Time Course of Auxin Stimulations of Growth

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ABSTRACT

Measurements of the time course of growth responses of corn coleoptile sections to pulses of auxin (10⁻⁵ M indoleacetic acid) establish that the growth rate changes in a regular pattern around the auxin pulse: a latent phase of 12 to 15 minutes is followed by an acceleration of growth rate lasting 15 to 20 minutes, after which a fairly steady rate is maintained. When the auxin source is withdrawn, there is an after-effect of about 15 minutes followed by a decay of growth rate, which reaches 50% decay after a further 15 to 40 minutes. The decay phase appears to be a function of the transport of auxin out of the sections. The 50% decay of growth for single cells is estimated at 30 minutes from the time of withdrawal of an exogenous supply of auxin. The regulation of growth by auxin is rapidly imposed or dissipated as auxin enters and exits, respectively, suggesting a facile association and dissociation of auxin with a growth-limiting site in the cell. It is proposed that the growth-stimulated state is dissipated at once when the transportable auxin has passed out of the cell.

Early work on the kinetics of auxin responses assumed that after auxin had been supplied to coleoptile or stem sections, growth promptly proceeded at a new linear rate (5). Bennett-Clark and Kefford (1) offered evidence that the auxin response may be neither immediate nor linear. It remained for Ray and Ruesink (10) to show that growth responses to auxin involved a lag period of 10 to 15 min. Since that time the lag period has been defined more precisely by several workers (4, 8, 11, 12) with the further additional fact that the growth rate begins to return to its endogenous rate at about 40 to 60 min after the removal of the auxin supply (3). The lag times for each of five types of plant growth regulators were found to be similar to the lag time for auxin (12). In the present study, the use of an auxinometer with very high resolution is utilized to describe the several component stages of the auxin-induced alterations of growth rate in corn coleoptile sections.

METHODS

The experiments were done with coleoptiles of corn seedlings (WF9 MST × B37) grown in vermiculite-filled pots standing in trays of water. The seedlings were grown in the dark (25 C) with 1 hr of red light each day, until the coleoptiles were 2.5 to 3.5 cm long. The standard experiment involved a 1-cm section cut from the coleoptile beginning 2 to 3 mm below the apex after removing the first leaf. Ten coleoptile sections were strung on a steel wire and placed in a tube which was continuously irrigated with phosphate buffer (10⁻⁵ M, pH 6.8) with or without indoleacetic acid (10⁻⁵ M IAA) as shown in Figure 1. Manipulations were done in dim green light, and the period of measurement was in darkness.

Elongation of the stack of coleoptile sections was measured with a Metripack position-sensing transducer (Brush Instruments, Clevite Corporation, Cleveland) as described by Warner and Leopold (12). The sensing axis was attached to a 2-cm needle, the tip of which rested on a piece of capillary tubing at the top of the stack of coleoptile sections. Displacement of the needle by the elongating sections (1 degree rotation = 100 mv output) was recorded on a Sargent recorder. The displacement of the recorder pen had been calibrated to the displacement of the sensing needle with a traveling microscope. The growth rate was taken as the slope of the recorded plot and was determined at 2- or 4-min intervals during the experiment.

Freshly cut coleoptile sections showed a high initial growth rate, and so in each instance sections were allowed to stand for about 30 min before treatments were begun, a precaution employed by other workers as well (4, 12).

The experiments on IAA transport were carried out by the method described by dela Fuente and Leopold (6), except that a high specific radioactivity of IAA-2-¹⁴C was used (10⁻⁵ M, 49 mc/mole). The same phosphate buffer was used as in the growth measurements and was incorporated into all agar blocks, with or without IAA. Radioactivity of the agar receptor blocks was determined by scintillation counting of alcohol extracts of the agar.

RESULTS

A comparison of the various phases of auxin-induced alterations in growth rate can be obtained by utilizing pulses of auxin of various durations. The results of four separate experiments utilizing pulses of 1- to 80-min duration are plotted together in Figure 2, arranged so that the time of beginning of the auxin pulse is set as time zero. The coleoptile sections had a very low endogenous growth rate at the time of auxin application, and in every instance there was a lag period of 12 to 15 min before the commencement of rate increases. The subsequent acceleration of growth continued for a period of about 15 min in each instance, followed by a settling to a constant rate if the auxin supply was continued for a long enough period, and then a decay of the growth rate. In each experiment in which the auxin supply was continued for extended periods of time, the growth rate was observed to overshoot the ultimate steady state for approximately 20 min, and then to drop back, as illustrated by the curve for the 80-min auxin treatment in Figure 2. This curve also shows a period of unaltered growth for about 15 min after the auxin supply was withdrawn before the growth rate began to decay.

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For the purposes of discussion, we will use the term “latent phase” in referring to the period after auxin application during which there is no apparent growth rate increase, “acceleration phase” for the period of increasing growth rate, “steady state” for the period of relatively constant, elevated growth rate, “after-effect” for the period of unaltered growth rate subsequent to the removal of the auxin supply, and “decay phase” for the period of declining growth rate.

If successive brief pulses of auxin are supplied to the same set of coleoptile sections, one can see the pulse of growth rate repeated, as in Figure 3. Following each auxin pulse, there was a latent phase of less than 15 min, followed by an acceleration phase of about 20 min duration, and then a decay phase of 40- to 60-min duration. The greater growth rate with the second pulse of auxin was found in all six repetitions of this experiment and is undoubtedly real. The decline in growth response with the third and fourth pulse was likewise consistently observed.

It is possible that the length of the various phases may be related to the equilibration of the auxin supply into and out of the coleoptile sections; if this is true, then one would expect to find differences as one varies the size of the sections used for the experiment. To investigate this possibility, 25-mm sections were cut from each coleoptile, and four such sections were utilized for each set-up, but in one case the 25-mm sections were left intact, in another they were cut into two 12,5-mm sections, and in the third they were cut into four 6,25-mm sections. In each instance they were given a 20-min pulse of auxin, and the growth rates were recorded as in Figure 4. The onset of the growth response was not appreciably different for the three lengths of sections, with the latent phase and the acceleration phases being about 15 and 20 min, respectively. The decay phase, however, was strikingly altered, the longest sections showing the slowest decay rate and the shortest sections the most rapid decay rate.

The fact that decay rates change with section length, as shown in Figure 4, suggests that one might be able to estimate the decay rate for sections of nearly zero length by extrapolation of the data for sections of various lengths. This would permit a rough approximation of the decay rate of the growth response for the single cell. In order to make such an extrapolation, the times for 50% decay after removal of the auxin source were made for all experiments carried out in this study (including four lengths of sections), and the average 50% decay times for each length is plotted in Figure 5 with the standard deviation indicated. The numbers of measurements involved in determination of each point varied from 19 (for 10-mm sections) to 3 (for 25-mm sections). While the variation for the 25-mm sections is very large, a progress line can be drawn through the variability ranges for each average, which extrapolates to the ordinate at about 30 min. We suggest, then, that as a rough approximation each cell experiences a 50% loss of auxin-stimulated growth at about 30 min after the auxin supply has been withdrawn.

The movement of the auxin out of the section would seem to be a logical determinant of the slope of the decay phase; then the application of an inhibitor of auxin transport should markedly slow the decay in growth rate. To investigate this possibility, sets of 10-mm sections were provided with an auxin supply for 1 hr, after which the after-effect and decay phases were followed with and without the presence in the bathing solution of 10⁻⁴ M 2,3,5-triiodobenzoic acid, a strong inhibitor of auxin transport (9). The results are plotted in Figure 6, showing that in each case there was an after-effect of roughly 15 min following the removal of the auxin, after which the decay phase set in. The decay of growth rate was markedly retarded by the application of the transport inhibitor.

In order to confirm that the TIBA³ was really inhibiting auxin transport out of the section as had been expected, a similar set of ten 10-mm coleoptile sections was inverted on an agar donor block containing 10⁻³ M IAA for 1 hr, after which the sections were placed erect between agar receptor blocks, which were renewed each 20 or 30 min (method of Leopold and Leopold [6]). In one group of 10 sections, the transport inhibitor had been incorporated into the agar of the receiver blocks (10⁻⁵ M TIBA). All receivers contained phosphate buffer (10⁻³ M, pH 6.8). The

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³ Abbreviation: TIBA: 2,3,5-triiodobenzoic acid.
FIG. 3. Growth responses of coleoptile sections to successive pulses of auxin. Each pulse was for 5 min, indicated by shaded bars on abscissa.

FIG. 4. Effects of section length on the growth rate following an auxin pulse of 20 min, indicated as shaded bar on abscissa. Four coleoptile pieces 25 mm long were assembled on the apparatus intact, cut into two 12.5-mm sections, or cut into four 6.25-mm sections. Results of such a test are shown in Figure 7 and establish that the TIBA treatment did in fact inhibit the transport of auxin out of the sections. The form of the decay curve for auxin transport is seen to be suggestive of the form of the growth decay curve (Figs. 1 to 4). Decay of auxin transport reached 50% at about 40 min after removal of the auxin supply, a timing similar to the decay curves for growth rates of coleoptile sections receiving auxin pulses of 1 hr or more.

DISCUSSION

The changes in growth rate with a pulse of auxin can be collectively described as a latent phase during which there is no increase in growth rate, an acceleration phase, and a steady state 188

FIG. 5. The time for 50% decay for the growth rate as a function of the length of coleoptile sections. Variability indicated as standard deviations.

FIG. 6. The after-effect and decay of growth rate after removal of the auxin supply. After continuous auxin supply for 1 hr (at time indicated by arrow) auxin medium was replaced by plain buffer solution (control) or a solution of buffer plus $10^{-5}$ M triiodobenzoic acid.
of growth rate. After the auxin supply has been removed, there is again a lag phase in which there is no change in growth rate (after-effect), and finally a decay phase which brings the growth rate back to an endogenous low value.

The latent phase includes the time required for the entry of auxin into the tissue plus whatever additional reactions may occur before the growth rate is first increased. There are several reasons to believe that the latent phase is principally an expression of the uptake and translocation of auxin. (a) Evans and Hokanson (3) observed that 2,4-D, which has a markedly slower transport rate, has a markedly longer latent phase than IAA. (b) Evans reported that esters of IAA, which enter tissues more rapidly than the acid, have markedly shorter latent phases. And (c) Zerk and Nissl (13) were able to reduce the latent phase to very short periods of time by using high concentrations of IAA and high temperatures to accelerate entry. The latent period for oat coleoptile sections is shorter than for corn (4), perhaps relating to the narrower dimensions and hence reader equilibration of IAA into the cells.

The acceleration phase can be construed to represent a period during which there is a continuous rise in the pool of limiting substances necessary for growth. The suggestion has been made by Evans and Ray (4) that the auxin stimulus may be mediated by the formation of some substrate which is limiting of growth and is used up during the growth processes. The kinetics of the growth response led them to expect a rapid turnover of such a limiting substance. They estimated that the half-life of the limiting substance must be in the range of a few minutes, since the steady state of growth rate is achieved within 30 min or less, and the half-life of a presumed limiting substance for this rate would be expected to be at least one-fifth the time needed for achieving the steady state. The enzymes in higher plants with the fastest turnover half-life have half-lives of several hours (e.g., 7), making it seem unlikely that the auxin stimulation involves the formation of enzymes which limit growth.

The decay phase has been shown in the present experiments to reflect the transport of the auxin supply out of the tissue, since shorter sections exhibit more rapid decay (Fig. 4), and inhibition of transport of IAA out of the sections by TIBA slows the decay (Fig. 5). Evans and Hokanson (3) observed that growth rates remained elevated for over 80 min after the withdrawal of 2,4-D, which they attributed also to its relatively weaker transport out of the tissue sections.

The decay phase is of special interest with respect to the mechanism of auxin regulation of growth, since it may provide evidence as to the persistence of the growth-stimulated state. From our efforts to estimate the 50% decay time of the auxin-induced growth stimulus (Fig. 5), we arrive at a rough estimate of 30 min as the value for a tissue of near-zero length, or the single cell. This value compares very closely with the time required for the 50% decay of the transportable auxin pool in the individual cell as estimated by dela Fuente and Leopold (6) for sunflower stem sections. In view of the striking similarity of the two values, we suggest that the mechanism of auxin stimulation of growth involves the continuous presence of transportable auxin in the cell, and that there is essentially no persistence of the stimulated state once the individual cell has secreted its transportable auxin pool. We have previously suggested a very close linkage between the acts of transport and growth stimulation (5).

A comment about the stoichiometry of the growth stimulus may also be in order. From Figure 2 it appears that the over-all growth pulse obtained with 80 min of auxin supply is much less than 80 times the pulse obtained with 1 min of auxin supply. Yet we know that uptake of auxin is essentially linear with time (2). The growth stimulations by auxin are more nearly proportional to the log of the auxin concentration than to the arithmetic concentration, and so, with increasing amounts of auxin entering the tissue, increasing amounts may become inaccessible to the growth-stimulating reactions and thus the lack of a linear relation between uptake duration and the growth response.

Since the completion of this manuscript, a paper has appeared by P. Penney (N. Z. J. Bot. 7: 290-301; 1969) describing the growth responses of stem sections to pulses of auxin. Her data show very clearly the lag, acceleration, and steady state growth phases for sections of pea and lupine.

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LITERATURE CITED