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THE EFFECT OF CHLORO-DERIVATIVES OF INDOLEACETIC ACID ON PLASMA MEMBRANE ELECTRON TRANSPORT AND PROTON EXCRETION

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ABSTRACT: As shown by Böttger and Hilgendorf [Plant Physiol. 86, 1038-1043 (1988)], the natural auxin indoleacetic acid in high concentrations (10 μM) inhibited both e^- and proton efflux by corn roots of undamaged plants measured with a pH-stat combined with a redoxstat. In this study we show that of various chloro-substituted indoleacetic acid derivatives only 4-chloro-IAA inhibited NADH oxidation and several derivatives stimulated trans-membrane hexacyanoferrate reduction in concentrations from 0.1nM to 10 μM in cultured carrot cells. Proton excretion by these cells was also stimulated by 5-, 6- and 5,7-chloro substituted indoleacetic acid, but the 4-chloro derivative inhibited H^+ excretion by the plasma membrane H^+-ATPase, while H^+ excretion in presence of hexacyanoferrate was stimulated. These divergent effects of chloro-substituted indoleacetic acid derivatives show that the trans-membrane redox system is not directly related to proton movement through the membrane in a 1:1 relationship.

Abbreviations. HCF III potassium hexacyano ferrate or potassium ferricyanide.

INTRODUCTION

Indole-3-acetic acid (IAA) is a growth hormone which induces rapid cellular responses in plant cells (Brummell and Hall, 1987). A hypothesis for the mechanism of auxin action according to Brummell and Hall (1987) is as follows: First, the auxin binds to a receptor in the plasma membrane, an act which promotes the inositol trisphosphate signal cascade (Boss and Morré, 1989) by which cellular Ca^{2+} is released. This results in the stimulation of protein kinases, which prepare an unknown protein to complex with auxin. The protein-auxin complex then induces m-RNA synthesis, which promotes cell wall loosening and leads to growth. These responses require at least 10-20 min for initiation and could, therefore, not be considered as the first plant response to auxin, whereas the addition of auxin to measurement of plasma membrane electron transport

reactions gives an immediate response in terms of stimulation of NADH oxidation or hexacyano ferrate reduction with associated increase in proton release.

Chlorinated auxins were chosen for the study of their effects on plasma membrane electron transport because they all show high biological activity, unless substituted in position 7 on the benzene ring unlike IAA itself (Pless et al., 1984). Since stimulation of plasma membrane electron transport by IAA and its chloro derivatives is a fast response, it could provide an initial mechanism for inducing the inositol trisphosphate signal cascade proposed by Brummell and Hall, (1987).

MATERIALS AND METHODS

Carrot cells were grown in suspension culture as previously described (Barr et al., 1985; and Barr et al., 1987). NADH oxidation was assayed spectrophotometrically with a DW-2A double beam spectrophotometer in the dual beam mode following a decrease of absorbance at 340nm with reference wavelength set at 430nm (Barr et al., 1985). Reactions were run at 24°C. The reaction mixture contained 25mM Tris-Mes, pH7, sucrose-salts solution (10mM KCl, 10mM NaCl and 10mM $CaCl_2$) and carrot cells (0.005gdrywt.). After a 3-min incubation period, 100μM NADH was added to start the reaction. Reaction rates were calculated using a millimolar extinction coefficient of 6.22. IAA or its chloro derivatives were added in concentrations indicated in figures.

Proton excretion was measured with a Corning combination pH electrode in a 5-ml water-jacketed cell at 24°C (Barr et al., 1987). The assembly was gently stirred on a magnetic stirrer during assays. The reaction mixture contained carrot cells (0.005gdrywt.), 25μM phosphate buffer, pH7, and IAA or its chloro derivatives added in ethanol. The basal rate of H^+ excretion was monitored for 5min, then 100μM ferricyanide was added to measure the contribution of protons from plasma membrane electron transport with ferricyanide as the impermeable electron acceptor on the outside of cells. Proton excretion rates were calculated from the addition of known amounts of HCl as described in (Barr et al., 1987).

IAA and NAA were purchased from the Sigma Chemical Co. (St. Louis, MO). The chloro derivatives of IAA were obtained from Dr. Böttger's laboratory, University of Hamburg, F.R.G.

RESULTS AND DISCUSSION

Indole-3-acetic acid (IAA) and α-naphthalene acetic acid (NAA) are known to promote growth in plant cells (Brummell and Hall, 1987), but the mechanism of auxin action is not clear and it may differ from species to species. The purpose of this study was to see if IAA and its chloro derivatives, as well as NAA, affected plasma membrane electron transport and H^+ excretion. A comparison of the effects of IAA, an auxin which can be oxidized by plant tissue (Pless et al., 1984; Reinecke and Bandurski, 1988; and Waldrum and Davies, 1981), with NAA, which cannot be oxidized, shows differences on cultured carrot cells: the rate of NADH oxidation is stimulated tenfold by NAA and only twofold by 0.01μM IAA (Figs. 1A, 1C). However, IAA in the same concentration had a greater effect on H^+ excretion than NAA (Figs. 1B, 1D). This may be significant, considering that the acid growth hypothesis, in which H^+ excreted by the plasma membrane H^+-ATPase may lead to cell wall loosening and increased growth (Hayashi, 1989; and Rayle and Cleland, 1982). The chloro derivatives of IAA (5-chloro IAA, 7-chloro IAA and 5,7-dichloro IAA) were tested to find out if they could act as

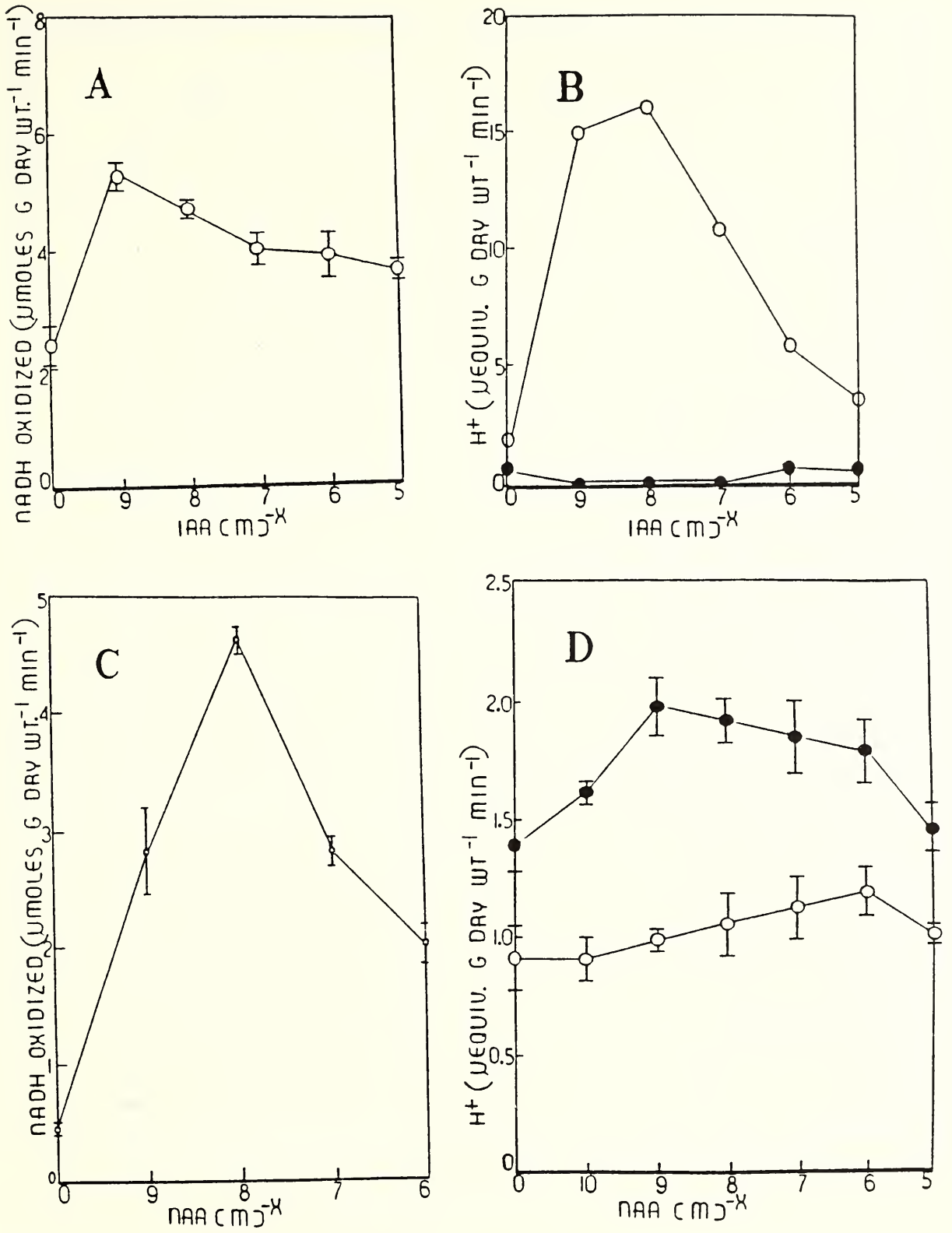

Figure 1. The effect of indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) on NADH oxidation and proton excretion by cultured carrot cells. A-rate of NADH oxidation at various IAA concentrations; B-rate of H^+ excretion without HCFIII (O) and with HCFIII (O) in presence of various IAA concentrations; C-rate of NADH oxidation with various NAA concentrations; D-rate of H^+ excretion without HCFIII (O) and with HCFIII (●) in presence of various NAA concentrations. NADH oxidation was assayed spectrophotometrically, H^+ excretion with a pH electrode, as described in Materials and Methods.

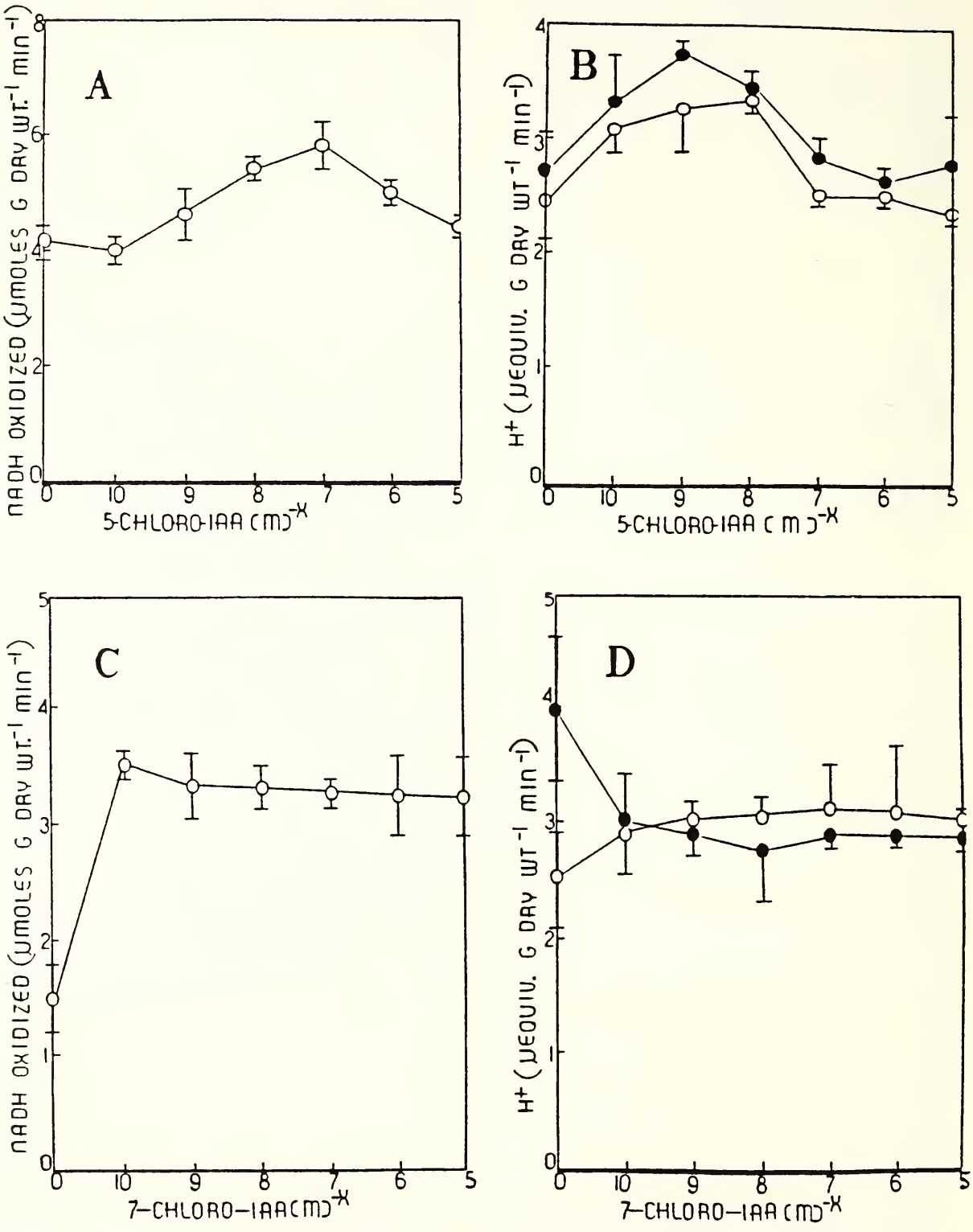

Figure 2. The effect of 5-chloro-indole-3-acetic acid (5-Cl-IAA) and 7-chloro-indole-3-acetic acid (7-Cl-IAA) on NADH oxidation and proton excretion by cultured carrot cells. A-rate of NADH oxidation at various 5-Cl-IAA concentrations; B-rate of H^+ excretion without HCFIII (O) and with HCFIII (●) in presence of various 5-Cl-IAA concentrations; C-rate of NADH oxidation at various 7-Cl-IAA concentrations; D-rate of H^+ excretion wihtout HCFIII (O) and with HCFIII (●) in presence of various 7-Cl-IAA concentrations. NADH oxidation was assayed spectrophotometrically, H^+ excretion with a pH electrode as described in Materials and Methods.

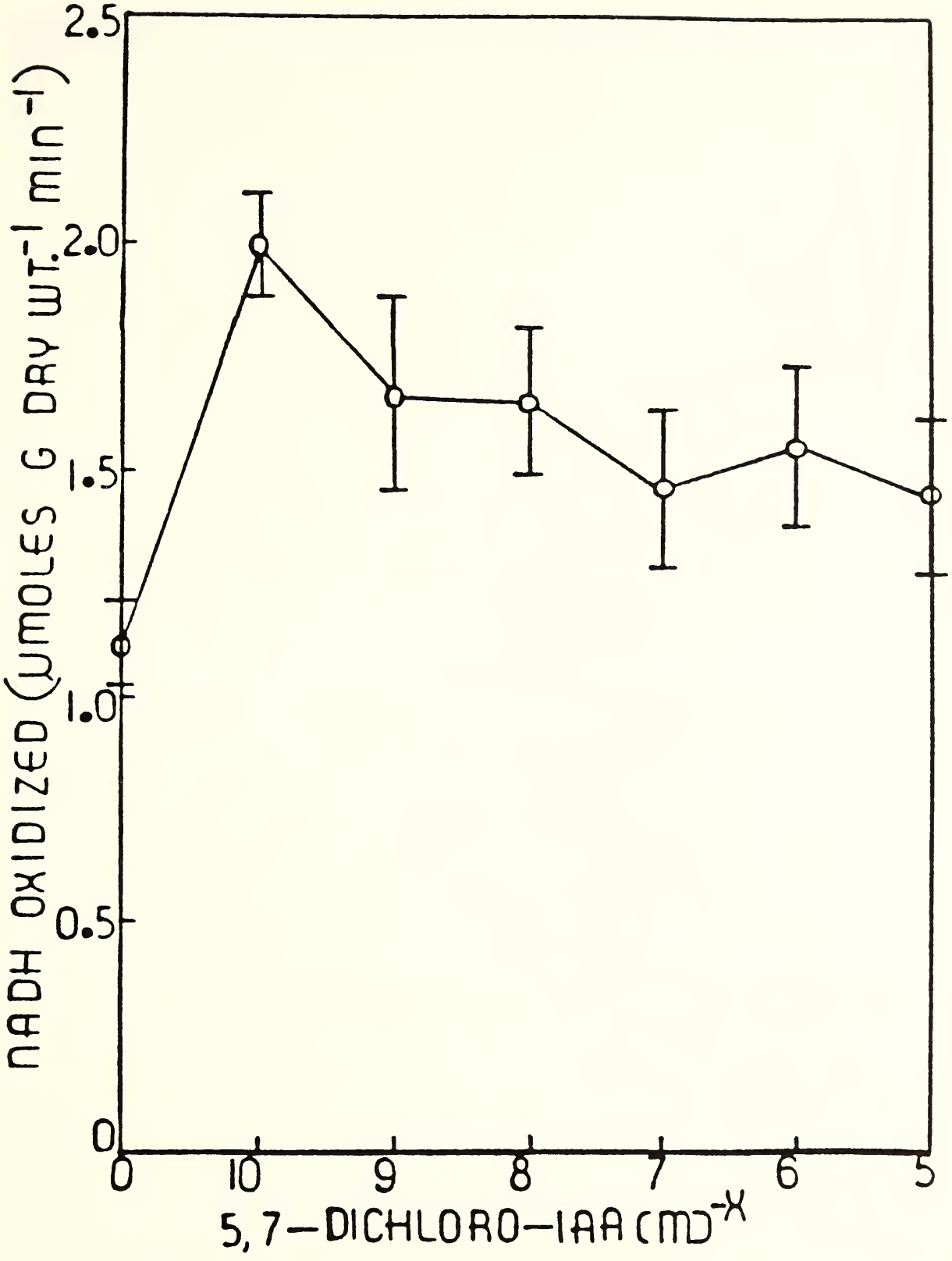

Figure 3. The effect of 5,7-dichloro-indole-3-acetic acid (5,7-Cl-IAA) on NADH oxidation by cultured carrot cells. NADH oxidation was assayed spectrophotometrically at various 5,7-Cl-IAA concentrations as described in Materials and Methods.

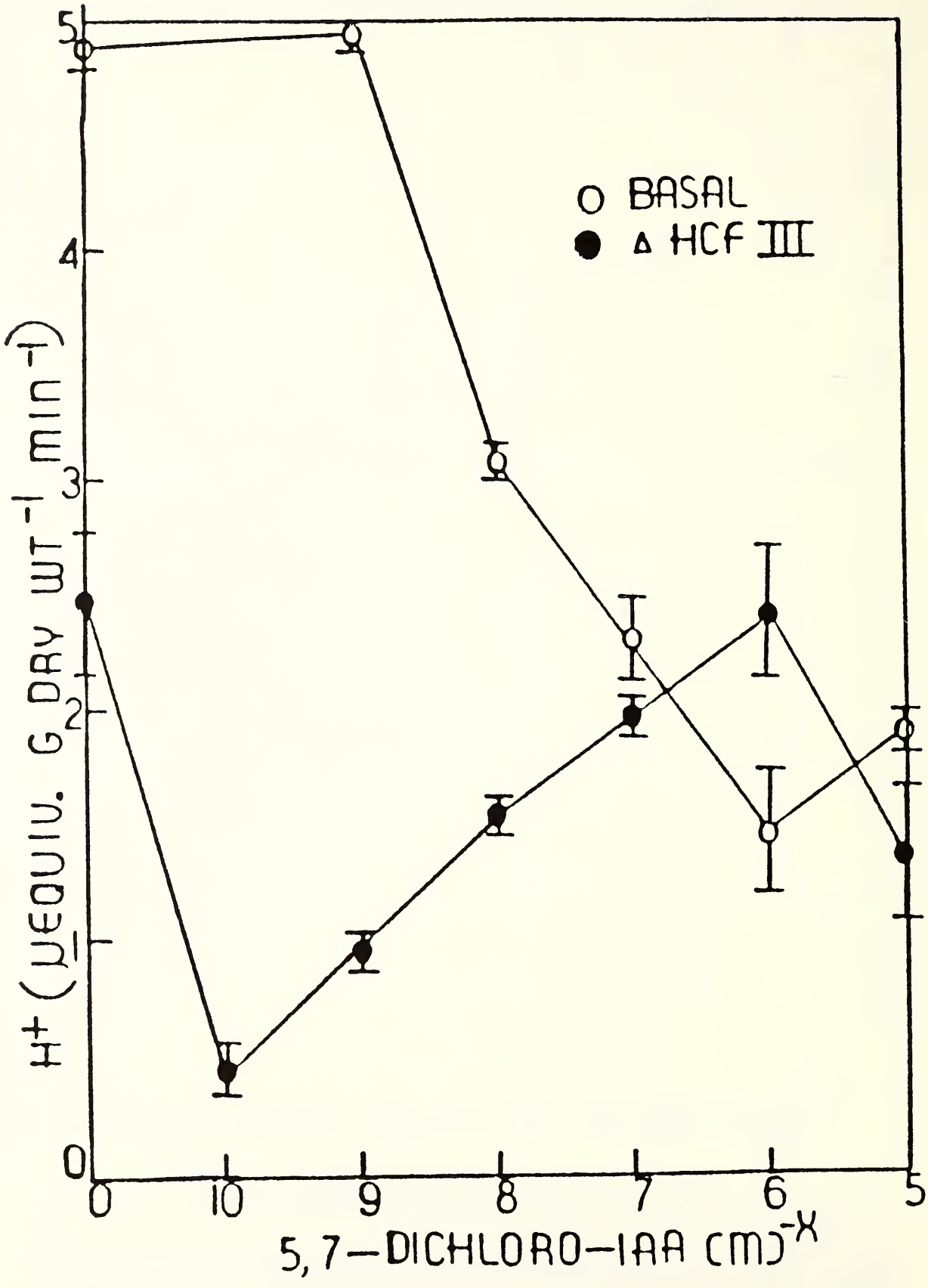

Figure 4. The effect of 5,7-dichloro-indole-3-acetic acid (5,7-Cl-IAA) on proton excretion by cultured carrot cells. H^+ excretion measured without HCFIII (O) and with HCFIII (●) at various 5,7-Cl-IAA concentrations, using a pH electrode as described in Materials and Methods.

growth regulators as their parent compound, IAA itself. Here, again, differences were noted in their mode of action. All three chloro derivatives stimulated NADH oxidation by carrot cells, but 5-chloro IAA was less effective than 7-chloro or 5,7-dichloro IAA (Figs. 2A, 2C, 3A). A thousand times higher concentration (0.1μM vs. 0.1mM) was necessary for 5-chloro IAA to achieve maximum stimulation of NADH oxidation (Fig. 2A). Differences in H^+ excretion were most notable in case of 7-chloro (Fig. 2D) and 5,7-dichloro IAA (Fig. 3B), both of which inhibited the basal rate of H^+ excretion, while 5-chloro IAA showed a slight stimulation of ATPase-mediated H^+ excretion (Fig. 2B). The H^+ excretion mediated through plasma membrane electron transport by carrot cells in presence of ferricyanide was not remarkable, showing only slight stimulation or inhibition.

Previous studies by our group on cultured carrot cells (Crane et al., 1983) or transformed versus non-transformed tobacco cells (Barr et al., 1985) have shown that transplasma membrane electron transport with HCF III as the impermeable electron acceptor respond to plant growth regulators and that an increase in proton excretion, aside from H^+ excreted by the plasma membrane H^+-ATPase, is seen when low concentrations of plant hormones are used (<1μM). Until the discovery of NADH oxidase from soybean hypocotyls (Barr et al., 1985), the stimulation of HCF III reduction by growth regulators was relatively small (about 25%), but stimulation of NADH oxidation can be 10X, as seen in Fig. 1C. Since the isolation of a hormone-sensitive NADH oxidase by Brightman *et al.* (Brightman et al., 1988), the stimulation of NADH oxidation by IAA and its chloro derivatives shown here can in the future be related to plant growth in general.

The stimulation by IAA and its chloro derivatives of NADH oxidation and H^+ excretion of cultured carrot cells seen here is contrary to Böttger and Hilgendorf (Böttger and Hilgendorf, 1988), who found inhibition of HCFIII reduction and H^+ excretion by intact maize seedling roots, when treated with IAA. HCFIII reduction by carrot cells in this study was not significantly affected by IAA or its derivatives (data not presented). These differences may be due to using tissue culture cells versus intact seedlings, or it may be a species-related difference. In an earlier study, Böttger and Lüthen (1986) found a correspondence between NADH oxidation and proton excretion by roots of *Zea mays*.

In summary, it is concluded that NAA, IAA and its chloro derivatives stimulate NADH oxidation by cultured carrot cells in low concentrations (0.1mM to 1μM), whereas only the H^+-ATPase generated protons are affected by these growth regulators. Unlike oxidation products of IAA metabolism, such as oxindole-3-acetic acid (Ernstsen et al., 1987; Henderson and Patel, 1972; and Nonhebel and Bandurski, 1984), which were found to be inactive as growth promoters, the chloro derivatives of IAA showed no inhibition of NADH oxidase activity. The inhibition of H^+-ATPase-excreted protons by 7-chloro and 5,7-dichloro IAA may also be significant, if the acid growth hypothesis is correct.

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