The electrical response of maize to auxins

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The electrical response of Zea mays coleoptiles and suspension cultured cells to several growth-promoting auxins (IAA, IBA, 2,4-D, 2,4,5-T, 1-NAA) and some of their structural analogues (2,3-D, 2-NAA) has been tested. In coleoptiles two typical electrical responses to IAA are observed: an immediate rapid depolarization, and a hyperpolarization following 7–10 minutes after the first external addition of IAA. Of the other tested compounds only 1-NAA significantly depolarized the cells, whereas all auxins as well as the analogues evoked delayed hyperpolarizations. In contrast, the suspension cells were not hyperpolarized by any of the tested compounds, but were strongly depolarized by IAA, 1-NAA, and to a lesser extent by 2-NAA. In these cells IAA and 1-NAA induced inwardly directed currents of positive charge which both saturated around 12 mA/m². The strong pH-dependence together with the half-maximal currents 0.49 μM IAA and 0.76 μM 1-NAA point to a symport of the anions with at least 2H⁺. The delayed plasma membrane hyperpolarization is a different response, and seems to be initiated by the protonated auxin species. In accordance with the current literature, it is interpreted as consequence of a stimulated proton extrusion. The finding that all tested compounds evoked a hyperpolarization, makes this response unspecific. It is concluded that a stimulation of proton extrusion is a necessary, but not sufficient step to induce elongation growth.

Introduction

Externally added IAA induces electrical responses in grass coleoptiles [1–6]. Although some of these effects have been known for some time, their importance for elongation growth is still a matter of considerable debate. Improved techniques and new ideas of auxin action enable us to reconsider older concepts and reevaluate some of the earlier data. In this context the acid growth theory [7,8] plays an important role, although some of its basic statements have been criticized lately [9,10].

Electrophysiological tests of auxin action during the elongation growth of coleoptiles are experimentally demanding, because the expanding tissue often terminates the measurements. In many instances a reimpalement is unsuccessful or leads to less reliable results. Moreover, quantitative measurements of electrical membrane current cannot be carried out on coleoptiles, mainly because of current leakage through the network of plasmodesmata and the possibility of occasional vacuolar impalements. To circumvent such problems, for some tests we have chosen suspension cultured maize cells which, because of their spherical form, allow quantitative measurements of the auxin induced electrical membrane current.

So far, electrophysiological work on auxin effects has mainly focussed on IAA. Using other growth-promoting auxins and some of their structural analogues, in this study we try to bring the electrical responses on a more general basis. We therefore attempt to analyze the carrier-mediated auxin uptake and the auxin-induced stimulation of the plasma membrane H⁺-ATPase.

Materials and Methods

Coleoptiles

Zea mays L. (Santos) seeds were soaked for 2 h in tap water. They were then placed on moist vermiculite and grown in the dark for 4–5 days at 24°C.

For electrophysiological experiments the coleoptile segments were excised and abraded with strips of emery cloth (Vitex KK14F No 392; VSM AG Hannover,
F. R. G.). They were then fixed upright in a Plexiglas chamber which was constantly perfused with the test medium (see below) and which allowed horizontal approach of the microelectrodes from both sides [11]. For these tests standard equipment (S-7000; WPI-Instruments, New Haven, CT, U.S.A.) was used [12–14]. Micropipettes were pulled vertically on a Getra instrument (Weilheim, F. R. G.) from borosilicate glass tubing with solid fiber (Hilgenberg), and were filled with 0.5 M KCl from the rear end by capillary displacement. The incubation time prior to the first impalement was generally 2 h. The tests showed that the segments produced a stable membrane potential about 1.5 h after incubation in the standard test solution.

Cell suspensions

The cultures were grown in Erlenmeyer flasks on a shaker at 60 rpm in the dark in Murashige-Skoog medium which was supplemented with 2,4-D. The cells were harvested by transferring them into the standard test medium which comprised 0.1 mM KCl, 0.1 mM NaCl, 0.1 mM CaCl₂ and a mixture of 5 mM Mes + Tris, adjusted to the respective pH.

For electrophysiological tests the cells were transferred into a flow-through chamber. Single cells or small cell clusters were sucked in a glass holding pipette, and were impaled from the opposite side. Prior to the impalement the cells remained for 3 h in this medium which was constantly stirred and aerated. The electrical membrane current was measured applying the one-electrode method by balancing the series resistance (including the microelectrode) to zero using a bridge circuit. Since the method relies on the precondition that the electrode resistance should be negligible towards the total membrane resistance of the cell measured on \( R_\text{el}/R_\text{m} = 1/1000 \), this condition has been checked carefully after the tests and the withdrawal of the electrodes. Current-voltage curves were obtained by feeding trains of rectangular current pulses into the spherical cells, and recording the pertinent changes in membrane potential. The auxin-induced current was extrapolated by graphical subtraction of the curve measured after auxin addition from the control I-V curve measured before the auxin treatment.

Cytosolic pH-tests were carried out with pH-selective microelectrodes, built as described by Felle and Bertl [15].

Intact vs. excised coleoptiles

In order to deplete intracellular IAA it is custom to remove the coleoptile tip several hours prior to the respective experiments. We did not compare IAA levels of intact and decapitated coleoptiles [16–19], but found that the ability of the coleoptiles to react to externally-added auxins was in no way affected by the removal of the coleoptile tip. The data to these tests are not shown.

Results

Maize coleoptile cells react to externally added IAA with two typical electrical responses: an immediate fast depolarization, and a delayed hyperpolarization which is observed 7–10 min after the IAA addition [20].

The fast reaction

Cells of maize coleoptiles as well as of suspension cultures respond to addition of external IAA with an immediate depolarization. For maize suspension cells this is shown in Fig. 1 together with the strong concentration and pH dependence. As demonstrated in Fig. 2, this response is completely different from the reaction to weak acids. For maize coleoptiles this depolarization is restricted to IAA and 1-NAA, whereas cells of maize suspension cultures are also depolarized by 2-NAA, although less substantial (data not shown). Also, the response to IAA is faster in suspension culture cells than in coleoptiles. The reason for these differences could be due to different affinities and specificities of the auxin carriers, but very likely also arise from the better accessibility of the tested compounds to single suspension cells. For both cell types the maximal auxin induced depolarization never exceeded 30 to 35 mV.

On the single spherical suspension cells the IAA-induced inwardly directed electrical current of positive charge has been measured. Two current-voltage (I-V) curves are shown in Fig. 3A. The control curve was measured before addition of IAA, the second one (+IAA) was taken in the presence of IAA and in the moment of maximal membrane depolarization \( E'_m \). At \( E'_m \), both curves have the same slope (= membrane conductance) which indicates that the observed depolarizations were not caused by an increase in membrane conductance. Graphical subtraction of both curves yields the IAA induced membrane current \( \Delta I_{\text{IAA}} \). This curve (dashed) is virtually voltage independent and as such is similar to I-V curves obtained in earlier work for hexoses and amino acids [12]. The same procedure carried out for the other IAA concentrations each, yields the saturation curve of Fig. 3B with a maximal

![Fig. 1. Depolarization of Zea mays suspension cells after the addition of the indicated IAA concentrations and at different external pH. W, return to control solution.](image-url)
IAA induced membrane current of 12.5 mA/m²; the half-maximal current is induced by 0.49 μM IAA. For 1-NAA a maximal current of 12.1 mA/m² has been extrapolated. This points to a carrier jointly used by both auxins, however, with a lower affinity for 1-NAA, according to the half-maximal current at 0.76 μM 1-NAA.

The delayed reaction

(a) Growth promoting auxins. A remarkable response of coleoptiles to IAA is the delayed hyperpolarization [3,21-24]. It occurs usually 7-10 min after the external addition of IAA and has been interpreted as an indication for the stimulation of the plasma membrane H⁺-ATPase. This idea is well supported by measurements of proton extrusion through coleoptiles [7,26], and it is assumed that this stimulation of the H⁺-ATPase is of central importance for elongation growth. To our knowledge, so far only IAA but neither other growth promoting auxins, nor their growth-inactive structural analogues have been tested as to their effectivity to elicit this delayed electrical response. For this reason we tested 1-NAA, 2,4-D, 2,4,5-T and IBA, and found that all evoked responses similar to IAA (Fig. 4), i.e. a delayed hyperpolarization which usually turned into an oscillation [13,24].

(b) Structural analogues. To our surprise, the analogues 2-NAA and 2,3-D of the strong auxins 1-NAA and 2,4-D respectively, also caused clear-cut hyperpolarizations (Fig. 5) and, as shown elsewhere, also acidified the external medium [26]. However, the hyperpolarizations were even more delayed (about 12 min), and the concentrations needed to evoke quantitatively comparable effects, were higher. Consequently, when 1-NAA or 2,4-D were added after 2,3-D and 2-NAA had already hyperpolarized the cells, the effect was enhanced. In Figs. 5A-D the effects of some auxin combinations are given: 5 μM 1-NAA + 5 μM 2-NAA, added after 10 μM 2-NAA, first depolarized and then further hyperpolarized the membrane; addition of 10 μM IAA then had only a minor effect (Fig. 5A). 10 μM 2-NAA exchanged for 10 μM 1-NAA, caused an immediate hyperpolarization, showing the typical proton pump overshoot usually observed after taking the de-

![Fig. 2. Comparison of the effects of acetic acid (Ac) and IAA at the indicated concentrations and external pH on membrane potential (E_m) and on cytosolic pH (pH_c) of abraded Zea mays coleoptile segments.](image)

![Fig. 3. Analysis of the IAA⁻ induced electrical current in Zea mays suspension cells. (A) Current-voltage curves, measured before (control) and after the addition of 10 μM IAA. The dashed curve represents the IAA-induced positively charged, inwardly directed electrical current (ΔI/IAA) which was obtained by graphical subtraction of the IAA-curve from the control (see text). E_m, resting potential before adding the IAA, Eₘᵢₙ, membrane potential in the presence of IAA at maximal depolarization. (B) Difference currents, as obtained by the method shown in (A), plotted against the external IAA (○) and 1-NAA (□) concentrations, as indicated. Each point represents the current measured at maximal depolarization in the presence of the respective auxin concentration.](image)

![Fig. 4. Comparison of the effects of different growth promoting auxins on the membrane potential of abraded Zea mays coleoptile segments. IBA, indolylbutyric acid; 1-NAA, 1-naphthylacetic acid; 2,4-D, 2,4-dichlorophenoxy acid; 2,4,5-T, 2,4,5-trichlorophenoxy acid.](image)
Fig. 5. Comparison of the effects of some growth promoting auxins, their inactive structural analogues (2-NAA, 2,3-D), and combinations thereof on membrane potential of abraded Zea mays coleoptile segments. 2-NAA, 2-naphthylacetic acid; 2,3-D, 2,3-dichlorophenoxy acid. Numbers in brackets denote concentration in µM. See text.

polarizing substrate (here 1-NAA) out (Fig. 5B). 5 µM IAA + 5 µM 2,4-D, added after 10 µM 2,4-D, depolarized the cell, but caused only weak further hyperpolarization (Fig. 5C). Finally, 2,4-D, exchanged for 2,3-D, clearly further hyperpolarized the cells (Fig. 5D).

(c) The effect of external pH: IAAH or IAA?. The acid growth theory emphasizes the importance of the external pH. In our experiments 10 µM IAA neither depolarized nor hyperpolarized the maize cells at pH 9. But already at pH 7.95 a 5 mV hyperpolarization was observed and at pH 6.1 the hyperpolarization was almost maximal with 28 to 30 mV. This raises the question for the effective IAA species, IAAH or IAA? Since from pH 7.95 to 6.1 the IAA− concentration just drops from 9.99 µM to 9.5 µM, whereas the IAAH concentration increases from 7 nM to about 70-fold, the binding site evidently recognizes IAAH. This is supported by corresponding tests carried out with different IAA concentrations but at constant pH 6. The data from both approaches only differ marginally and are shown as dose-response curves in Fig. 6.

Discussion

The depolarization, a weak acid effect?

Clearly, the results given in Figs. 1 to 3 cannot be interpreted as weak acid effects on the plasma membrane [27]. In Fig. 2 the action of acetic acid and IAA on maize coleoptiles are directly compared. At an external pH 5, 1 mM acetic acid and IAA both induced similar shifts in cytosolic pH, which is evidently the result of the protonated weak acids entering the cells and releasing protons according to the higher cytosolic pH [15,28]; electrically however, IAA and acetic acid cause different responses: whereas IAA at first strongly depolarizes the cells, acetic acid causes an immediate hyperpolarization. At pH 4.8 the different mode of action of acetic acid and IAA is even more evident. Whereas 10 µM IAA immediately depolarizes the cells followed by the hyperpolarization typical for coleoptiles, the same amount of acetic acid has no measurable effect. It should be emphasized, however, that weak acids do have effects on membranes and can indeed lead to depolarizations. Extensive work on the action of fatty acids carried out by Frachisse et al. [28] have revealed that weak acids induce time-variant changes in membrane conductance, which however were detectable only at much higher concentrations than those of the auxins used here.

The carrier-mediated auxin uptake: an electrophoretic IAA / proton symport?

The data from Figs. 1 and 3 clearly point to a saturable electrophoretic auxin uptake mediated by a carrier [29–32]. In parallel, IAA pH-dependently also enters the cell in its protonated form. Applying radioisotope techniques, it has always been difficult to sharply separate this unspecific intrusion of IAAH from the carrier mediated transport. In measuring the IAA-induced depolarization and the corresponding electrical membrane current we detect the carrier-mediated IAA uptake only; the half-maximal IAA-induced current of 0.49 µM IAA (Fig. 3B) almost corresponds with the 0.3 µM given by Hertel [32] for Cucurbita. Three observations point to a proton/IAA− symport: (1) IAA− induced positive charge moves into the cell; (2) this current is saturable; (3) the electrical responses are strongly pH-dependent. But, which species of IAA (IAAH or IAA−) is symported with how many H+? If
we decide on an IAAH/nH ÷ symport, basically 1 H +
would be sufficient to induce a membran2 depolariza-
tion and an inwardly directed positive current. In the
tests carried out at pH 7.3 and 6.0 (Fig. 1), respectively,
large differences of depolarizations (and membrane cur-
rents) are observed. For example, at pH 7.3, 1 µM IAA
depolarizes the plasmalemma by 5 mV, but by 22 mV at
pH 6. Calculating the concentrations of both species
within this pH range, we find that [IAA−], does not
change significantly, whereas [IAAH], does so by a
factor of 20; this would point to IAAH as the sym-
ported species. But at pH 7.3 only 3 nM IAA are
protonated. This would not only require an unusually
high affinity of the carrier for IAAH, but is also not
warranted by the measured half-maximal current of
0.49 .aM IAA (Fig. 3B). This means that the pH sensi-
tivity is not due to an increase in the transported
substrate, but rather to more H ÷ at lower pH. We
therefore favour IAA− as transport substrate and sug-
gest that the auxin influx carrier cotransports IAA−
with at least 2 H ÷.

The specificity of the uptake carrier

Apparently, not all growth promoting auxins induce
an electrical current. For both Zea mays coleoptiles and
suspension cells we found that IAA and 1-NAA were
the only compounds to induce a significant electrical
current, whereas the other tested auxins, even at concen-
trations of 10 µM, had no effect in coleoptiles and at
the most minor effects in suspension cells. This is in an
interesting contrast to the results obtained by Sabater
and Sabater [33], and Hertel [32] who found in binding
studies in Lupinus and Cucurbita, respectively, that the
uptake carrier had a much higher affinity for IAA and
2,4-D (and 2-NAA) than for 1-NAA. Rubery [34] found
in crown gall cells, that beside IAA also 2,4-D had a
saturable uptake component. We are not excluding the
possibility that the nondepolarizing compounds are also
bound by this uptake carrier, but we are certain that in
maize they are not transported electrophotically. It is
conceivable, however, that the auxin uptake carriers of
monocots and dicots possess different specifici-
lar reactions of the coleoptiles, viz. a delayed hyper-
polarization (Fig. 5), as well as proton extrusion [36].
Since we found in auxiliary experiments that these
compounds did not stimulate the elongation growth of
maize coleoptiles at concentrations 10 µM or lower
(data not shown), our observations indicate that beside
the auxin-induced acidification of the cell wall space
other processes must take place as well to induce growth.
Clearly, to evoke responses which were quantitatively
comparable to the responses to IAA, higher concen-
trations of 2-NAA and 2,3-D had to be added which,
however, were still below the level needed to cause
trivial weak acid effects.

An auxin-binding protein (ABP) has been identified
by Löbler and Klümö in maize [37] which, after bind-
ing IAA, supposedly activates a signal transduction
chain, finally leading to elongation growth. Our data
indicate that this cascade is forked. Since both growth-
active auxins and growth-inactive structural analogues
seemingly stimulate the H ÷-ATPase (hyperpolarization,
proton extrusion), we must assume that they all bind
first to the same structure (possibly the described ABP),
a process which appears too unspecific to induce growth.
On the other hand, when the H ÷-ATPase is deactivated,
no elongation growth takes place. This indicates that
the auxin-induced H ÷-ATPase stimulation and subse-
quently external acidification is a necessary, but not a
sufficient condition to stimulate growth. We therefore
suggest that the auxin-induced stimulation of the plasma
membrane H ÷-ATPase (proton extrusion) is a prere-
quisite for the growth induction, e.g., by stimulating
transport of cell wall constituents or precursors thereof.
Since many transport processes across the plasma mem-
brane of plant cells are proton-driven, interruption of
this proton turnover necessarily leads to severe inhibi-
tion of such membrane transport [38].

(b) Suspension culture cells. None of the tested auxins
hyperpolarized these cells, nor was proton extrusion
stimulated. The observation that FC rapidly stimulated
proton extrusion (data not shown) proved that the
H ÷-ATPase was indeed active. It appears that apart
from the uptake carrier there is no structure to recog-
nize auxins in connection with a H ÷-ATPase stimula-
tion, as observed in coleoptiles. One explanation is that
by origin the suspension culture cells are mesophyll cells
and as such do not respond to IAA with elongation
growth, but react otherwise to it (i.e. cell division).
These cells may possess the same signal transduction
chain(s) as the coleoptiles, but cannot be activated
because the receptor (APB) is either missing or not
expressed.

Concluding remarks

Since all tested auxins as well as their structural
analogues apparently stimulate the H ÷-ATPase in cole-
optiles, it appears that the activating binding site is rather unspecific. It may well be that we shall find many more non-growth promoting compounds with the ability to induce a delayed stimulation of the H'\nATPase, and it should be interesting to find out why they activate only part of the signal chain but do not stimulate growth. We must assume that one or more internal binding sites \[39,40\] are more specific and filter out the growth promoting compounds. Also, the function of the highly specific influx carrier remains unclear yet. IAAH (and 1-NAAH) enter the cells pH-dependently due to their weak acid character, and at the physiological external pH of 4.8 to 5 could easily be accumulated by a factor of 200 to 300. Since our data are in favour of an electrophoretic IAA uptake, the membrane potential of about \(-90\) mV (at pH 4.8, the natural pH of the apoplast; \[26\]) adds to the proton gradient across the plasma membrane of roughly 2.5 units (\(-150\) mV) to form an electrochemical proton gradient of \(-240\) mV, and hence a thermodynamic driving force of \(-385\) mV for IAA\(^-\). It is possible that the high affinity for IAA\(^-\) together with the increased driving force give the cell a possibility to keep external IAA concentration low in order to maintain high sensitivity for IAA.

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