Investigations on the Mechanism of the Brassinosteroid Response

VI. EFFECT OF BRASSINOLIDE ON GRAVITROPISM OF BEAN HYPOCOTYLS

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WERNER J. MEUDT
United States Department of Agriculture, Agricultural Research Service, Agricultural Research Center-West, Plant Hormone Laboratory, Beltsville, Maryland 20705

ABSTRACT

Brassinosteroids are steroidal lactones of plant origin that promote growth of a number of plant systems, and particularly the growth induced by auxins. Biologically active brassinosteroids (BR) also promote the growth of gravitropic hypocotyls of 7-day-old light grown Phaseolus vulgaris when gravistimulated. Brassinolide-mediated promotion of curvature of gravistimulated internodes occurs in the absence of exogenously supplied indole-3-acetic acid (IAA). This is in contrast to the BR-promoted bending of vertically positioned bean hypocotyls, which is dependent upon exogenous IAA. Brassinosteroid treatment increased the graviperception of young internode tissues and the bending of the gravistimulated sections as well as the subsequent reversal of bending after the sections were placed vertically. These results indicate that BR sensitizes bean hypocotyls to gravistimulation and potentiates the action of a growth factor that induces gravitropic growth.

Brassinolide is a steroidal lactone first isolated from rape (Brassica napus L.) pollen in 1970 (14) and chemically identified in 1979 (7). A total of 11 biologically active analogs of brassinolide have since been isolated primarily from immature parts of a variety of plants (1-4, 8, 11, 15, 19-22). Although a physiological function for BR has yet to be assigned, they stimulate growth when applied exogenously to green plant tissues (13, 14, 17). The action of BR is tissue specific. It sensitizes young, actively growing tissues to exogenous IAA (13) without apparently affecting auxin uptake and/or auxin transport (6). The objective of this study was to further characterize the action of BR using a relatively fast growth response that requires no exogenous auxin supply. Here, BR was used to determine if this steroid affects the rate of gravitropic response (i.e., bending) and if it interacts with IAA in this graviresponse as it does in IAA-induced growth (13). The data presented suggest that biologically active BR potentiates gravitropic-mediated growth responses and that the action of BR is independent of exogenous auxin.

MATERIALS AND METHODS

Plant Material. Bean seeds (Phaseolus vulgaris L. cv Bush Burpee Stringless purchased from the Meyer Seed Co., Balti-

1 Mention of a trademark, proprietary product, or vendor does not constitute a guarantee of warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
Brassinosteroid Effect on Bending of Gravistimulated Hypocotyls. As in the initial experiments, bean hypocotyls responded to gravitropic stimulation by bending upward (Fig. 1). Treatment with biologically active BR (BR-1105) during gravistimulation of isolated bean hypocotyl sections enhanced the bending response. Specificity of the molecular structure of this enhancement was apparent, because the biologically inactive structure (28,3β-dihydroxy BR-940) could not mimic the effect of BR (Fig. 1). Both control sections and sections treated with BR-940 curve about 40° during the initial 3 h of gravistimulation. Data in Figure 1 also show that sections treated with active BR-1105 remain flexible and straighten after sections were placed vertically (arrow), as indicated by decreasing angles observed when recovery occurred. Curvature of control sections and sections treated with the inactive form remained relatively fixed and did not recover.

Concentration Response Curve. Brassinosteroid-induced stimulation of the gravitropic response is a function of the log concentration of BR. About 70 nM BR yielded a significant increase in bending over the control sections (Fig. 2). Higher concentrations of BR-1105 caused greater bending. All measurements were made after 1 h of gravistimulation. The highest concentration tested in these experiments was 6.8 μM which induced about the same bending in 1 h as 6.8 nM after 3 h.

Effect of BR on the Threshold Response to Gravitropic Stimulation. Under the experimental conditions used, isolated bean hypocotyls do not bend unless they receive at least 30 min of gravistimulation. The apices of sections are displaced at a rate that increases linearly with increasing length of gravistimulation (Fig. 3). Sections treated with about 70 nM BR, in contrast, start to bend within 15 min of gravistimulation. Extrapolation of the data indicates that the threshold response for control sections is 30 min and for BR treated sections is about 7 min of gravistimulation. In this experiment, the sections were placed upright after the indicated times of gravistimulation, and the recoveries (straightening) of the sections recorded. Data presented in the right panel of Figure 3 show that the rate of recovery was also stimulated by BR while the recovery of control sections was slow and minimal. After 60 min, control sections showed only about a 20% recovery from the curved condition while BR treated sections recovered 100%.

**RESULTS**

Brassinosteroid Effect on Bending of Gravistimulated Hypocotyls. As in the initial experiments, bean hypocotyls responded to gravitropic stimulation by bending upward (Fig. 1). Treatment with biologically active BR (BR-1105) during gravistimulation of isolated bean hypocotyl sections enhanced the bending response. Specificity of the molecular structure of this enhancement was apparent, because the biologically inactive structure (2β,3β-dihydroxy BR-940) could not mimic the effect of BR (Fig. 1). Both control sections and sections treated with BR-940 curve about 40° during the initial 3 h of gravistimulation. Data in Figure 1 also show that sections treated with active BR-1105 remain flexible and straighten after sections were placed vertically (arrow), as indicated by decreasing angles observed when recovery occurred. Curvature of control sections and sections treated with the inactive form remained relatively fixed and did not recover.

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Auxin-BR Interaction. It was demonstrated previously (13) that BR can change the sensitivity of bean hypocotyls to exogenous auxin. Also, it is implied from the literature that the gravitropic response is controlled by auxin (16). Thus, in the next set of experiments I tested the possibility that gravitropic stress sensitizes sections to auxin and whether a combination of gravitropic stimulation and BR treatment would enhance the reported auxin effect on gravitropism. Data in Figure 4 indicates that added auxin in the presence and absence of BR does not increase gravitisted bending of bean internodes. Data in Figure 5 were obtained from gravitropically bent internode sections (i.e. sections given 3 h of gravitropic stimulation prior to auxin treatment) and show that IAA applied to the lower (L), convex side of gravitisted sections caused no further bending. While the lower side of the sections was insensitive to auxin, the upper (U) concave side did respond to auxin treatments resulting in greater bending. The sensitivity of the concave side of auxin was, however, no greater than the sensitivity of tissues not previously exposed to gravitropism (6, 13).

Action of BR and Auxin on Omnilaterally Gravistimulated Bean Internode Sections. The primary objective of this experiment was to look further for possible changes in sensitivity of bean internode sections to auxin under conditions in which the sections were not allowed to bend during gravitropic stimulation by exposing all cells omnilaterally to gravity. Second, the experiment was designed to test BR for possible growth-promoting activity in these sections in the absence and presence of auxin. Under these conditions the sections did not curve. After these treatments, all of the sections were subsequently transferred to scintillation vials and treated either with filter paper disks containing 0.1 nmol IAA or with disks containing 2.08 nmol BR. The results show that gravistimulated sections do not differ much, if any, from nongravistimulated sections in their sensitivity to IAA and/or to BR, and that there is no change in the apparent synergistic response between IAA and BR (Figs. 6 and 7).

DISCUSSION

The response of plants to gravistimulation is well documented in the literature (16). For the most part, growth induced by gravistimulation has been assumed to be caused by an increase in auxin levels in the lower side of responding tissues. The mechanism by which such an asymmetrical distribution of auxin would occur is not clear, although involvements of differential auxin biosynthesis (18), differential metabolism (i.e. conjugation) (5) or lateral auxin movement (16) have been proposed. It is difficult to reconcile the results presented in this paper (i.e. the increased curvature of internodal tissue induced by BR during gravitropic stimulation) simply with an asymmetric distribution of auxin unless BR promotes lateral auxin transport which is doubtful (6). In these studies a uniform treatment with BR
followed by gravitropic stimulation does give a large increase in stimulation of growth over controls.

Auxin does not seem to be limiting bending, because a uniform treatment with auxin did not stimulate gravitropic-induced curvature over the control. Auxin application also had very little effect, if any, on gravistimulated hypocotyls treated with BR (Fig. 4). This implies that gravistimulated hypocotyls are sensitive to BR but not to exogenous auxin. The results are interpreted to mean that auxin is not limiting. The mechanism of BR-enhanced gravitropic-induced growth seems to differ from BR-enhanced, auxin-induced growth. In the former, BR acts in the absence of applied IAA. These data suggest that the action of BR is independent of exogenous auxin in gravitropically stimulated growth. However, one cannot rule out the possibility that BR interacts with endogenous IAA (9, 10), and that BR reduces the need for a presumptive high threshold concentration of IAA that might be required for a gravitropic response, by increasing or decreasing the receptivity of tissues to endogenous IAA. The data presented in this paper are interpreted to indicate that, at least in bean tissues, BR may have primary functions in regulating the sensitivity of tissues to respond to growth stimulants such as auxin and gravitropic stimulators that induce curvature.

LITERATURE CITED