Loss of Recovery Capacity of Plasmalemma K+ Influx after Cutting in Chlorsulfuron Pretreated Maize Roots

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ABSTRACT

Active K+ influx was studied in apical segments from maize (Zea mays L., hybrid lines XL 342) and pea (Pisum sativum L. var Laxton superbo) seedlings pretreated with the herbicide chlorsulfuron (2-chloro-N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl)benzenesulfonamide).

Even though both plants were sensitive to chlorsulfuron, a strong inhibition of K+ uptake only was evident in maize root segments after 12 hours pretreatment with 10 micromolar chlorsulfuron. The inhibition was revealed only when maize root segments were washed for 2 hours before uptake measurements. This was done in order to recover K+ influx inhibited by cutting injury. Consequently, we demonstrated that roots from chlorsulfuron pretreated maize seedlings lost the capacity to recover from cutting injury by washing. By contrast, K+ influx in pea roots was not inhibited by chlorsulfuron because pea roots notoriously do not exhibit the 'washing' effect.

Chlorsulfuron (2-chloro-N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl)benzenesulfonamide) is the active ingredient in Du Pont 'Glean'. This new herbicide is primarily for weed control in small grain cereals and it is used at extremely low concentration (10–40 g active ingredient/ha for weed control in wheat) (9).

Previous studies on the mode of action of chlorsulfuron indicate that it acts by inhibiting plant cell division (12). This effect can be observed in corn root tips within 2 h of treatment and at a concentration as low as 28 nm (0.001 ppm).

Photosynthesis, respiration, RNA, and protein synthesis are initially unaffected under conditions where plant cell division is strongly inhibited. Higher concentrations 500 µM are required to inhibit these processes (2). It has also been demonstrated that chlorsulfuron does not inhibit plant cell division by a direct inhibition of DNA synthesis (13). Hence, at present, the primary site of chlorsulfuron action is unknown. Several herbicides are claimed to produce toxicity via a direct or indirect effect on plasma membrane (3, 14). The main function of plasma membrane is the control of ion exchange between the cytoplasm and the space external to the cell. Transport processes through the plasmalemma play a key role in the functioning of the cell and their dysfunction can be used as an indication of a primary action of the herbicide on the plasmalemma (7).

In this work we compare the K+ transport system capacity of root segments from control and chlorsulfuron pretreated maize seedlings, in order to investigate whether the plasmalemma is actually disturbed by chlorsulfuron.

Preliminary results reveal a strong inhibition of K+ uptake in chlorsulfuron pretreated roots but only after 'washing' the segments.

It is known that corn responds to a variety of injuries (cutting, rubbing, cold, or osmotic shock) by a rapid blockage of electrogenic H+ pumping with a resultant decline in cell potential and K+ influx. With washing, a gradual recovery of these physiological attributes has been demonstrated (1, 5, 8). Our experiments are carried out in order to demonstrate that roots from chlorsulfuron pretreated maize seedlings lose the capacity to recover the damage induced by cutting shock at K+ transport level.

MATERIALS AND METHODS

Plant Materials. Maize seeds (Zea mays L., hybrid lines XL 342) were supplied by the Dekalb Center (Chiarano, Italy). Pea seeds (Pisum sativum L. var Laxton superbo) were from a local seed supplier. Maize and pea seeds were continuously rinsed with tap water for 3 h and germinated on filter paper wetted with 0.5 mM CaSO4 at 27°C for 4 d (maize) and 5 d (pea) in the dark.

Chlorsulfuron Treatment. Seedlings were transferred to filter paper wetted with CaSO4 fresh solution with or without 10 µM chlorsulfuron for 12 h unless otherwise specified. Chlorsulfuron was kindly supplied by Du Pont (Wilmington, DE). The chlorsulfuron was of analytical grade.

Washing Procedure. Apical segments (0.6 cm) of primary maize and pea roots from seedlings pretreated with or without chlorsulfuron were collected and washed for 3 h in 0.5 mM CaSO4 solution, using 4 g fresh weight of tissue/L washing media at 30°C.

Influx Experiments. Influx determinations were made using 150 mg samples of excised roots incubated in 10 ml of 0.2 mM CaCl2 plus 0.1 mM phosphate (K+ salt, pH 6) labeled with 86Rb for 30 min. 86Rb was supplied by Amersham International Limited (Amersham U.K.) and the specific radioactivity was 289 mCi/mmol. KCl was added to the standard solution at a concentration 0.7 mM unless otherwise indicated. The experiments were run in a thermostatically regulated water bath at 30°C with shaking for 30 min. At the end of the incubation, the roots were briefly rinsed with 15 ml of ice-cold distilled H2O, washed for 15 min at 0°C with 20 ml unlabeled incubation solution, and finally rinsed with 20 ml of the same solution. The roots were suspended in Lumagel (Lumac B.V. Holland) and the radioactivity was counted with a liquid Scintillation Counter (LKB Wallac 1212 Ultradeta). Three series of independent experiments were carried out giving highly reproducible results. Data reported in the Tables and Figures refer to a single typical experiment in three replicates.

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RESULTS AND DISCUSSION

K⁺ influx through the plasmalemma was studied in 0.6 cm root apical segments from 3-d-old maize seedlings grown 12 h in the presence or absence of chlorsulfuron, before uptake measurements. Table I shows that chlorsulfuron pretreatment strongly inhibited K⁺ uptake both at low (0.7 mM) and high (10 mM) KCl external concentration. In order to investigate this effect of chlorsulfuron on K⁺ influx, four different chlorsulfuron concentrations were applied to corn roots for 12 h. At the concentration of 10 nM to 10 μM chlorsulfuron the resulting K⁺ uptake inhibition ranged from 12 to 40% with respect to the untreated roots (Fig. 1).

Moreover, the time course of the inhibition is such that at 10 μM chlorsulfuron, the inhibitory effect is evident after 5 h of pretreatment, reaching a maximum after 13 h and remaining stable thereafter (Fig. 2).

Because previous experiments with corn root tips (12) indicated that chlorsulfuron inhibits plant cell division, we performed our experiments only on apical root segments. To test whether

Table I. Effect of Chlorsulfuron on K⁺ (⁴⁺) Uptake in Maize Apical Root Segments

<table>
<thead>
<tr>
<th>KCl External Conc. (mM)</th>
<th>K⁺ (⁴⁺) Uptake</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>+ 10 μM Chlorsulfuron</td>
</tr>
<tr>
<td>0.7</td>
<td>2.88 ± 0.070</td>
<td>1.49 ± 0.092</td>
</tr>
<tr>
<td>10</td>
<td>7.93 ± 0.160</td>
<td>4.48 ± 0.100</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of chlorsulfuron different concentrations on K⁺ (⁴⁺) uptake after 12 h of pretreatment.

Table II. Effect of Chlorsulfuron on K⁺ (⁴⁺) Uptake at 0.7 mM KCl Concentration in Pea Root Segments

To test whether

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K⁺ (⁴⁺) Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh control</td>
</tr>
<tr>
<td>A</td>
<td>2.43 ± 0.068</td>
</tr>
<tr>
<td>B</td>
<td>2.52 ± 0.051</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of 10 μM chlorsulfuron after different hours of pretreatment on K⁺ (⁴⁺) uptake in maize root apical segments. Transport inhibition is expressed as per cent of the transport obtained in the control. Standard errors ranged from 3 to 8%.

Fig. 3. K⁺ (⁴⁺) influx in different root sections (□) from control roots, (■) from chlorsulfuron pretreated roots. Standard errors ranged from 2 to 5% of the values reported.

Fig. 4. Effect of washing for varying time periods on K⁺ (⁴⁺) influx in corn root segments excised from plants grown for 12 h before measurements in CaSO₄ (—) or in CaSO₄ + 10 μM chlorsulfuron (——).

the herbicide action on K⁺ uptake is peculiar to the dividing cells or to other regions of the root, we divided the maize roots into segments of 0.3 cm each. Figure 3 shows that all segments of chlorsulfuron pretreated roots show the same K⁺ uptake, while the maximum chlorsulfuron inhibition of K⁺ influx occurs in the segment corresponding to the elongation zone (0.3–0.6 cm from the apex) due to the fact that in control roots an increased uptake is observed with a maximum in the segment corresponding to the elongation zone.

It is known that cutting corn roots into segments causes an inhibition of K⁺ uptake (5). To eliminate this effect, the so-called washing of the roots is a widely used procedure (4). The ability of K⁺ uptake to recover by washing in maize root segments is
greatest near the apex and declines with increasing distance from the apex (10). Our data reported on control roots in Figure 3 are consistent with this observation. On the contrary, K⁺ uptake values in chlorsulfuron pretreated roots don’t exhibit any difference along the root and they all were lower than control values.

These data could be explained by hypothesizing that the chlorsulfuron pretreated roots are not able to recover from cutting injury during the washing treatment. For this hypothesis to be valid chlorsulfuron inhibition of K⁺ uptake should not appear in intact roots, or in a plant, such as pea, whose roots do not exhibit ion uptake enhancement by washing after cutting (11). In fact, the experiments performed on intact roots of maize seedlings show no inhibition of K⁺ uptake after chlorsulfuron treatment (2.55 ± 0.112 K⁺ μmol·g⁻¹ fresh weight h⁻¹ in control tissues and 2.69 ± 0.090 K⁺ μmol·g⁻¹ fresh weight h⁻¹ in pretreated tissues). Similarly, pea root segments do not show either enhancement of K⁺ uptake after 4 h of washing or inhibition by chlorsulfuron treatment (Table II). A further indication of the validity of this hypothesis can be found in the observation that maize root segments, showing an increase of K⁺ uptake at increasing washing times, exhibit a recovery from the injury of up to 100% of the initial value, while chlorsulfuron pretreated roots show an increase of only 20% within 1 h (Fig. 4).

In our opinion the chlorsulfuron pretreatment does not inhibit K⁺ uptake but the ability of K⁺ uptake to recover following inhibition by cutting. Washing is the essential step to bring out the inhibition which is not peculiar to the zone of the dividing cells but, according to our results, extends along the whole root.

The mechanism by which chlorsulfuron inhibits the recovery capacity could be found in the biochemical processes associated with cutting injury. Cutting causes a rapid increase in membrane permeability followed by an increase in free cytosol Ca²⁺ (15), and a rapid inhibition of H⁺ extrusion and K⁺ uptake probably through ATPase inactivation (6, 16). The inhibition of active transport is gradually relieved by washing. We hypothesize that in chlorsulfuron pretreated roots the alteration of membrane function is not reversed by washing. Studies are in progress on the biochemical steps involved in the loss of recovery capacity.

LITERATURE CITED