposed that endogenously synthesized putrescine could be transported across plant cell membranes into the apoplasm and, subsequently, translocated via the xylem to other tissues or organs.

Although very little is known of the mechanism(s) and kinetics of putrescine transport across plant membranes, we (7) reported that the concentration-dependent kinetics for putrescine uptake across the plasmalemma in roots of maize seedlings could be resolved into a single saturable component (K_m = 120 μM, V_{max} = 0.397 μmol·g fresh weight^{-1}·h^{-1}), which we hypothesized to be a protein-mediated process. Similarly, a number of animal studies have shown putrescine uptake to be dominated by a saturable protein-mediated transport system with a K_m value ranging between 1.1 and 155 μM (for review, see ref. 23). In addition, we (11) found that other divalent cationic compounds, including the diamine cadaverine and the herbicide paraquat, competitively inhibit putrescine uptake in maize roots, whereas the tetramine spermine appeared to be a noncompetitive inhibitor. Studies with animal tissues have also indicated that paraquat shares the same transport system with putrescine (3, 25). This competitive interaction was suggested to be due to a similar charge distribution (0.6–0.7 nm) between the two amino groups for the two divalent cationic compounds (22). This hypothesis also explains our reported (11) competitive interaction between cadaverine and putrescine, because cadaverine has a charge distribution similar to that of putrescine, differing only by an additional carbon between the two amine groups. In contrast, spermine is a much larger molecule with a charge distribution (1.6 nm) considerably greater than that of putrescine (18).

Although it seems reasonable that organic molecules containing two similarly spaced monovalent cationic sites may compete for transport with putrescine, it is also possible that it is a nonspecific cation transport system. In support of this view, Pistocchi *et al.* (20) provided evidence suggesting that Ca^{2+} competitively inhibited the transport of putrescine across the plasmalemma of carrot (*Daucus carota* L.) cell cultures, whereas La^{3+} noncompetitively inhibited putrescine uptake. Oddly, high concentrations (>50 mM) of monovalent cations, Na^+ and K^+, were also considered to competitively inhibit the uptake of putrescine in carrot cell cultures (6). In reciprocal studies in which maize root segments were used, de Agazio *et al.* (6) reported that putrescine and other polyamines competitively inhibited the uptake of K^+. However, subsequent work by the same authors (5) indicated that the putrescine-induced reduction in K^+ uptake was not due to a direct

effect of putrescine but to the phytotoxic effect of the degradation products of putrescine oxidation, *e.g.* H_2O_2 and possibly oxygen radicals.

In plants, metabolic inhibitors appeared to have little if any effect on putrescine uptake (2, 20), suggesting that putrescine uptake in plants is not energy dependent. In contrast to the limited reports in plant tissues, several studies of fungi (4, 26) and animal tissues (3, 17, 25) have shown putrescine transport to be sensitive to metabolic inhibitors, indicating energy-dependent uptake of the diamine in these systems.

Thus far, reports of the effects of cations and metabolic inhibitors on putrescine transport in plants have considered only the effects of varying concentrations of the inorganic cation or inhibitor of interest on the uptake of a single concentration of putrescine. These experiments are difficult to interpret because they do not differentiate between an effect on the putative protein-mediated transport system and the cell wall component. Furthermore, they do not describe the effect of the cation or inhibitor on the kinetics of putrescine transport. Consequently, the present study was designed to determine the specificity of the putrescine transport system by examining the effect of mono- and polyvalent cations on putrescine uptake in roots of intact maize seedlings. In addition, we investigated the effect of metabolic inhibitors and sulfhydryl reagents on the kinetics of putrescine transport in maize roots. Results indicate that Ca^{2+}, Mg^{2+}, and La^{3+} noncompetitively inhibit putrescine uptake. In addition, sulfhydryl reagents had little to no effect on putrescine transport, whereas metabolic inhibitors and K^+ only partially inhibited putrescine uptake. The inhibitory effect of K^+ and CCCP[1] was closely correlated to the depolarization of the E_m. This latter point suggests that the driving force for putrescine uptake is the transmembrane electrical potential across the plasmalemma.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* L. cv 3377 Pioneer) seeds were surface sterilized in 0.5% NaOCl and germinated in the dark on filter paper saturated with 0.2 mM $CaCl_2$. After 3 d, germinated seedlings were selected for uniform growth, transferred to polyethylene cups with perforated bottoms (two to three seedlings per cup), and then covered with black polyethylene beads. Cups were then placed into precut holes in the covers of black plastic boxes containing 5 L of aerated 0.2 mM $CaCl_2$ solution. The seedlings were grown at 25°C under light intensity of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seedlings (5 d old) were used in all experiments.

Uptake Studies

Intact maize seedlings were placed in Plexiglas wells containing 60 mL of a solution consisting of 5 mM Mes-Tris (pH 6) and 0.2 mM $CaCl_2$. In experiments in which the interactions between putrescine and Ca^{2+} were compared, the concentration of $CaCl_2$ was altered accordingly. Uptake solutions were gently bubbled with air. Appropriate concentrations of unlabeled putrescine and treatment cation were added to each well 20 min before addition of 1.2 μCi (0.02 μCi/mL) of [^{3}H] putrescine, 2.0 μCi (0.033 μCi/mL) $^{45}Ca^{2+}$, or 3.0 μCi (0.05 μCi/mL) $^{86}Rb^+$. Metabolic inhibitors and the impermeant sulfhydryl reagent PCMBS were added to the uptake solution 30 min (and 2 h for CCCP) before the introduction of radiolabeled putrescine. Maize roots were exposed to the penetrating, covalently binding sulfhydryl reagent, NEM, for 5 min before the start of the experiment. Following a 20-min incubation in labeled uptake media, the solution in the wells was vacuum withdrawn, and uptake wells were refilled with ice-cold desorption solution consisting of 5 mM Mes-Tris (pH 6) and 5 mM $CaCl_2$. After two 7.5-min desorptions (15 min total desorption time), seedlings were removed from the wells, and roots were excised, blotted, weighed, and oven dried. We (7) have shown previously that this uptake-desorption regimen allows for the quantification of unidirectional putrescine influx across the root-cell plasmalemma. Dried roots were oxidized in a biological oxidizer (Harvey Instruments, Patterson, NJ), and trapped ^{3}H was measured in a Beckman LS5000TD liquid scintillation counter. From previous work (7), HPLC analysis of radiolabeled polyamines, extracted from maize roots following absorption from solution containing [^{3}H]putrescine, confirmed putrescine to be the primary radiolabeled compound. The data, combined from several uptake experiments, showed that putrescine represented 69.4% (SD 0.7%) of the total radiolabel in the root. Because there were no significant differences among experiments or among treatments, radioactivity in oxidized roots was adjusted for total putrescine after multiplying by 0.69.

Electrophysiological Studies

An intact maize seedling was placed in a Plexiglas chamber, which was attached to the stage of an Olympus compound microscope mounted on its back, on the surface of a vibration-damped table (Kinetic Systems, Inc.). Seedlings were allowed to equilibrate for 30 min in 0.2 mM $CaCl_2$ and 5 mM Mes-Tris buffer (pH 6). E_m was measured using a WPI model KS-750 amplifier and microelectrodes (tip diameter = 0.5 μm) made from single-barreled borosilicate glass tubing and filled with 3 M KCl (adjusted to pH 2 to reduce tip potentials). The reference electrodes, also 3 M KCl-filled micropipettes, were placed in the solution bathing the seed to minimize contamination of the solution bathing the root with K^+ diffusing from the reference electrodes. Root cells were impaled with the microelectrode using a hydraulically driven Narashige micromanipulator mounted on the microscope stage. The effect of 20 μM CCCP and 50 μM KCl was determined on the E_m of cells of the root epidermis and cortex approximately 2 cm from the root apex.

RESULTS

Interaction between Putrescine and Inorganic Cations

We (7) recently reported that the concentration-dependent kinetics for putrescine uptake into roots of maize seedlings were nonsaturating but could be resolved into a saturable

[1] Abbreviations: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; E_m, membrane potential; NEM, *N*-ethylmaleimide; PCMBS, *p*-chloromercuribenzene sulfonic acid.

component, hypothesized to be protein-mediated uptake, and a linear component, determined to be cell wall-bound putrescine not removed during the desorption period. In these experiments, we considered the possibility that Ca^{2+} may interact with the transport of the diamine, putrescine. Consequently, we examined the effect of Ca^{2+} on the uptake of 100 μM putrescine.

Results (Fig. 1A) indicate that increasing Ca^{2+} levels significantly reduced the uptake of putrescine in maize roots. In a solution containing 2 mM Ca^{2+}, putrescine uptake was reduced by 88% compared with uptake in 0.05 mM Ca^{2+}. Very little additional inhibition was measured at 5 mM Ca^{2+} (data not shown). To further show that this inhibitory effect was on putrescine transport across the plasmalemma, we measured putrescine translocation from roots to maize shoots after a 2-h accumulation of [^{3}H]putrescine (100 μM) in roots in the presence of 0.2 or 2.0 mM Ca^{2+}. Root exposure to the high Ca^{2+} concentration significantly inhibited the accumulation of putrescine in roots and subsequent translocation to shoots (Table I). Because the Casparian strip should presumably prevent the movement of putrescine from the bathing solution to the shoot via a purely apoplasmic route, we considered these results to reflect an inhibition in the transport of putrescine into the root cell symplasm and not a direct effect on putrescine translocation.

Table I. *Putrescine Translocation in Maize Seedlings following a 2-h Accumulation of [^{3}H]Putrescine in a Solution Containing 100 μM Putrescine, 5 mM Mes-Tris buffer (pH 6), and 0.2 or 2.0 mM Ca^{2+}*

Radioisotope in roots and shoots was determined immediately following a 2-h loading of [^{3}H]putrescine and a brief wash in deionized water. Values represent means of nine replicates. Numbers in parentheses are percentages ^{3}H at 2.0 mM Ca^{2+} compared with values at 0.2 mM Ca^{2+}.

Ca^{2+}	Putrescine Absorbed/Translocated			Total ^{3}H in Shoot
	Total	Root	Shoot	
Mm	*nmol putrescine g root fresh wt^{-1}*			%
0.2	442	399	42.9	9.7
2.0	138 (31)	128 (32)	9.9 (23)	7.2
LSD (0.05)	74	48	8.3	

The inhibitory effect of Ca^{2+} on putrescine uptake was similar to the effect of putrescine on Ca^{2+} uptake in a reciprocal experiment (Fig. 1B). This suggested that Ca^{2+} and putrescine either occupy similar charge sites on the surface of the plasma membrane or that they competitively inhibit the transport of each other, as was suggested by Pistocchi *et al.* (20) using carrot cell cultures. To test these proposed interactions, we examined the effect of varying Ca^{2+} levels on the concentration-dependent kinetics of putrescine uptake. Increasing Ca^{2+} concentrations dramatically inhibited the saturable component (predominant between 25 and 200 μM putrescine) of putrescine uptake (Fig. 2), whereas the slope of the linear components (predominant between 400 and 1000 μM putrescine) was only gradually reduced as the concentration of Ca^{2+} increased. Between 0.05 and 2 mM Ca^{2+}, the slope of the linear component decreased by 25%, which probably was the result of increased competition for cell wall- and membrane-binding sites. Over this same Ca^{2+} concentration range, however, the V_{max} for the saturable component for

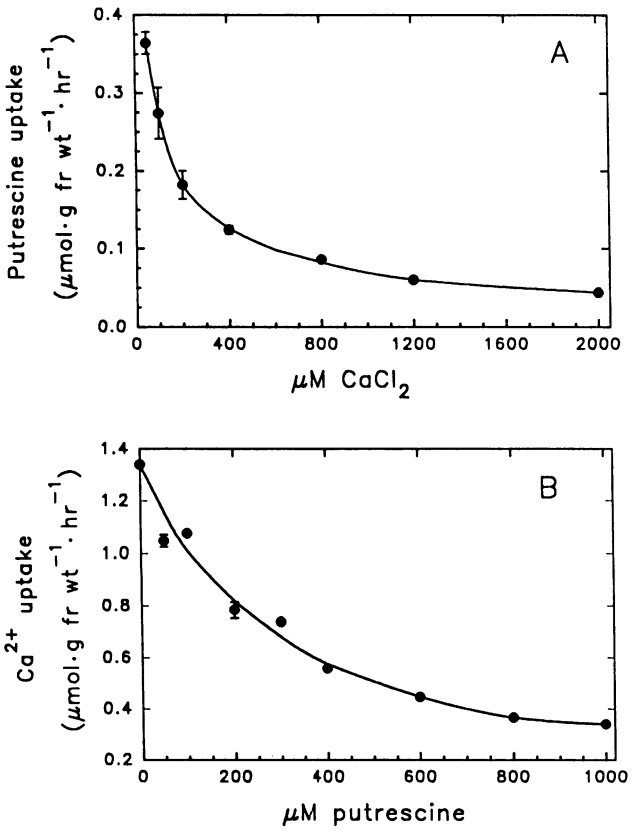

Figure 1. Effect of increasing concentrations of $CaCl_2$ on the uptake of 100 μM putrescine (A) and putrescine on the uptake of 200 μM Ca^{2+} (B). Uptake was for 20 min in either [^{3}H]putrescine or $^{45}Ca^{2+}$ and was followed by a 15-min desorption in ice-cold 5 mM $CaCl_2$ + 5 mM Mes-Tris (pH 6). Each data point represents the average of four replicates ± SD. fr wt, Fresh weight.

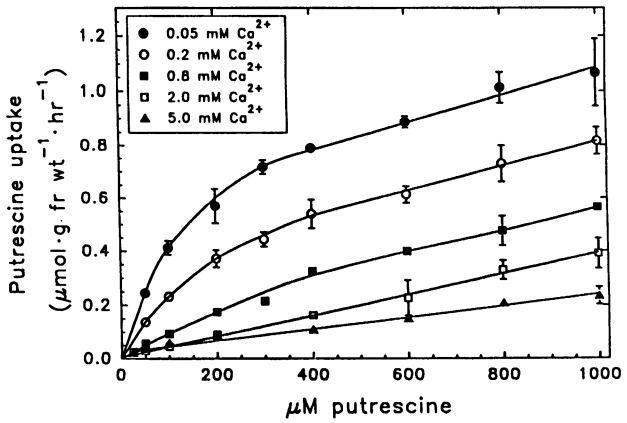

Figure 2. The influence of varying calcium concentrations in the uptake solution on the concentration-dependent kinetics of putrescine influx into intact maize roots. Data points represent the average of four replicates ± SD. fr wt, Fresh weight.

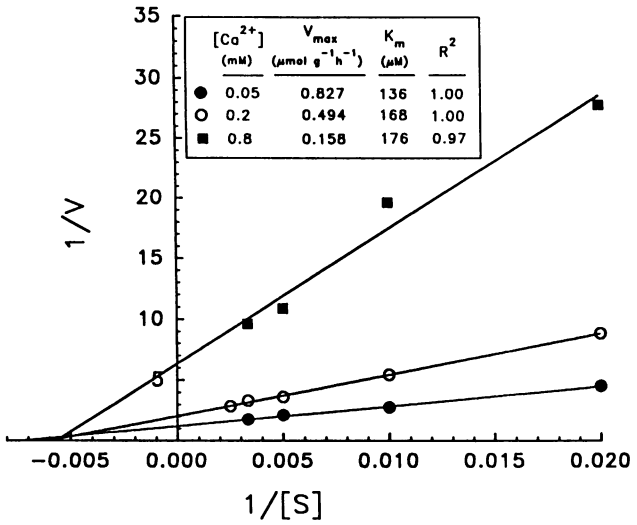

Figure 3. Lineweaver-Burk transformation of putrescine influx data from Figure 2 showing the effects of 0.05, 0.2, and 0.8 mM Ca^{2+} on the saturable component for putrescine transport.

putrescine uptake decreased by 98%, from 0.579 to 0.010 μmol·g fresh weight^{-1}·h^{-1}. Increasing the Ca^{2+} concentration from 2 to 5 mM continued to reduce the slope of the linear component by an additional 45%, again suggesting that Ca^{2+} and putrescine, to some degree, compete for similar cell wall- and membrane-binding sites.

Lineweaver-Burk transformation of the data for the saturable components of putrescine uptake indicate that Ca^{2+} noncompetitively inhibits the transport of putrescine into maize roots (Fig. 3). This finding supports the view that putrescine and Ca^{2+} do not share the same transport system. To further show that putrescine uptake across the plasmalemma is not mediated by a Ca^{2+} transport system, we examined the effect of the organic Ca^{2+} channel blocker, verapamil, on both Ca^{2+} and putrescine uptake. The inhibitory effect of verapamil on 0.2 mM Ca^{2+} transport was concentration dependent (Fig. 4). At 400 μM verapamil, Ca^{2+} uptake

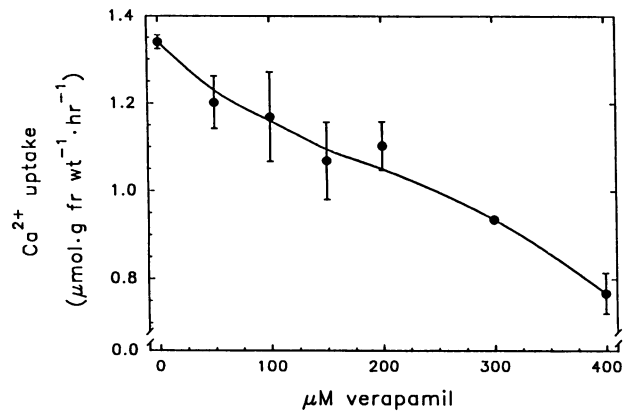

Figure 4. Influence of increasing verapamil concentrations on Ca^{2+} uptake (0.2 mM Ca^{2+}) in maize roots. Data points are the average of four replicates ± SD. fr wt, Fresh weight.

Table II. *Effect of Verapamil, KCl, CCCP, KCN, NEM, and PCMBS on the V_{max} of the Saturable Component for Putrescine Uptake*

Radiolabeled putrescine was determined after a 20-min uptake period. The linear component associated with cell wall binding was subtracted from total uptake values. Data represent the averages of four replicates.

Treatment	Duration of Pretreatment	V_{max}
	min	*% control*
0.4 mM verapamil	20	100
0.5 mM KCl	20	30
20 μM CCCP	30	52
20 μM CCCP	120	46
0.5 mM KCN	30	40
0.3 mM NEM	5	103
2.0 mM PCMBS	30	61

was inhibited by 43%. A similar concentration of verapamil had no effect on the saturable component for putrescine uptake (Table II). These results provide additional evidence to support the view that Ca^{2+} and putrescine do not share a common transporter.

In addition to Ca^{2+}, we also investigated the effect of other polyvalent cations, including Mg^{2+} and La^{3+}, on the concentration-dependent kinetics for putrescine uptake. Maize roots were exposed to 1.8 mM Mg^{2+} in addition to 0.2 mM Ca^{2+} for a total inorganic divalent cation concentration of 2.0 mM. Results (Fig. 5) were similar to those obtained with 2.0 mM Ca^{2+} (Fig. 2). Although Mg^{2+} nearly completely abolished the saturable component for putrescine uptake, it had little effect on the linear (cell wall) component. The effect of 0.2 mM La^{3+} was similar to that of 1.8 mM Mg^{2+} and 2.0 mM Ca^{2+} (Fig. 5).

From a previous report by de Agazio *et al.* (6), it was suggested that putrescine acted as a competitive inhibitor of K^+ uptake. In this study, we examined the effect of 0.5 mM

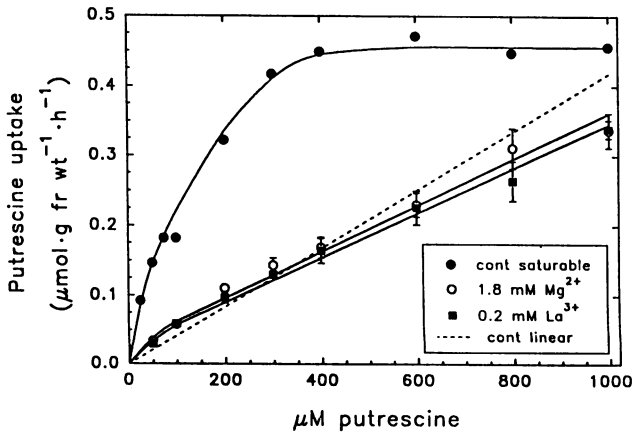

Figure 5. Influence of 1.8 mM Mg^{2+} and 200 μM La^{3+} on the kinetics of putrescine uptake in maize roots. ●, Saturable component for putrescine uptake in the control (cont) (0.2 mM Ca^{2+} in the uptake solution); -----, linear uptake in the control (cont), which is due primarily to cell wall binding. Data points for Mg^{2+} and La^{3+} treatments are the average of four replicates ± SD. fr wt, Fresh weight.

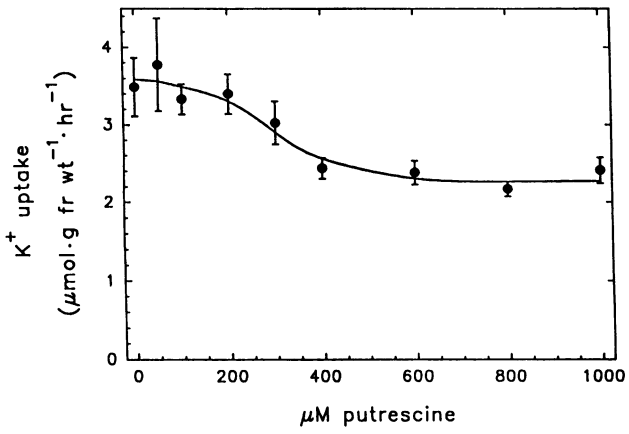

Figure 6. Potassium ($^{86}Rb^+$) uptake (in 50 μM K^+) in roots exposed to varying concentrations of unlabeled putrescine. All data points are the average of four replicates ± SD. fr wt, Fresh weight.

K^+ on the concentration-dependent kinetics of putrescine uptake in intact maize roots. Results indicated that K^+ partially inhibited (70%) the saturable component of putrescine uptake (Table II) but had no significant effect on the linear component (data not shown). In contrast, increasing putrescine concentrations had little effect on the uptake of 50 μM K^+ (Fig. 6). No inhibition in K^+ uptake was measured between 50 and 200 μM putrescine, and concentrations from 400 to 1000 μM resulted in no more that 33% inhibition in K^+ transport.

Effect of Metabolic Inhibitors and Sulfhydryl Reagents on Putrescine Uptake

Although animal studies have repeatedly demonstrated putrescine uptake to be sensitive to metabolic inhibitors, particularly CN^- (3, 17, 25), studies in which plant tissues were used indicate that putrescine uptake is not energy dependent (2, 20). However, a number of problems associated with these plant studies have led us to speculate that perhaps limited putrescine uptake was occurring under control conditions in these systems (7). Consequently, we examined the concentration-dependent kinetics of putrescine uptake following exposure to two metabolic inhibitors, an uncoupler (CCCP) and a respiratory inhibitor (KCN). Additionally, we examined the effect of two sulfhydryl reagents, PCMBS and NEM (5 min exposure), on putrescine uptake.

The inhibitory effect on the saturable component of putrescine uptake was similar regardless of the metabolic inhibitor or the pretreatment time (Table II). The V_{max} of the saturable component was inhibited by 48 and 60% following a 30 min pretreatment with 20 μM CCCP and 0.5 mM KCN, respectively. A 2-h pretreatment with 20 μM CCCP did not dramatically reduce putrescine uptake (54% inhibition) beyond that measured after a 30-min pretreatment. Sulfhydryl reagents had little effect on putrescine transport. A 5-min pretreatment in 0.3 mM NEM did not effect the uptake of putrescine into maize roots, whereas a 30-min pretreatment in 2 mM PCMBS caused only a 39% inhibition in the saturable component for putrescine uptake. Regardless of the treatment, no significant differences were measured in the slope of the linear component associated with cell wall binding (data not shown). Interestingly, a 30-min pretreatment with 0.5 mM KCN had no more inhibitory effect on putrescine uptake than did a similar concentration of KCl (Table II). Based on this result and the similar response of maize roots to 30-min and 2-h pretreatment with 20 μM CCCP, we hypothesized that putrescine transport was not directly coupled to ATP levels in the root. Alternatively, we considered that perhaps the reduction in the V_{max} by KCl, KCN, and CCCP was more directly related to the transmembrane electrical potential across the plasmalemma. It is widely recognized that K^+, CCCP, and CN^- depolarize the E_m across the plasmalemma (12, 16). In electrophysiological measurements, we found that 50 μM K^+ rapidly depolarized the E_m of maize root cortical cells by 64 mV (Fig. 7). Similarly, 20 μM CCCP caused a 101-mV depolarization of the E_m within 10 min of exposure but had little effect thereafter (70 additional minutes).

DISCUSSION

Evidence for Noncompetitive Inhibition of Putrescine Transport by Inorganic Cations

In petals of *Saintpaulia ionantha* Wendl., Pistocchi *et al.* (19) demonstrated that Ca^{2+} concentrations as high as 1.7 mM stimulated putrescine uptake. However, in a subsequent report (20) of a study in which carrot cell suspensions were used, they reported that Ca^{2+} stimulated putrescine uptake by 35% at 1 μM but inhibited uptake at concentrations between 50 μM and 1 mM. Based on these results, they suggested that Ca^{2+} competitively inhibited the transport of putrescine across the plasmalemma. Similarly, Davis and Ristow (4) reported Ca^{2+} to be a competitive inhibitor of putrescine uptake in the filamentous fungus *Neurospora crassa.* Although our results also show that increasing concentrations of Ca^{2+} or putrescine inhibit the uptake of the other ion, this inhibitory effect does not appear to be due to competition for either a Ca^{2+} channel (Fig. 4, Table II) or a putrescine transport system (Fig. 3).

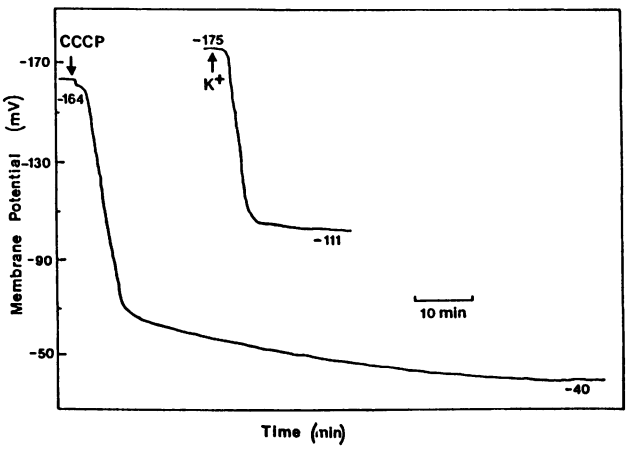

Figure 7. Representative time course of the response of the root cortical cell E_m of intact maize roots grown in a low concentration of salt (initial conditions: 0.2 mM $CaCl_2$ + 5 mM Mes-Tris buffer [pH 6]) to exposure to solutions containing either 20 μM CCCP or 50 μM KCl.

Consequently, we hypothesize that putrescine is not transported across the plasmalemma by a nonspecific divalent cation transport system. In contrast, the specificity of the system facilitating putrescine influx appears to be more closely related to the charge distribution and/or charge density of putrescine and other divalent cations (11). Thus, it appears more likely that the inhibitory effect of Ca^{2+} on putrescine uptake is due to shielding or binding of Ca^{2+} to sites on the membrane surface, which could include the diamine transport protein. In support of this view, Kaur-Sawhney and Galston (13) reported that the action of the tetramine spermine on Chl senescence was greatly reduced in the presence of Ca^{2+}. They concluded that the cationic property of Ca^{2+} interfered with the binding properties of polyamines.

The noncompetitive inhibition of putrescine uptake does not appear to be specific to Ca^{2+}. Other polyvalent cations, including Mg^{2+} and La^{3+}, also dramatically inhibited the saturable component for putrescine uptake. However, the trivalent La^{3+} was more effective in blocking putrescine uptake than either Ca^{2+} or Mg^{2+}. La^{3+} at 200 μM almost completely eliminated the saturable transport system for putrescine, whereas a similar concentration of Ca^{2+} resulted in significant putrescine uptake (Fig. 5). In carrot cell cultures, Pistocchi *et al.* (20) also found La^{3+} to inhibit putrescine uptake to a much greater degree than did Ca^{2+} at all concentrations between 10 and 500 μM. The ability of La^{3+} to dramatically inhibit the saturable component for putrescine uptake at a much lower concentration than was necessary for divalent cations is probably due to the increased capacity of La^{3+} to bind to or shield negatively charged membrane-binding sites. In support of this hypothesis, Kinraide *et al.* (14) found that higher valency cations ($C^{3+} > C^{2+} > C^{+}$) reduced the toxicity of externally applied Al^{3+}, La^{3+}, and H^{+} by reducing the cell surface activity of these ions.

Driving Force for Putrescine Transports

Kochian and Lucas (15) reported the saturable component for maize root K^+ uptake to be sensitive to a 5-min treatment with 0.3 mM NEM and slightly less sensitive to a 20-min treatment in 2 mM PCMBS. As a result, they suggested that saturable K^+ uptake involved sulfhydryls at both the exterior and interior surface of the plasmalemma. In contrast, we found that a similar exposure to 0.3 mM NEM had no effect on the saturable component for putrescine uptake. Although a 30-min treatment in 2 mM PCMBS inhibited saturable putrescine uptake by 39%, this is probably due to an increase in the cation concentration in the uptake solution, because PCMBS exists as a monosodium salt. Consequently, it does not appear that putrescine transport depends on sulfhydryls at either surface of the plasmalemma.

Previous reports suggested that metabolic inhibitors have little if any effect on putrescine uptake in carrot cell cultures (20) or in petals of *S. ionantha* (19). In carrot cultures, concentrations as high as 0.5 mM CCCP, NEM, dicyclohexylcarbodiimide, diethylstilbestrol, or Na-orthovanadate had no significant effect on putrescine uptake. Only 0.5 and 1.0 mM 2,4-dinitrophenol-treated cells exhibited a slight (20–34%) reduction in uptake of the diamine. In maize roots, however, we found that 0.5 mM KCN inhibited the V_{max} of putrescine uptake by 60% (Table II). Interestingly, this level of inhibition was similar to that obtained with 0.5 mM KCl. Consequently, CN^- does not appear to have an additive effect on the K^+-induced inhibition in putrescine uptake. A 20 μM exposure to the uncoupler CCCP for 30 min resulted in a 48% inhibition in saturable V_{max}. Because a much longer exposure (2 h) to the same concentration of CCCP caused only a slightly greater inhibitory effect, we speculated that putrescine uptake in maize roots is probably not ATP dependent.

Although putrescine uptake was not directly correlated with the time of exposure to CCCP or the addition of CN^-, there did appear to be a correlation between putrescine uptake and the transmembrane electrical potential in maize roots. Both CCCP and K^+ caused a significant depolarization in the E_m of maize root cortical cells (Fig. 7). Despite a dramatic CCCP- and K^+-induced reduction in the E_m, it is important to note that a significant downhill electrochemical gradient for putrescine uptake would still exist. Thus, it is not surprising that an E_m of −40 mV following exposure to CCCP would only partially inhibit putrescine uptake. In fungi (4, 26) and animal (3, 17, 25) cultures, putrescine uptake was also sensitive to metabolic inhibitors, particularly CN^-. Although putrescine transport was suggested to be an energy-dependent process in these systems, no direct relationship was established between putrescine uptake and the electrical potential across the plasma membrane.

Based on the results presented here and by Hart *et al.* (11), we hypothesize that the putrescine transport system is competitively inhibited by divalent cations (*i.e.* cadaverine and paraquat) where the +2 charge results from distinct monovalent amine groups that are separated by the same atomic dimensions as in putrescine. However, putrescine uptake is noncompetitively inhibited by other polyvalent cations, including Ca^{2+}, Mg^{2+}, La^{3+}, and spermine, which do not share a similar charge distribution. This noncompetitive effect is apparently due to charge shielding, binding to the putrescine transport protein, or to interactions with the lipid bilayer. In addition, putrescine uptake appears to have little or no sensitivity to sulfhydryl reagents but is significantly, although not completely, inhibited by K^+ and metabolic inhibitors. The similarity in the response to K^+ and metabolic inhibitors appears to be correlated with a depolarization of the E_m, suggesting that the transmembrane electrical potential across the plasmalemma of maize roots acts as the driving force for putrescine uptake.

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