

THE EFFECT OF ELECTRON DONORS ON TRANSMEMBRANE FERRICYANIDE REDUCTION AND ASSOCIATED PROTON EXCRETION BY CULTURED CARROT CELLS

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INTRODUCTION

The natural electron donor to transplasma membrane electron transport with impermeable potassium ferricyanide as the electron acceptor can either be NADH (Barr, 1987, 1988; Barr, *et al.*, 1987; Böttger and Lüthen, 1986; Böttger, *et al.*, 1985; Bown and Crawford, 1988; Bown, *et al.*, 1988; Craig and Crane, 1981; Federico and Giartosio, 1983; Ivankina and Novak, 1980; Krüger and Böttger, 1988; Neufeld and Bown, 1987) or NADPH (Cakmak and Marschner, 1988; Lass, *et al.*, 1986; E. Marré, *et al.*, 1988; M.T. Marré, *et al.*, 1988; Rubinstein, *et al.*, 1984; Sijmons, *et al.*, 1984a, 1984b; Ze-sheng, *et al.*, 1984). Since either nucleotide is impermeable to living cells, since there is a NADH oxidase on the outside of cells that is stimulated by plant hormones (Brighman, *et al.*, 1988) or not (de Luca, *et al.*, 1984; Lin, 1982, 1984; Pupillo, *et al.*, 1986), and since NADH can chemically reduce ferricyanide, a new procedure for studying electron donor effects on cultured carrot cells was devised. It consisted of preloading these cells with electron donors for 15, 30, 45, or 60 min and washing any non-internalized donor off before assaying ferricyanide reduction and associated H^+ excretion. Such a procedure yielded good results with differences among electron donors.

MATERIALS AND METHODS

Carrot cells were grown in 250 ml Erlenmeyer flasks on 50 mls of culture medium as previously described (Barr, 1987). Transmembrane ferricyanide reduction and H^+ excretion assays were also performed as described therein.

Incubation of cells with four electron donors—NADH, sodium ascorbate, H_2O_2 , and $MnCl_2$ —was as follows: 5 ml aliquots of washed carrot cells suspended in sucrose-salts solution (0.1 M sucrose, 10 mM KCl , 10 mM $NaCl$ and 10 mM $CaCl_2$) were transferred to 15 ml centrifuge tubes and the required concentrations of electron donors were added. The tubes were mixed briefly on a Vortex and incubated for 15, 30, 45, and 60 min on a reciprocal shaker. Two min before the end of each incubation period, the tubes were filled with an additional 10 ml of sucrose-salts solution, mixed by stirring with a spatula, and centrifuged for 2 min in a table model International centrifuge at 1,500 RPM (about 200 x g) at room temperature. After centrifugation, the supernatant was discarded, and the cells were suspended in 5 ml of fresh sucrose-salts. Aliquots (0.1 ml) of these cells were used for simultaneous ferricyanide reduction and H^+ excretion assays. With practice, a single experiment with four separate incubation times could be conducted in 60 ± 10 min. However, in this study, each donor was used in three different concentrations, although only the significant data are reported here.

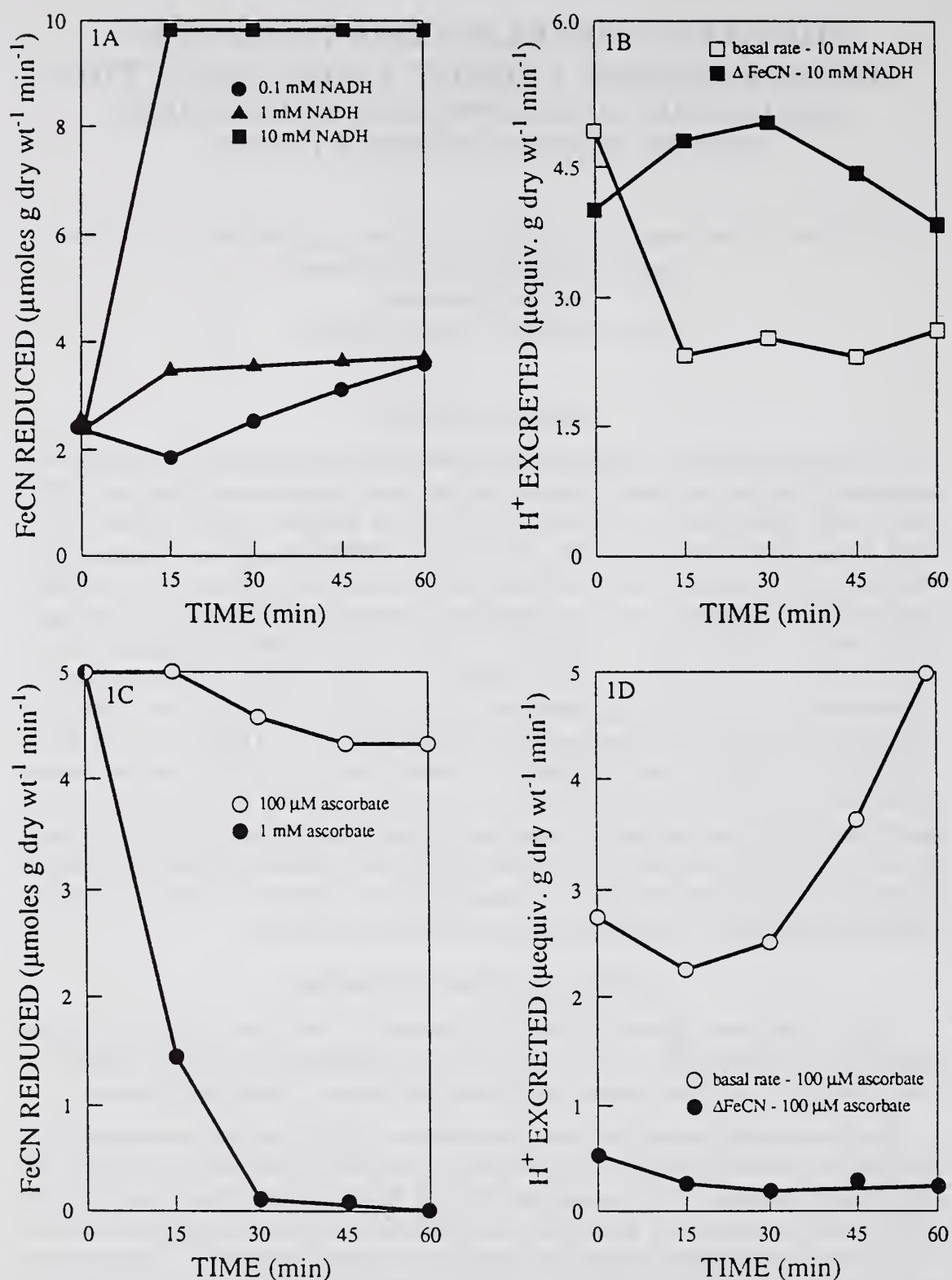


FIGURE 1. The effect of electron donors on transmembrane redox reactions and associated proton excretion by cultured carrot cells. **A.** The effect of NADH on ferricyanide reduction. **B.** The effect of NADH on H^+ excretion. **C.** The effect of sodium ascorbate on ferricyanide reduction. **D.** The effect of sodium ascorbate on H^+ excretion. Carrot cells were preloaded with electron donors. Excess donor was washed off after 15, 30, 45, or 60 min before assays for ferricyanide reduction or H^+ excretion. Assays are described in **Materials and Methods**.

RESULTS AND DISCUSSION

Transmembrane redox reactions involve electron donation to the electron transport chain by internal electron donors, NADH (Barr, 1987, 1988; Barr, *et al.*, 1987; Böttger and Lüthen, 1986; Böttger, *et al.*, 1985; Bown and Crawford, 1988; Bown, *et al.*, 1988; Craig and Crane, 1981; Federico and Giartosio, 1983; Ivankina and Novak, 1980; Krüger and Böttger, 1988; Neufeld and Bown, 1987) or NADPH (Cakmak and Marschner, 1988; Lass, *et al.*, 1986; E. Marré, *et al.*, 1988; M.T. Marré, *et al.*, 1988; Rubinstein, *et al.*, 1984; Sijmons, *et al.*, 1984a, 1984b; Ze-sheng, *et al.*, 1984). Both nucleotides are impermeable to plant plasma membranes, so that exogenous NADH can only activate an outside NADH oxidase (de Luca, *et al.*, 1984; Lin, 1982, 1984; Pupillo, 1986), which leads to stimulation of ferricyanide reduction and associated H^+ excretion by cultured carrot cells (Figures 1A and B). Sodium ascorbate, a presumed permeable electron donor to whole cells, in a low concentration (100 μM), slightly inhibited ferricyanide reduction and associated H^+ excretion, while the basal rate of H^+ excretion due to the action of the plasma membrane H^+ -ATPase was stimulated (Figures 1C and D). Hydrogen peroxide, another permeable electron donor, stimulated ferricyanide reduction (Figure 2A), but inhibited H^+ excretion in presence of ferricyanide (Figure 2B). Proton excretion by the H^+ -ATPase was slightly stimulated by hydrogen peroxide. This result is unexpected by a model of tight coupling between transmembrane ferricyanide reduction and associated H^+ excretion, but the relationship between these two processes is, probably, more complicated than a simple 1:1 relationship. A controversy still exists in the plant plasma membrane literature on this point. Originally, a tight coupling between ferricyanide reduction and H^+ excretion was suggested (Barr, 1987, 1988; Barr, *et al.*, 1987; Böttger and Lüthen, 1986; Böttger, *et al.*, 1985; Bown and Crawford, 1988; Bown, *et al.*, 1988; Craig and Crane, 1981; Federico and Giartosio, 1983; Ivankina and Novak, 1980; Neufeld and Bown, 1987), but lately various studies have shown (Lass, *et al.*, 1986; E. Marré, *et al.*, 1988; M. Marré, *et al.*, 1988; Rubinstein, *et al.*, 1984; Ze-sheng, *et al.*, 1984) that a more indirect relationship may exist in some cases, where only electrons cross the plasma membrane, but the H^+ liberated upon oxidation of NADH inside the cell may be deposited in a special membrane domain from which the H^+ -ATPase is activated by plasma membrane redox protons (Morré, *et al.*, 1986). Manganous chloride used as an electron donor to plasma membrane redox inhibited both ferricyanide reduction (Figure 2C) and redox-associated H^+ excretion in a concentration of 1 mM (Figure 2D); this concentration also inhibited 75% of the H^+ -ATPase excreted protons (Figure 2D). Thus, various electron donors, preincubated with carrot cells for 15, 30, 45, and 60 min and washed off before assays, evoked different responses from cells: (1) stimulation of both ferricyanide reduction and associated H^+ excretion (NADH); (2) inhibition of redox and redox-associated H^+ excretion, while stimulating the basal rate of H^+ excretion by the plasma membrane H^+ -ATPase (ascorbate); (3) stimulation of ferricyanide reduction, but inhibition of redox H^+ excretion, while stimulating H^+ -ATPase-mediated protons (hydrogen peroxide); and (4) inhibition of ferricyanide reduction and H^+ excretion by redox reactions and H^+ -ATPase (manganous chloride). The above results show that the relationship between transmembrane redox and associated H^+ excretion in relation to the action of the plasma membrane H^+ -ATPase is not a simple one.

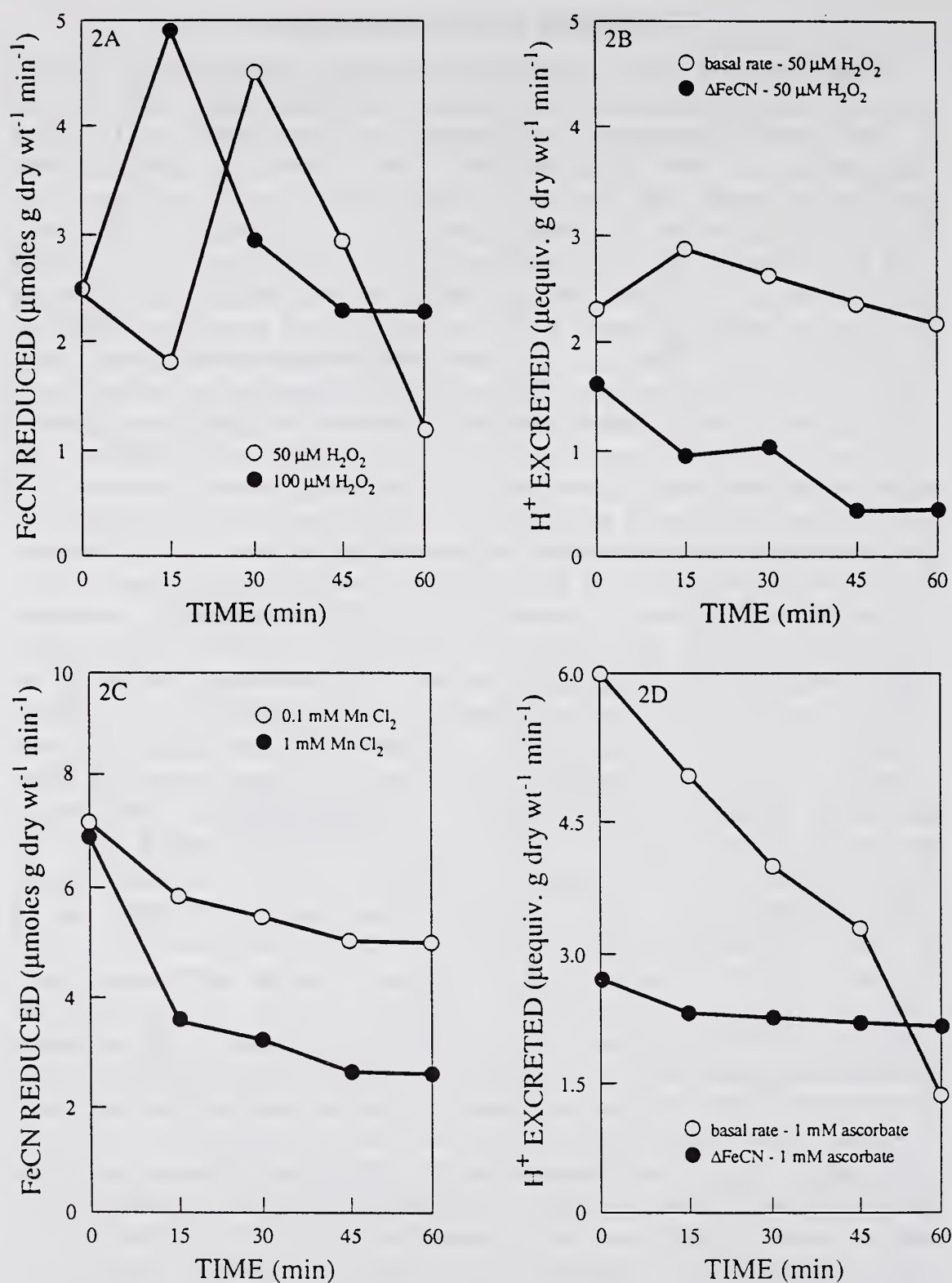


FIGURE 2. The effect of electron donors on transmembrane redox reactions and associated proton excretion by cultured carrot cells. **A.** The effect of H_2O_2 on ferricyanide reduction. **B.** The effect of H_2O_2 on H^+ excretion. **C.** The effect of MnCl_2 on ferricyanide reduction. **D.** The effect of MnCl_2 on H^+ excretion. Carrot cells were preloaded with electron donors. Excess donor was washed off after 15, 30, 45, or 60 min before assays for ferricyanide reduction or H^+ excretion. Assays are described in **Materials and Methods**.

ACKNOWLEDGMENTS

This research was supported by NIH Grant PO1 CA-36761 to F.L.C. The authors also thank Mrs. Bobby Taylor for providing the illustrations.

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