Hormonal Control of a Transplasmamembrane Electron Transport System in Plant Cells

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Introduction

Evidence has been presented for the presence of an electron transfer system in the plasma membrane of plant cells (1, 2). The system has been proposed as a transmembrane system that transfers electrons from internal reducing compounds to electron acceptors located external to the cell. Addition of external electron acceptors results in net proton release from the cells. The extracellular reduction of ferricyanide has been shown not to be due to the release of a reducing compound from the cell or to ferricyanide (FeCN), which is a membrane impermeable acceptor, penetrating or being transported into the cells.

The presence of plasma membrane electron transfer seems to be a ubiquitous feature of the plasma membrane of all organisms (7). Electron transfer in mammalian plasma membrane is well established and shows hormone control for FeCN reduction, both with isolated membranes when NADH-FeCN reductase is measured and with intact cells when external FeCN reduction (without NADH addition) is measured (3, 6, 9). A system for electron transfer to external acceptors has been described by Crane and collaborators using yeast (4). Here we present results showing a hormonal control of plasma membrane electron transfer in carrot cells.

Materials and Methods

A carrot cell culture line, obtained from Drs. P. M. Hasegawa and R. A. Bressan of the Purdue Horticulture Department, was grown in liquid suspension culture. Cells were harvested in the logarithmic phase of growth and washed with "assay solution": 0.1 M sucrose containing 0.01 M each of NaCl, KCl, and $CaCl_2$. The suspension was subsequently aerated and aliquots used as needed. Potassium ferricyanide (FeCN) was obtained from Fisher Scientific Co., Tris and Mes buffers were obtained from Sigma Chemical Co., sucrose from Mallinnckrodt. 2, 4-Dichlorophenoxyacetic acid (2, 4-D) was obtained from Nutritional Biochemicals Corporation. Fusicoccin was a gift of Professor Alessandro Ballio of Cittá Universitaria, Rome. An Aminco DW-2a spectrophotometer was used to measure FeCN reduction at 420 nm, using the dual beam mode with a reference at 500 nm. The FeCN concentration used was 0.4 mM. The sample was stirred continuously with a magnetic stirring assembly. A millimolar extinction coefficient of 1.0 was used for FeCN.

For the fusicoccin experiment cells were washed with growth medium without 2,4-D and placed in medium without 2,4-D for 8 hours, after which the growth medium was replaced by 0.1M sucrose. Cells in 0.1M sucrose with 0.02 M $CaCl_2$ and 0.05 M Tris-Mes, pH 7.0, were incubated with fusicoccin in the medium, added as an ethanolic solution, for 1 minute before FeCN addition. The percent effect after incubation with ethanol for 1 minute was substracted from the percent fusicoccin effect. The fusicoccin effects in Table I were obtained in the same way, except the cells were not incubated in medium without 2,4-D, but were washed twice with

TABLE I. *Fusicoccin Stimulation of Ferricyanide Reduction*

Fusicoccin (M)	10^{-9}	10^{-7}	10^{-5}	6×10^{-5}
Stimulation (%)	+ 0.4	+ 5.2	+ 37.7	+ 60.4

Cells were incubated 2.5 minutes with fusicoccin.
A rate was taken subsequent to FeCN addition.

"assay solution". Cells were incubated with fusicoccin for 2.5 minutes before FeCN addition.

2,4-D experiments were performed in "assay solution" with 0.013 M Tris-Mes buffer, pH 7.0. 2,4-D effects were obtained by measuring FeCN reduction over time and adding an aqueous solution of 2,4-D made up as the sodium salt, after the rate of FeCN reduction was observed to rise on aeration. The percent effect of 2,4-D was calculated from the maximum change observed after 2,4-D addition. In Table II 2,4-D was added in dimethylsulfoxide (DMSO) and the change on addition of DMSO to a second identical aliquot was subtracted from the 2,4-D effect. There was no large effect observed of the DMSO on FeCN reduction rate in the second control aliquot. The average value ± standard deviation is reported.

Results

Figure 1 shows fusicoccin stimulation of FeCN reduction by whole carrot cells after a 1 minute incubation, with two points for each concentration tested. The stimulation begins at about 10^{-7}M and maximum stimulation of about 20% at 10^{-4}M. The increase in stimulation with concentration appears to level off between 10^{-6}M and 10^{-4}M, as compared to 10^{-8}M to 10^{-6}M.

Table I shows fusicoccin stimulation of FeCN reduction after a 2.5 minute incubation. The stimulation again appears at about 10^{-7}M. The stimulation is higher throughout and does not saturate at the highest concentration, possibly a reflection of an effect from the high ethanol levels used for that concentration on metabolism or membrane permeability.

TABLE II. *2, 4-D Inhibition of Carrot Cell FeCN Reduction 2, 4-D Concentration 2×10^{-5}M*

Trial #	Effect (%)
1	- 14.9
2	- 13.6
3	- 25.5
4	- 32.5
5	- 16.9
	Average -20.7 ± 8.0

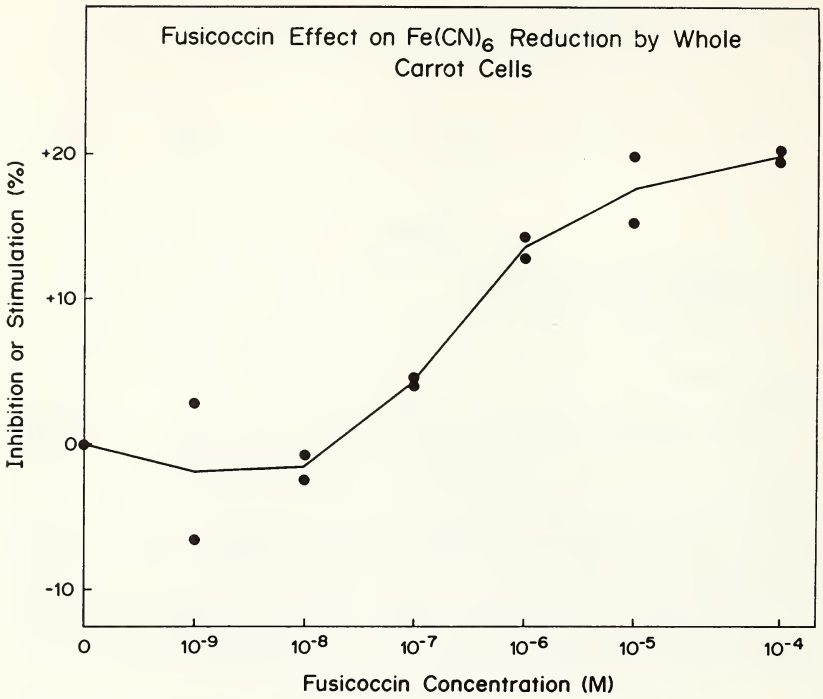

FIGURE 1 *Fusicoccin stimulation of ferricyanide reduction by carrot cells.*

Figure 2 shows the rise in FeCN reduction observed on aerating cells washed free of growth medium. 2,4-D at 1.8 x 10^{-5}M decreases this rate, beginning within a few minutes and continuing for an hour, when the rate of reduction is near the pre-rise level.

Table II is a summary of five of the types of experiments seen in Figure 2, using a 2,4-D concentration of 2 x 10^{-5}M, where the maximum inhibition ranges from 13.6 to 32.5 percent. The average is 20.7 percent inhibition.

Discussion and Conclusions

Results just presented indicate hormone control of plasma membrane electron transport in plant cells. Very little is known about how fusicoccin brings about its effects, but an electron transfer system in the plant plasma membrane could be one site of action in addition to a postulated stimulation of a plasma membrane H^+-ATPase. The stimulation of electron transfer by the toxin fusicoccin might exert important metabolic and control effects on the cell and be responsible for the changes in membrane potential and for the proton release that have been reported to be associated with fusicoccin. The redox system could interact with other plant hormones, or, if it is an NADH dehydrogenase, change the NADH/NAD^+ level in the cytosol.

The mammalian plasma membrane has a transmembrane NADH dehydrogenase (3). Results indicative of a transmembrane electron transfer system in plant cells have previously been presented (1) and this system shows the ratio of protons

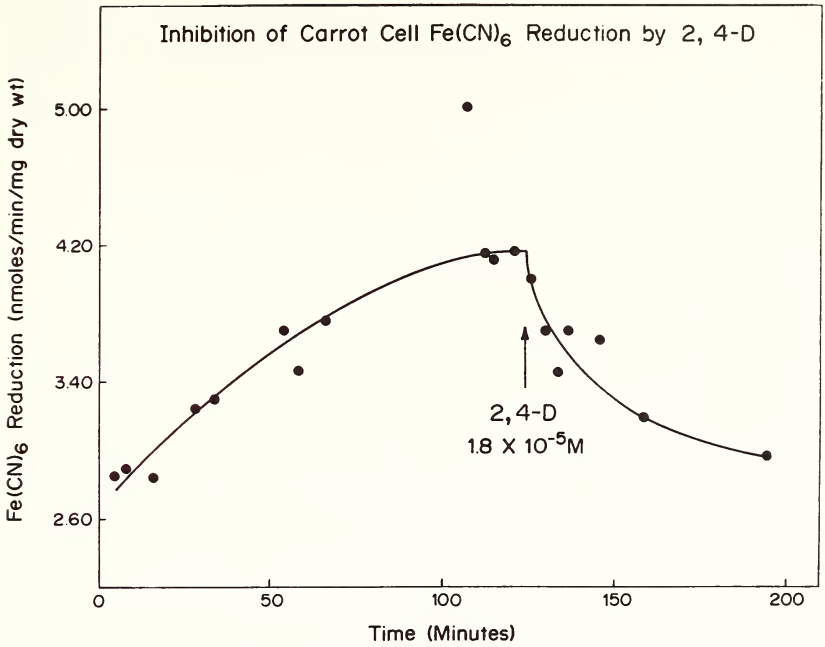

FIGURE 2 *2,4-D inhibition of whole cell ferricyanide reduction.*

released to FeCN reduced to be greater than one, which could hyperpolarize the cell membrane and suggests coupled proton transport, as in chloroplasts and mitochondria.

The 2,4-D inhibition observed in figure 2 and table II might be interpreted as the inhibition of an electron transfer system that is operating at a stimulated level. The stimulation could be due to the removal of 2,4-D from the cell, by outward diffusion from the cell as the cells are grown with 2,4-D at 2 x 10^{-6}M as the only growth regulator needed for growth in culture. The 2,4-D might inhibit the electron transfer system, allowing the cells to maintain a normal level of activity to oxygen, ferric iron or other acceptors. IAA (indoleacetic acid) also inhibits the rise in FeCN reduction rate.

We see opposite effects of fusicoccin and IAA for FeCN reduction by root tissue derived carrot cells and opposite effects have been seen by others on growth of corn root segments and lentile roots (5, 8). Here, in contrast to stimulatory effects seen by some on coleoptile and stem growth, fusicoccin stimulates and auxin inhibits FeCN reduction. Fusicoccin failed to stimulate growth in corn roots above 10^{-5}M, similar to Figure 1 of this study, where fusicoccin appears to approach a saturating concentration at 10^{-5}M.

In conclusion, the results demonstrate the control of plasma membrane electron transfer evidenced by fusicoccin stimulation and 2,4-D inhibition in carrot cells. Both auxins and fusicoccin are believed to have the plasma membrane as one of their sites of action. This system may be one means by which plant cells mediate their response to plant hormones and may explain in part the results observed with

fusicoccin. The basis of the responses of plants to hormones are poorly understood or unknown and there may be complex interactions of other hormones with this transmembrane electron transport system. This system may be one mode by which hormones bring about their interrelated action and varied effects, which include control of cell growth and development.

Acknowledgments

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