Rapid Hormone-induced Hyperpolarization of the Oat Coleoptile Transmembrane Potential

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ABSTRACT

The effects of the plant growth substances indoleacetic acid (IAA) and fusicoccin on the transmembrane potential of Avena coleoptile cells (at 27-29 °C) were studied. Fusicoccin caused hyperpolarization of the membrane potential which started after a lag of less than 20 seconds, and which on average reached -49 mv at an external K⁺ concentration of 1 mm and -75 mv at 0.1 mm K⁺. IAA caused a hyperpolarization of -25 mv starting after a lag of 7 to 8 minutes. These results suggest that fusicoccin and IAA both activate electrically H⁺ extrusion.

In this study, we have examined the time course of IAA and FC-induced changes in the PD and have shown both compounds induce hyperpolarization which appears to be related to the proton excretion found earlier.

MATERIALS AND METHODS

The plant material consisted of 15- to 20-mm sections cut from 25- to 32-mm long coleoptiles of Avena sativa, cv. Victory. The plants were grown and the sections were harvested as described earlier (1), except that the manipulations and all experimental procedures were carried out under fluorescent illumination. After removal of the leaves, the sections were peeled (19) and before use were preincubated for a minimum of 1 hr and a maximum of 3 hr in a solution containing 1 mm MES-tris (pH 6), 0.1 mm CaCl₂, and 0.1 or 1 mm KCl.

Transmembrane potential differences were measured with pairs of 3 m KCl-filled glass microelectrodes and the electronic equipment described earlier (10). Segments were mounted horizontally in the apparatus illustrated in Figure 1A of reference 17, and 2.5 to 3.5 ml of basic solution was added. In most cases, the measuring electrode was inserted through the external longitudinal wall of a cell from the first mesophyll layer, although in some cases, cells from the second mesophyll layer were used. When the PD had stabilized sufficiently, IAA or FC was added to bring the final concentration to 10 μM, a concentration which causes maximal H⁺ excretion (1, 4). Throughout the experiments, the PD was continuously recorded. Membrane resistances were measured before and after addition of the hormones, using a single electrode technique. The PD measured here is that for the outside vacuole, but probably reflects the PD at the plasma membrane since the PD across the tonoplast is believed to be small (10).

Three problems concerning these experiments need to be mentioned. First, the rapid growth of the sections, especially in response to FC, often caused sufficient bending of the electrode that it either broke, or leakage around the electrode and rapid depolarization took place. This could sometimes be counteracted by lateral displacement of the tissues or by inserting the electrodes deeper into the cell. A number of runs, however, were terminated prematurely and are not included in these results. Second, the experimental setup did not allow aeration of the solution or mixing following addition of the hormones, with the result that localized concentrations of hormones may have differed for short periods from the values given. Finally, the temperature in the room where the apparatus was housed and the solutions that were used was at 27 to 29 °C, a temperature considerably above that used in H⁺ excretion and K⁺ uptake studies (22-23 °C) (5, 6).

RESULTS

In the absence of hormones, a PD that averaged -109 mv was found in the presence of solutions having 1 or 0.1 mm KCl. The effects, in individual cases, of IAA and FC on the PD of Avena

1 This research was supported by United States Energy Research and Development Administration Contract AT(45-1)-2225-T19 to R. E. C. and National Science Foundation Grant BMS 74-18627 to N. H. B. A. P. was supported by a grant from the Dutch Organization for the Advancement of Pure Scientific Research (Z. W. O.) for his stay at Washington State University.
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4 Abbreviations: FC: fusicoccin; PD: transmembrane potential difference.
coleoptile cells are illustrated in Figure 1 and the data are summarized in Table I. In many cells there was a slight depolarization of 2 to 5 mv during the first 1 to 2 min after addition of IAA. After a lag that averaged 7 to 8 min, the PD then began to hyperpolarize and an average hyperpolarization of 26 mv occurred within 20 to 30 min. In contrast, FC induced hyperpolarization which began after a lag of generally less than 20 sec and which reached −49 mv after only 5 to 8 min when the external HCl level was 1 mm. A reduction in the KCl level to 0.1 mm had little effect on the membrane potential in the absence of hormones, but increased the FC-induced hyperpolarization to 75 mv. Addition of FC to auxin-treated sections caused a hyperpolarization of −22 mv in addition to the existing auxin-induced hyperpolarization.

This hyperpolarization could, in theory, be due to changes in resistance of the membranes to passive ion movement causing a change of the diffusion potential or an increase of the basic electrogenic mechanisms rather than to any increase in electrogenic ion flow. However, measurement of the resistance before and after addition of either hormone showed no consistent or large change in the resistance. The change in PD apparently was not due to a change in the external pH in response to the hormones, since a reduction in the pH of the external solution from 6 to 5.15 caused the PD to depolarize by only 4 mv (data not shown).

**DISCUSSION**

This paper provides the first time course for the effect of auxin on the membrane potential of plant cells. We have found that auxin causes a hyperpolarization of the PD starting after a lag of 7 to 8 min. This lag is less than that reported with *Avena* coleoptiles for auxin-induced growth (10-14 min) (6, 11) or H⁺ excretion (14 min) (6), but it must be remembered that these PD experiments were run at 27 to 29°C while the growth experiments were conducted at 25°C and the H⁺ efflux experiments at 22 to 23°C. Since the lag for auxin-induced growth is known to decrease with an increase in temperature (11), one would expect the lag prior to the change in PD to be shorter than 10 min. In previous experiments with *Avena* coleoptiles, auxin-induced hyperpolarization after 2 hr (9) and depolarization of the PD after longer periods (14) have been found.

The time course for FC-induced hyperpolarization of the PD recorded here for *Avena* coleoptiles resembles those published for squash cotyledons (16), barley roots (18), and corn roots (7). In each case, hyperpolarization began after a lag of less than 1 min, but considerable differences exist in the amount of hyperpolarization and the speed with which it was reached. For example, a hyperpolarization of about −50 mv was reached in squash cotyledons after 30 min (16), while the same amount of hyperpolarization took only about 12 min in barley roots (18) and 5 to 8 min in *Avena* coleoptiles (Table I). In both *Avena* coleoptiles and barley roots (7), the hyperpolarization of the PD correlates well with the time course of hormone-induced H⁺ efflux and growth promotion (4), and in both of these tissues the amount of FC-induced hyperpolarization is reduced when the external K⁺ concentration is increased.

Both IAA and FC stimulate the uptake of K⁺ into *Avena* coleoptiles (5, 13). The passive equilibrium potential for K⁺ is given by $E_K = 59 \log K/K_i$. In the experiments here, $E_K$ would be about −85 mv, appreciably below the membrane potential of −109 mv. Thus, there is a driving force inward for K⁺ of 24 mv prior to treatment. The hyperpolarization induced by FC or IAA increases the inward driving force on K⁺ and might be sufficient to account for at least some of the K⁺ influx. However, this completely fails to account for H⁺ extrusion, since the electropotential gradient for H⁺ efflux will be uphill. From this it must be inferred that H⁺ excretion is an active process. Certainly the hyperpolarization is not consistent with a neutral K⁺/H⁺ exchange as proposed by Haschke and Lüttge (13) for the IAA effect. It is consistent with the hypothesis that IAA activates a proton pump such as a plasmalemma-bound ATPase (12) in which H⁺ is actively extruded and K⁺ moves in accord with the electrical gradient. Pitman et al. (18) have made this suggestion with respect to the effect of FC on barley roots.

A more specific model based on a carrier mechanism—not excluding an influence of the electrical gradient—has been proposed (5). In this model, the hormones might activate a carrier which transports protons outward and which then cycles back carrying a cation, in which case the PD remains unchanged, or cycles back without a cation, in which case the PD is hyperpolarized. Conditions which lead to a lower K⁺ uptake rate, such as a lower external K⁺ concentration, should result in a greater hyperpolarization of the PD. This is shown to occur with FC, supporting the earlier suggestion (5) that FC activates such a carrier system. The small and slow effect of auxin on K⁺ uptake compared with its effect on H⁺ excretion (5) makes it unlikely that IAA is acting in this fashion, although this possibility cannot be eliminated on the basis of the present evidence.

Results with IAA from longer experiments (3-15 hr) under somewhat different conditions suggest that the results obtained here are transient, i.e. hold for 0 to 2 hr. Longer term studies indicate that IAA-treated *Avena* coleoptile cells become depolarized by about 12 mv or more, accumulate about 30 to 100% more K⁺ than controls, and show 1.6-fold more K⁺ passive efflux from the cytoplasmic compartment while the vacuolar efflux is only slightly affected (13).
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