Effect of Exogenous Putrescine, Spermidine, and Spermine on K⁺ Uptake and H⁺ Extrusion through Plasmamembrane in Maize Root Segments

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ABSTRACT

The action of exogenous polyanamines (putrescine, spermidine, and spermine) on 'washing' and fusicoccin-stimulated K⁺ uptake and H⁺ extrusion through the plasmamembrane in maize (Zea mays, hybrid line Pienus S 516) root apical segments was studied. The results showed that polyanamines inhibit the washing-stimulated K⁺ influx and H⁺ extrusion without interfering with K⁺ uptake and H⁺ extrusion stimulated by fusicoccin. Spermidine appeared to be the most effective in inhibiting K⁺ uptake and H⁺ extrusion while putrescine showed a smaller inhibiting action with respect to the others. The analysis of kinetic constants indicated that the polyanamines behave as competitive inhibitors with respect to K⁺.

The naturally occurring polyanamines elicit a variety of physiological responses ranging from promotion of growth to regulation of senescence (1, 2, 8, 16). When exogenously applied to ageing and detached tissue, polyanamines interact with membranes inducing changes which, in turn, lead to retardation of senescence. In fact, exogenous polyanamines are able to preserve Chl retention in thylakoid membranes of barley chloroplasts (14), to stabilize oat leaf protoplasts against lysis (7), or to reduce membrane permeability in discs of beet root storage tissues (13). However, to date, it is not yet clear whether the polyanamines associate unspecifically with negatively charged phospholipids because of their polycationic nature (15) or whether their interaction with cellular macromolecules is more specific (3, 17). Recently it was demonstrated that polyanamines selectively rigidify the surface of the plasma membrane, reducing fluidity of microsomal membrane from primary leaves of bean, and that some of the physiological effects of polyanamines could reflect membrane rigidification rather than a true physiological response (15).

As transport processes of anions, cations, and organic substances through the plasma membrane is its main function, it is interesting to know whether polyanamines, interacting with the membrane, can interfere with these processes. It was demonstrated that high external concentrations of Ca²⁺ ions, which, like polyanamines, are able to rigidify the membranes, decrease K⁺ uptake in wheat and cucumber (4) and ATPase activity in barley roots (5). A relationship between the inhibition of both K⁺ uptake and ATPase activity and Ca²⁺ induced reduction in mobility of the lipid polar head groups was suggested (4, 5).

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The purpose of this research was to study the effect of polyanamines on K⁺ influx and H⁺ extrusion in apical root segments of Zea mays. K⁺ influx and H⁺ extrusion were stimulated both by a 'washing' procedure and then by the fungal phytotoxin fusicoccin, after being inhibited by 'cutting' (10, 11). Results are reported that indicate a specific action of polyanamines on K⁺ influx and H⁺ extrusion, reflecting in our opinion, a physiological role of polyanamines at the plasma membrane level.

MATERIALS AND METHODS

Plant Material. Maize seeds (Zea mays L., hybrid line Pienus S 516) were supplied by the Dekalb Center (Chiarano, Italy). Maize seeds were continuously rinsed with tap water for 12 h and germinated on filter paper wetted with 0.5 mM CaSO₄ at 27°C in the dark for 3 d.

Washing Procedure. Tipis root apical segments (1 cm) of primary maize roots were collected and washed for 2 h in 0.5 mM CaSO₄ solution using 4 g fresh weight of tissue/L washing solution in a thermostated bath at 30°C in the dark.

Polyamine Treatment. Root segments after the washing procedure were treated with polyanamines (putrescine, spermidine, and spermine, chloride forms dissolved in 0.5 mM CaSO₄ solution) for 1 h in the dark in a thermostated bath at 30°C with shaking. Polyanamines were used at a concentration of 1 mM, unless otherwise indicated.

Influx Experiments. K⁺ influx determinations, measured with 86RbCl as tracer, were made using 150 mg samples of excised roots in 0.1 mM phosphate (K⁺ salt, pH 6) + 0.2 mM CaSO₄ as previously reported (6). 86RbCl (specific activity 1.24·10⁵ Bq g⁻¹) was supplied by Amersham International Limited, Aneresham, Bucks., U.K. For the determinations of kinetic constants 1 mM Tris-MES buffer + 0.2 mM CaSO₄ (pH 6.0) was used and the polyanamines were added during K⁺ uptake. The calculation was done using a linear regression program which gave best fit estimates for K⁺ and Vₘₐₓ. In the experiments with fusicoccin a concentration of 10 μM was used during K⁺ uptake.

H⁺ Extrusion Experiments. Samples of 150 mg of root apical segments were washed for 2 h and then transferred into 5 ml of aerated 1 mM Tris-MES buffer (pH 6.0) + 0.2 mM CaSO₄ with or without putrescine, spermidine, and spermine at a final concentration of 1 mM. After addition of polyanamines, the pH of the samples was adjusted to 6.0 by Tris, 10 mM. Samples were allowed to equilibrate for 30 min at 30°C and then KCl was added to a final concentration of 20 mM. After 15 min from the addition of KCl, fusicoccin was added to a final concentration of 10 μM. The H⁺ concentration changes were measured by titrating in a Radiometer TTT80 with 0.2 mM NaOH. During titration, N₂ was bubbled through the liquid. The H⁺ extrusion was measured
15 min after adding KCl and 1 h after fusicoccin treatment, using different samples.

Data reported in the tables and figure refer to a single typical experiment in three replicates. Three series of independent experiments were carried out giving reproducible results.

RESULTS

K⁺ influx through the plasma membrane was measured in root apical segments from 3-d-old maize seedlings washed for 2 h in CaSO₄ solution and pretreated for 1 h with different concentrations of putrescine, spermidine, and spermine in separate solutions. Washing of root apical segments was necessary to reactivate the K⁺ transport system strongly inhibited by 'cutting' (10).

Figure 1 shows the effect of different concentrations of putrescine, spermidine, and spermine on K⁺ uptake. Spermidine appears to be the most effective inhibitor over the entire range of concentration. The resulting K⁺ uptake inhibition increased from 11% at 0.5 mM to 82% at 20 mM concentration. Spermine exhibits a smaller inhibiting effect while putrescine shows a slight but reproducible activation at 0.5 mM followed by an inhibiting effect lower than those of the other polyamines.

In Table I the values of the kinetic constants $K_m$ and $V_{max}$ for K⁺ uptake in the presence and absence of polyamines are reported. The concentration range of KCl was between 2 and 50 mM. The increase in the value of apparent $K_m$ with no change of the value of $V_{max}$ in the presence of polyamines suggests that the mechanism of inhibition is competitive.

Since the activation of K⁺ uptake induced by fusicoccin is additive to that induced by washing, the effect of the polyamines on K⁺ uptake stimulated by fusicoccin was examined in washed root apical segments. The results show that polyamines do not inhibit the stimulation by fusicoccin of K⁺ uptake (Table II).

The effect of polyamines on proton extrusion induced by washing and then by fusicoccin was investigated, since this process is involved in the transport of cations through the plasma membrane (12). The experiments were carried out in the presence of high concentrations of KCl (20 mM) which is known to enhance H⁺ extrusion (9). Table III shows that, similar to the results of the K⁺ uptake experiments, polyamines inhibit H⁺ extrusion in washed root segments without interfering with the extrusion stimulated by fusicoccin. Also in this case putrescine exhibits a smaller inhibiting effect than those of spermidine and spermine.

DISCUSSION

It has been recently suggested that the decrease of plasma membrane fluidity caused by polyamines is due to a nonspecific effect measured when they act as polyvalent cations and associate with the negatively charged head groups of the bilayer phospholipids (15). In this context the inhibition, induced by polyamines, of K⁺ uptake and H⁺ extrusion through the plasma membrane reported in this paper could be explained as a consequence of the rigidification of membrane, in analogy with Ca²⁺, which at high external concentrations is known to reduce the mobility of lipid polar head groups (5) and to inhibit K⁺ uptake (4) and ATPase activity (5). On the other hand, the membrane rigidification previously reported was evident at high concentration (50 mM) while at 1 mM, the concentration used in our experiments. It was found that putrescine did not show any effect and spermidine and spermine decreased membrane fluidity by only 10% (15), it is unlikely that the inhibition of K⁺ uptake of about 50%, found at 1 mM, can be ascribed only to a nonspecific membrane rigidification. Our results support the different hypothesis that the effect of polyamines on K⁺ uptake and H⁺ extrusion is to be ascribed to a specific interaction of polyamines with the plasma membrane. In fact, the analysis of kinetic constants indicate that polyamines behave as competitive inhibitors of K⁺. Moreover, polyamines inhibit preferentially K⁺ uptake and H⁺ extrusion activated by washing and have no effect on that activated by fusicoccin. A nonspecific effect on plasma membrane, such as rigidification, would alter indiscriminately the biochemical functions of membrane.

In conclusion we suggest that the inhibiting effect of exogenous polyamines on K⁺ uptake and H⁺ extrusion through plasma membrane reflects a true physiological role. In fact, we would hazard the hypothesis that the endogenous polyamines are in-

![Figure 1](image-url)  
**Fig. 1.** Effect of different concentrations of polyamines on K⁺ (⁸⁶Rb) uptake. (○), Control; (●), putrescine; (△), spermidine; (□), spermine. Data reported refer to a single typical experiment in three replicates. Standard errors did not exceed 5%.

<table>
<thead>
<tr>
<th>Treatment (5 mM)</th>
<th>$R$</th>
<th>$K_m$ (mM ± SD)</th>
<th>$V_{max}$ (μmol g⁻¹ fresh wt h⁻¹ ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.99</td>
<td>6.06 ± 0.18</td>
<td>11.76 ± 0.29</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.99</td>
<td>8.69 ± 0.44</td>
<td>12.05 ± 0.48</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.99</td>
<td>22.22 ± 1.00</td>
<td>13.80 ± 0.62</td>
</tr>
<tr>
<td>Spermine</td>
<td>0.96</td>
<td>12.50 ± 0.44</td>
<td>9.09 ± 0.34</td>
</tr>
</tbody>
</table>
volved in the response of the root to cutting by selectively blocking the basal K⁺ uptake and H⁺ extrusion.

Further studies are in progress on this suggestive hypothesis and its relative implications.

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LITERATURE CITED