Putrescine Uptake in *Saintpaulia* Petals

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**ABSTRACT**

Putrescine uptake and the kinetics of this uptake were studied in petals of *Saintpaulia ionantha* Wendl. Uptake experiments of [\(\text{^3}H\)] or [\(\text{^{14}}C\)] putrescine were done on single petals at room temperature at various pH values. The results show that putrescine uptake occurs against a concentration gradient at low external putrescine concentration (0.5–100 micromolar) and follows a concentration gradient at higher external putrescine concentrations (100 micromolar to 100 millimolar). 2,4-Dinitrophenol and carbonyl cyanide-m-chlorophenylhydrazone, two uncouplers, had no effect on putrescine uptake. Uptake rates were constant for 2 hours, reaching a maximum after 3 to 4 hours. Putrescine uptake depended markedly on the external pH and two maxima were observed: at low external concentrations of putrescine, the optimum was at pH 5 to 5.5; at higher concentrations the optimum was at pH 8.

Much evidence suggests that ubiquitous polyamines such as putrescine, spermidine, and spermine are involved in plant growth and development and can be considered as a new class of plant growth substances (5, 12). A recent article on the positive effect of polyamines in apple fertilization showed that polyamine spraying increased fruit-set and yield per tree and fruit growth (9, 10). This induced us to examine the uptake and translocation of exogenous putrescine by apple corymb in short-term experiments. Isolated young leaves and flowers displayed the capacity to absorb this compound. Furthermore, long-term experiments on entire corymbs demonstrated that apple fruitlets and young leaves can synthesize polyamines and translocate putrescine via the peduncle (3). In other plant systems such as etiolated pea seedlings, little radioactivity was transported to shoots and roots from the cotyledons when radioactive putrescine or spermidine were injected (25).

At the present no data have been available on the mechanism of polyamine uptake, although many studies have been carried out on the mechanism of amino acid uptake (e.g. 7, 18, 23). The purpose of this research was to study the rates and the mechanism of putrescine uptake from the surrounding medium into petals of *Saintpaulia ionantha*. The effects of concentration, pH, and metabolic inhibitors were also investigated in order to obtain some data as to the mechanism involved.

**MATERIALS AND METHODS**

**Plant Material.** Plants of *Saintpaulia ionantha* Wendl were grown in the Botanical Garden. The experiments were performed at room temperature (20°C) and in continuous light (2000 lux). Thepetal epidermis was thin, without stomata and cuticle. Petals chosen were of the same size and age.

**Uptake Experiment.** Uptake experiments were done with single petals (weight, 50–60 mg; surface area, 2.0 ± 0.3 cm\(^2\)). Each petal was floated in a watchglass with the whole surface (usually the upper) in contact with the incubation medium. This consisted of radioactive putrescine (37 KBq in 5 \(\mu\)l [\(\text{^{14}}C\)] putrescine, 69.19 GBq/mmol or 7.4 KBq [\(\text{^{14}}C\)] putrescine, 4.03 GBq/mmol) and 100 \(\mu\)l of distilled H\(_2\)O (pH 5–5.5 unbuffered or at pH values ranging from 4 to 10). Some experiments were performed with 10 mm citric acid/trisodium acetate buffer (pH 5.5). At the end of the incubation time the petals were washed several times in tap water. They were then ground in 2 ml 0.1 N HCl and centrifuged for 10 min at 3,000g in a Sorvall Superspeed centrifuge (rotor SS 34). After centrifugation, 0.1 ml of the supernatant was mixed with 4 ml of scintillation fluid and radioactivity was determined in a Beckman scintillation counter with automatic quench compensation. The radioactivity in the pellet after 3,000g centrifugation ranged between 4% and 8% of total radioactivity according to the time course of the uptake experiment and was not considered in later experiments.

A preliminary experiment was done with intact flowers by placing a drop of [\(\text{^3}H\)] putrescine (37 KBq in 5 \(\mu\)l) on the central portion of each petal and leaving it for intervals of 10, 20, 30, and 60 min; the petals were excised at the end of the experiment and treated as described above.

Some uptake experiments were performed on leaf discs from 3-month-old *Nicotiana glauca* R.GRAH. Leaf discs (1 cm diameter, weight 20–22 mg) were punched with a cork borer from mature leaves. The discs were floated in watchglasses with the upper surfaces in contact with the incubation medium (20 \(\mu\)l of 7.4 KBq [\(\text{^{14}}C\)] putrescine and 100 \(\mu\)l of 50 mm unlabeled putrescine chloride) for 1 h, in the light (3000 lux) on a shaker.

**Fractionation.** *Saintpaulia* petals exposed for 1 h to 27.8 KBq of [\(\text{^{14}}C\)] putrescine in 50 mm unlabeled putrescine at pH 8 were ground in a mortar in 5 ml of medium containing 0.25 mm Tris-acetate (pH 8) and 2 mm EDTA according to Dohrmann et al. (11). The homogenate was fractionated by three consecutive centrifugations: 500g for 10 min, 9,000g for 20 min and 98,000g for 40 min at 4°C. The respective pellets were washed, recentrifuged, and resuspended in the grinding medium and 0.1 ml of each fraction was placed in scintillation fluid to determine radioactivity.

**Polyamine Analysis.** Polyamines were extracted, separated, and detected by the method of direct dansylation described by Smith and Best (22) using precoated silica gel 60 TLC plates with concentrating zone with ethylacetate:cyclohexane (2:3, v/v) as the solvent. Spots were scraped from the plates, extracted with acetone on a Vortex mixer, and centrifuged. Fluorescence was measured with a spectrofluorimeter (emission 505.5 nm, excitation 360 nm) and results compared with dansylated standards.

**ADC, ODC, and SAMDC Assay.** In preliminary experiments,

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\*Supported by CNR, Italy. Special grant I.P.R.A. Subproject 1.4. Paper No. 311.

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2 Abbreviations: ADC, arginine decarboxylase; ODC, ornithine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; PCA, perchloric acid; DNP, 2,4-dinitrophenol; CCCP, carbonyl cyanide-m-chlorophenylhydrazone; Pu, putrescine; Sd, spermidine; Sm, spermine.
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Fig. 1. Time course of [3H]putrescine uptake from the upper surface of petals in Saintpaulia flowers. 37 KBq of [3H]putrescine in 5 μl were supplied on the central portion of the petals. Each point represents the mean ± SD of five different measurements.

Fig. 2. Effect of pH on [3H]putrescine uptake at low external concentration (0.5 μM). Saintpaulia petals were floated, upper surface facing down, on 5 μl (37 KBq) of [3H]putrescine in 100 μl of water at pH values ranging from 4 to 10 during 30 min. The experiment was repeated five times with similar results. The figure represents a single experiment.

ADC and ODC activities were measured at various pH values ranging from 7.1 to 8.8 in Tris-HCl buffer. ODC activity showed a maximum at pH 8.1 and decreased rapidly at pH values above and below this. ADC activity showed a gradual increase up to pH 8.8. Petals (about 1 g fresh weight) were ground in a mortar in the assay buffer containing 50 μM EDTA, 25 μM pyridoxal phosphate, 2.5 mM 1,4-DTT, 100 mM Tris-HCl. The homogenate was then centrifuged at 26,000g for 10 min at 0°C. ODC activity was determined by incubating 0.5 ml of the supernatant, previously filtered through paper filter, with 14.8 KBq in 20 μl of L-[U-14C]ornithine (124 GBq/mmol). The ADC activity was determined in the same manner by using, instead of ornithine, 14.8 KBq in 20 μl of L-[U-14C]arginine (10.4 GBq/mmol). SAMDC activity was assayed following two methods: the method of Suresh and Adiga (24) at pH 7.6 and the method previously used for ADC and ODC activity at pH 8.1. In the former assay, petals were ground in a mortar in 0.2 M Tris-HCl (pH 7.6) buffer containing 5 mM 1,4-DTT, 5 mM Mg-acetate, and 0.1 mM pyridoxal phosphate, and then centrifuged at 26,000g for 10 min at 0°C. SAMDC activity was determined in the same manner as ADC and ODC activity, by using 9.25 KBq in 25 μl of S-adenosyl-L-[carboxyl-14C]methionine (2.22 GBq/mmol). About 35% higher 14CO2 release was obtained with the method of Suresh and Adiga (24) compared to the method used for ODC and ADC activity. In all cases, the test tubes containing the reaction mixture were capped with special rubber stoppers fitted with center wells, each containing 0.2 ml of protosol. The reaction was allowed to proceed for 2 h at 37°C in a water bath and was terminated by the injection of 0.2 ml of 6% (w/v) PCA. After an additional 60 min of shaking, the protosol was removed from the center well and placed in a scintillation counter.

Fig. 3. Effect of pH on [14C]putrescine uptake at high external concentration (50 μM). Saintpaulia petals were floated, upper surface facing down, on 20 μl (7.4 KBq) of [14C]putrescine in 100 μl of water at different pH values for 1 h. The experiment was repeated two times. The figure represents a single experiment.

Fig. 4. Time course of [3H]putrescine uptake from upper and lower surfaces of Saintpaulia petals. 37 KBq in 5 μl of [3H]putrescine were present in 100 μl of water at pH 5.5. The continuous line (-----) represents the curve obtained from experimental data (O, upper surface; ■, lower surface); the dotted line (----) represents the theoretical curve calculated by a cubic polynomial function with the method of least squares. Each point represents the average ± SD of three separate experiments.

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A similar pattern was observed in the same concentration range of putrescine at pH 8, but with different values of uptake. One mM putrescine in the external solution had a toxic effect on the petals. The data analyses with the linear expressions of the Michaelis-Menten equation according to Lineweaver-Burk (19) and Eadie-Hofstee (14) (Table I) were performed on a computer using a linear regression program which gave best fit estimates for $K_m$ and $V_{max}$. The $K_m$ and $V_{max}$ values change with the external pH and are of the same order of magnitude as those found for lysine uptake in excised barley roots (10) and for arginine and lysine uptake in barley leaf slices (9). The distribution of putrescine into subcellular fractions of *Saintpaulia* petals is shown in Table II. Putrescine was localized mostly in the supernatant of the 98,000g centrifugation step which presumably represents soluble fraction of cytoplasm and vacuoles. In addition, the 500g fraction contains a moderate amount of putrescine.

Metabolic inhibitors were used to determine whether putrescine uptake was due to active transport or not. DNP and CCCP, two uncoupler factors, were separately added at concentrations of 0.01 and 0.1 mM to the incubation medium containing 0.5 mM or 50 mM putrescine for time intervals ranging from 30 min to 3 h. The inhibitors tested had no effect on uptake of putrescine in *Saintpaulia* petals. To test further whether polyamine uptake was an active mechanism, we examined the effect of the same inhibitors on putrescine uptake by a system which is more active in phosphorylation such as leaves from *N. glauca*. The leaves were preincubated for 1 h in the presence of the two inhibitors, each at concentrations of 0.01 and 0.1 mM, which were also present in the incubation medium containing 50 mM putrescine; putrescine uptake, measured after a 1 h incubation, was not affected. Table III shows ADC, ODC, and SAMDC activities and the endogenous polyamine content in petals after treatment with 50 mM putrescine with respect to the untreated control. All the enzymatic activities measured were inhibited by added putrescine, while polyamine content, especially putrescine, increased. Like putrescine uptake, endogenous polyamines in untreated petals and putrescine content in petals exposed to 50 mM putrescine decreased with increasing numbers of flowers per plant.

### DISCUSSION

Based on the determination of free putrescine in the intracellular pool which was approximately 103 nmol/g fresh weight at 0 time (Table III) and assuming that 1 g fresh weight is roughly 90% H$_2$O, the intracellular concentration of putrescine (114 μM)

#### Table II. Distribution of Supplied Putrescine in Differential Centrifugation Fractions of *S. ionantha* Petals

<table>
<thead>
<tr>
<th>Putrescine Distribution</th>
<th>nmol/g fresh wt</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>500g pellet</td>
<td>178.1</td>
<td>12.7</td>
</tr>
<tr>
<td>9,000g pellet</td>
<td>96.9</td>
<td>6.9</td>
</tr>
<tr>
<td>98,000g pellet</td>
<td>29.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1100.6</td>
<td>78.4</td>
</tr>
</tbody>
</table>

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FIG. 5. Putrescine uptake over a wide concentration range. A, $10^{-3}$ to $10^{-1}$ M; B, $10^{-3}$ to $10^{-1}$ M. 7.4 KBq in 20 μl of [14C]putrescine were added to 100 μl of an unlabeled putrescine solution at various concentrations; the experiment was carried out for 1 h at pH 5.5. Each point represents the mean ± SD of at least three different experiments.

### RESULTS

Preliminary experiments showed that the rate of putrescine uptake from the upper surface of petals of *Saintpaulia* was constant for 1 h (Fig. 1).

Putrescine uptake depended markedly on the external pH and two maxima were observed: at low external concentration of putrescine, the optimum was at pH 5 to 5.5 (Fig. 2); at higher concentration of putrescine, the optimum was at pH 8 (Fig. 3). The external pH did not change during the time course of the experiment. The optimum pH for uptake of basic amino acids has been reported to vary with different plant materials and to range between pH 4 and 8 (6, 13, 18, 23). Putrescine and cadaverine uptake in mouse brain slices (17) increased with increasing pH whereas the uptake of amino acids decreased with increasing pH.

In the solution buffered at pH 5.5, putrescine uptake decreased by 60% in *Saintpaulia* petals as compared with the unbuffered control at the same pH during a 1 h experiment. Putrescine uptake was slightly higher at the lower surface than at the upper surface (Fig. 4). The decrease in uptake at the upper surface occurring at 4 h could be due to petal senescence. Putrescine uptake also declined with increasing number of flowers per plant.

At pH 5.5 the rate of putrescine uptake over a 0.01 to 100 mM concentration range was linear at the lower external concentrations (Fig. 5A) whereas it slowed down at the higher concentrations (Fig. 5B) producing a curve typical of saturation kinetics.

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### Table I. Kinetic Constants for Putrescine Uptake in *S. ionantha* Petals

<table>
<thead>
<tr>
<th>pH</th>
<th>Putrescine Concentration</th>
<th>Analysis</th>
<th>$R^2$</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (μmol g$^{-1}$ fresh wt h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>$10^{-5}$ to $10^{-1}$</td>
<td>Lineweaver-Burk</td>
<td>0.99</td>
<td>8.63</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eadie-Hofstee</td>
<td>0.88</td>
<td>12.11</td>
<td>4.55</td>
</tr>
<tr>
<td>8</td>
<td>$10^{-4}$ to $5 \times 10^{-2}$</td>
<td>Lineweaver-Burk</td>
<td>0.99</td>
<td>2.4</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eadie-Hofstee</td>
<td>0.82</td>
<td>3.0</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Correlation coefficient.*
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Table III. Enzymic Activities and Endogenous Levels of Polyamines in S. ionantha Petals

Enzymic activities and endogenous polyamine content were determined in untreated petals and in petals supplied with 50 mm putrescine for 1 h.

<table>
<thead>
<tr>
<th>Enzymic Activities</th>
<th>Polyamine Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control +50 mm putrescine</td>
<td>Inhibition</td>
</tr>
<tr>
<td>ADC</td>
<td>0.63</td>
</tr>
<tr>
<td>ODC</td>
<td>0.96</td>
</tr>
<tr>
<td>SAMDC</td>
<td>3.08</td>
</tr>
</tbody>
</table>

exceeded by at least 200-fold the putrescine concentration in the medium (0.5 μM) used for the experiments on pH- and time-dependent uptake (Figs. 2 and 4). Similarly, with 10 μM putrescine in the medium the intracellular putrescine concentration was 10- to 11-fold greater than in the medium and putrescine was accumulated against a concentration gradient up to 100 μM external putrescine concentration (Fig. 5A). By increasing the external concentration of putrescine (100 μM to 100 mM), the uptake followed the concentration gradient and saturation seemed to occur only at higher levels.

The high concentration of putrescine absorbed during the 1 h experiment, about 30 times higher than the endogenous level in the controls and 6% of 50 mM exogenous putrescine supplied, raises many questions on the significance of the putrescine within the cell. The appreciable difference between endogenous levels of spermidine and spermine in petals treated with exogenous putrescine and those of the controls suggests a utilization of putrescine as a precursor for polyamine synthesis. Not only ADC and ODC but also SAMDC were inhibited by about 50% in petals which had absorbed putrescine. The inhibition of ODC and ADC activities could be due to the regulation of these enzymes by putrescine and their inactivation as demonstrated for ODC by numerous authors in animal systems (e.g. 2). This fact suggests a certain regulatory capacity of isolated S. ionantha petals like other plant systems, such as undetached apple fruitlets and young leaves in which the putrescine absorbed was metabolized to spermidine and spermine (3). We have not observed diamine oxidase activity, but the high concentration of putrescine in petals could suggest a low level of activity of this enzyme. Thus, an explanation is that the putrescine absorbed was mostly compartmented in vacuoles as indicated in etiolated pea seedlings (4).

Up to now the only data for polyamine uptake were obtained using mouse mammary gland cells (15), mouse brain slices (17), and undifferentiated and differentiated NB-15 neuroblastoma cells (8). Spermidine uptake between 10 and 50 μM showed an apparent $K_m$ value of 0.47 μM (15) in mammary gland cells while the $K_m$ for putrescine had a value ranging from 2.75 to 28 μM in neuroblastoma cells (8). More recently, uptake of spermidine by cowpea prooplasts has been shown, but only after 20 h incubation (16).

With regard to the mechanism of putrescine uptake, this could be due to an ATP-independent carrier-mediated transport system and thus it could be a passive process. In fact, in secretory granules of adrenal medulla, an electrogenic carrier for catecholamines was identified that could partly explain the large internal concentration of catecholamines (1).

The biphasic effect of pH on putrescine uptake by Saintpaulia petals could be explained by uncharged putrescine at pH 8 and by the positively charged putrescine at acidic pH values. The label in the cell wall fraction could be due to the binding with the acidic groups of cell wall components. Although polyamines have an effect on the stabilization of cell membranes (20), probably as result of their binding to the phospholipid component of the membranes, little putrescine was found in the membrane fraction presumably because little putrescine was protonated at pH 8. At pH 8, most of the putrescine may pass through the membranes and it is probably compartmented in vacuoles. We also suggest that putrescine, at acidic pH, may be acetylated prior to its passage through the membrane, which could alter the cationic nature of its positive charges.

Recent studies have shown that acetylation and deacetylation of polyamines are important metabolic pathways which control intracellular polyamine levels and activity (21).

In conclusion, putrescine can be easily taken up into plant cells; this uptake is pH-dependent, occurs partly against a concentration gradient, and the rate of uptake is constant for a few hours.

Acknowledgment.—We wish to thank Professor Sergio Cocucci for suggestions and criticisms.

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