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EFFECT OF OCTYLGUANIDINE ON THE GROWTH OF ETIOLATED BARLEY SEEDLINGS

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Key Word Index—*Hordeum vulgare*; Gramineae, barley; growth inhibition; octylguanidine.

Abstract—The effect of octylguanidine on the growth of intact etiolated barley seedlings was tested. Inhibition of root and shoot growth was observed and this inhibition was partially relieved by addition of K^+ to the culture medium. Octylguanidine probably inhibits growth of roots and shoots by interfering with the transport of K^+ across the cell membrane.

INTRODUCTION

It has been shown that octylguanidine (OG) [1] is an effective inhibitor of the uptake of K^+ in excised barley, oat and corn roots [2–4] and it has been suggested that this inhibitor acts at the cell surface by interfering with the function of the ATPase of the plasma membrane [3]. It has also been reported that OG increases the permeability of the cell membrane of onion epidermis monolayers to water and methyl urea [5]. These findings suggested that OG affects the transport properties of membranes. In the present work, the action of OG on the growth of intact etiolated barley seedlings was studied. The effect of OG on the respiration of excised barley roots was also studied, to determine whether the action of OG on the transport and permeability properties of the membrane could be due to a primary effect on other metabolic systems.

RESULTS

The effect of OG on the length of roots and shoots of barley seedlings as a function of time is shown in Fig. 1. From the second to the fifth days the increment in length of controls was linear. OG (30 μM) added at the beginning of the experiment severely inhibited the elongation of roots; this effect was only partially reversible; growth resumed after the plants treated with the inhibitor were washed with water and transferred to inhibitor-free solutions, but the rate of growth was much lower than that of roots that had not been exposed to OG. The inhibitory action of OG was also observed after roots had undergone substantial growth. Only a slight inhibitory effect of OG on shoot growth was observed (Fig. 1B).

Figure 2 shows the effect of OG (30 μM) on the fresh and dry weights of roots and shoots of barley seedlings grown for 6 days. For comparative purposes the root and shoot lengths are also shown. Seedlings exposed to OG had smaller fresh and dry weights of roots than the controls. The results are expressed as % of the length and weight of roots and shoots incubated in the absence of OG. The results correlate with those of Fig. 1A, since

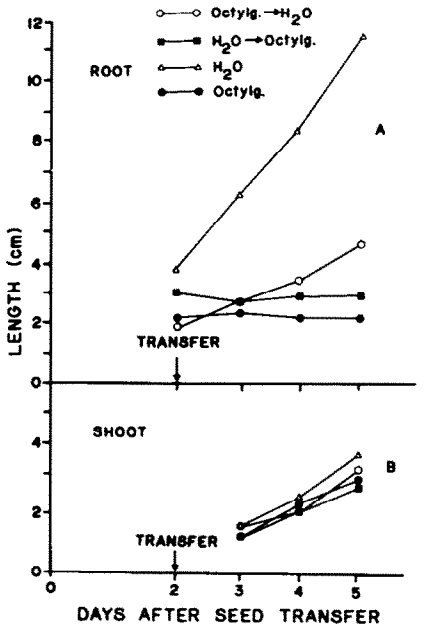

Fig. 1. Effect of OG (30 μM) on the elongation of barley seedlings. Two batches of plants (eight plants each) were grown in H_2O and two similar batches in OG soln for 2 days. At this time, one of the batches grown in H_2O (■—■) was transferred to OG containing soln. Another batch that had grown in the presence of OG was washed and transferred to H_2O (○—○). (▲) indicates growth of seedlings in media from which OG was omitted in the preincubation and incubation periods. (●—●) indicates growth of seedlings in the presence of OG in the preincubation and incubation periods. Mean values of 24 seedlings. (A) Root length. (B) Shoot length.

washing of the roots results in greater values for length and fresh and dry weights. In agreement with the results of Fig. 1B it was found that the effect of OG was much lower in shoots than in roots.

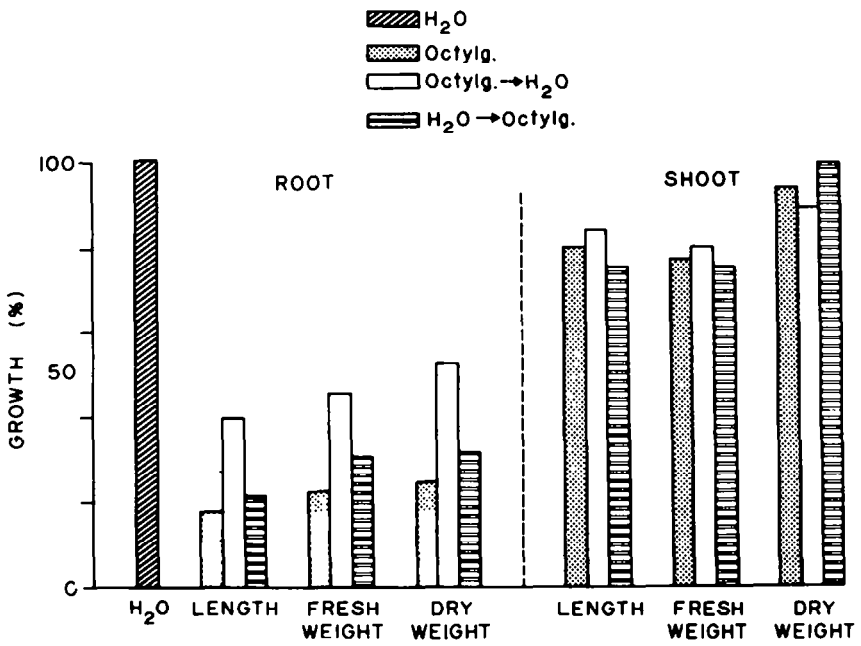

Fig. 2. Effect of OG (30 μM) on the length, fresh and dry wts of 6-day old barley seedlings. Experimental conditions as indicated in Fig. 1. Mean values of 24 seedlings.

The effect of different concentrations of OG on the growth of barley seedlings is presented in Fig. 3. Inhibition of root growth took place at all concentrations of OG that were tested. At the lower concentrations the inhibitor had a greater effect on the elongation than on the fresh and dry weights. Half maximal inhibition of root elongation was attained with about 5 μM OG. For shoot growth, OG is less inhibitory than for root growth, indeed

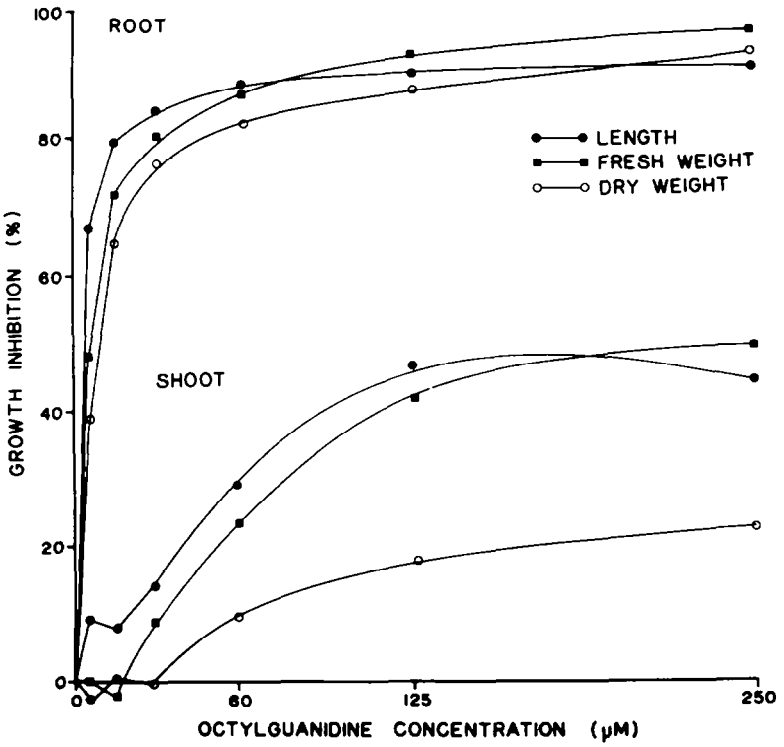

Fig. 3. Effect of different concns of OG on the length and fresh and dry wts of 6-day-old barley seedlings. OG was added at transplanting time. Mean value of 24 seedlings.

concentrations of OG at least one order of magnitude higher than in roots are required to induce half maximal inhibition of growth in shoots.

Since OG inhibits the absorption of K^+ by isolated roots [2–4] it was considered that this effect could account for the action of OG on growth. Therefore experiments were devised to determine whether K^+ reverse the inhibitory action of OG on growth. Except with 2 mM of potassium chloride in the medium, the growth of roots was not affected (Fig. 4). The data also show that increasing concentrations of K^+ in the growth media partially reversed the action of 5 μM OG on root growth; at 15 μM OG, K^+ induces, a very small reversal of the inhibitory OG action, as observed in the weight measurements. It is interesting that in shoots, although the action of OG is less dramatic than in roots, K^+ overcomes to a large extent the inhibitory action of OG.

OG was a more effective inhibitor of growth (Fig. 3) than of K^+ transport [2–4]. This suggests that inhibition of growth was the result of a primary OG action on cellular processes other than K^+ transport. The effect of OG on the respiration of excised roots was therefore studied. Oxygen consumption by roots pretreated or incubated with different OG concentrations showed that this compound did not affect respiration. Results suggest that OG was not accumulated by the cell, otherwise inhibition of respiration would have been observed. Therefore, it may be inferred that OG acts at the level of the cell membrane.

DISCUSSION

It has been shown that OG inhibits growth of roots and shoots. The inhibition of root growth is partially relieved by adding K^+ to the culture medium, while that of shoots is fully overcome by K^+. This indicates that in shoots the action of OG is strictly related to K^+ action. In view of the data in previous reports [2–4] it is more likely to be due to an interference with the K^+ transport. In roots, the action of OG seems to be more complex. Indeed the concentrations of OG required to inhibit growth and K^+ transport are different (5 μM for half maximal inhibition

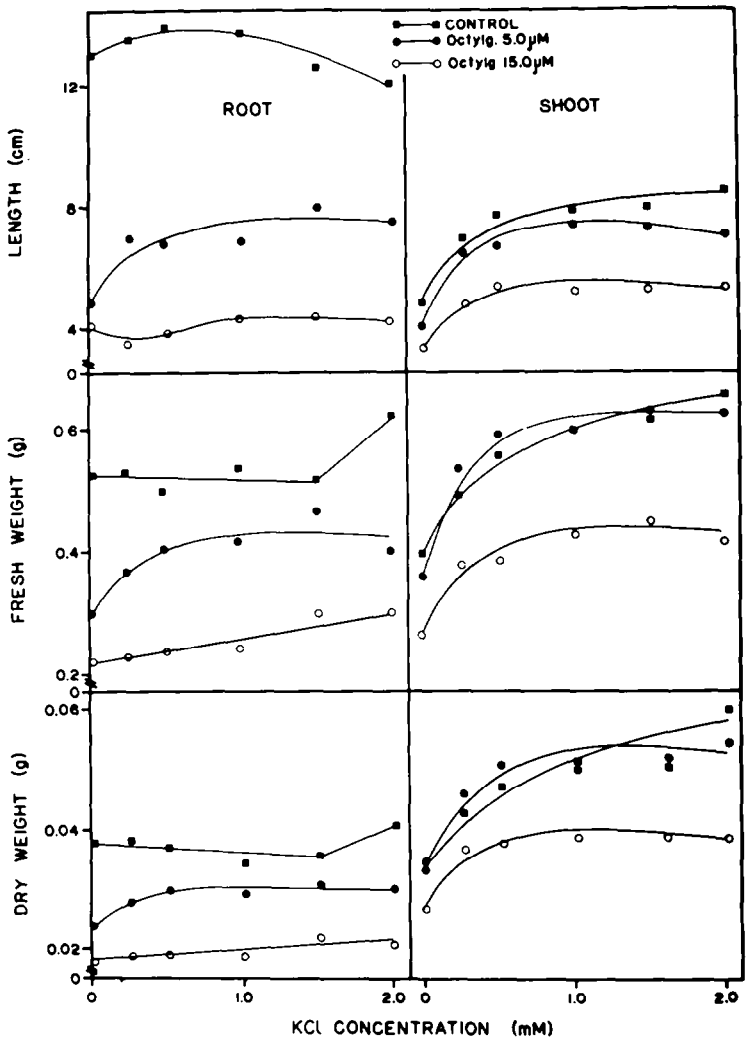

Fig. 4. Influence of increasing external K^+ concn on the growth of 6-day old barley seedlings treated with two OG concns (5 and 15 μM). Growing solns contained $CaSO_4$ (1 mM). Salts were added at transplanting time. Mean values of 24 seedlings.

of growth and 50 μM for transport) and the inhibition of the former process is only partially reversed by K^+. These differences indicate that, in roots, growth inhibition is due to an effect on K^+ transport plus an additional action on other metabolic process. However it should be pointed out that the measurements of K^+ transport were made for incubation time in minutes, whilst the growth experiments required a period of hours. Over short periods of time OG may not accumulate to a significant extent in cells, since it did not inhibit tissue respiration. This suggests that the inhibition of K^+ transport over the short absorption time is due to an effect of OG at the cell surface, while the inhibition of growth results from inhibition of K^+ absorption, as well as interference with oxidative phosphorylation [3]. Thus the time dependent interaction of the inhibitor with its target may account for the differences in concentration required to affect the process. Nevertheless the data obtained clearly indicate that octylguanidine inhibits growth of roots and shoots, probably by interfering with K^+ transport across the cell membrane.

EXPERIMENTAL

Growth experiments. Barley seeds (*Hordeum vulgare* var. Apizaco MV-72) were washed × 3 with H_2O, soaked for 24 hr in continuously aerated H_2O and rinsed again × 3 with H_2O. Eight seeds were transferred to a plastic screen covered with cheesecloth. This was supported by a 150 ml glass beaker that contained the culture solns; the corners of the cheesecloth were in contact with the soln. The seedlings were grown in the dark at 24° ± 3° under continuous aeration. The composition of the culture solns and subsequent treatment varied in each expt. The culture soln had an initial pH of 7. After transfer to the plastic screen, seedlings were harvested at different periods of growth. At harvest, the plants were separated into shoots and roots. The roots were rinsed with H_2O and blotted with paper towel. The length and the fresh and dry wts (60°) of roots and shoots were determined. Expts were repeated at least three times. Calculated standard deviations were lower than 5% of each data point.

Respiration experiments. For assay of oxygen consumption the following protocol was used. Seedlings of barley were grown in the dark under continuous aeration in a soln of 0.5 mM $CaSO_4$ as described earlier [6]. Roots of 6-day old seedlings were excised and rinsed several times with cold H_2O. Fifty one segments of 5 cm length (segment with 1 apical cm and 4–6 basal cm were discarded) were pooled for a single respiration experiment. Oxygen uptake was measured with a Clark electrode (Yellow Springs) at 30° in a water-jacketed 30 ml beaker which contained 20 ml of the incubation medium in two different conditions. In one, various concns of OG (30–500 μM) were added to respiring roots. In another, roots were preincubated with OG at 30° in a vol. of 100 ml for various times (1–10 min) and continuously shaken in a Dubnoff incubator. The roots were then removed from the inhibitor soln, rinsed with H_2O and transferred to the oxygraph vessel. Oxygen uptake was recorded under constant and vigorous stirring. Roots were removed from the vessel, washed with cold H_2O, blotted with paper towel and weighed. All results refer to fresh wt.

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