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THE ROLE OF CALCIUM ACCUMULATION AND THE CYTOSKELETON IN THE PERCEPTION AND RESPONSE OF *COPRINUS CINEREUS* TO GRAVITY

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ABSTRACT

The role of Ca^{2+} in the gravitropic perception and/or response mechanism of ***Coprinus cinereus*** was examined by treating stipes with inhibitors of Ca^{2+} transport and calmodulin. Inhibitors had no effect on gravity perception but significantly diminished gravitropism. It is concluded that, under the conditions tested, Ca^{2+} is not involved in gravity perception by ***Coprinus*** stipes, but does contribute to transduction of the gravitropic impulse. The results would be consistent with regulation of the gravitropic bending process requiring accumulation of Ca^{2+} within a membrane-bound compartment. Treatment of stipes with an actin inhibitor caused a significantly delayed response, a result not observed with the Ca^{2+} inhibitors. This suggests that cytoskeletal elements may be involved directly in perception of gravity by ***Coprinus*** stipes while Ca^{2+}-mediated signal transduction may be involved in directing growth differentials.

INTRODUCTION

Control of cell Ca^{2+} accumulation has been implicated in the gravity perception mechanism of plants /1, 2/. There is polar Ca^{2+} redistribution upon gravistimulation in maize roots /2, 3/ and Ca^{2+} chelators have been used to eliminate gravity sensing in plants /2, 4/. Few attempts have been made to assess the role of Ca^{2+} in fungal gravitropism /5/. Hence the role of Ca^{2+} in the gravitropic perception and/or response of ***Coprinus cinereus*** was examined in a number of ways: by treating stipes with a Ca^{2+} channel blocker, verapamil; a Ca^{2+} ionophore, A23187, which equalises both intra- and extracellular Ca^{2+} gradients; an extracellular Ca^{2+} chelator, BAPTA; and calmidazolium, an inhibitor of calmodulin-mediated Ca^{2+} uptake, using concentrations known to eliminate gravitropism and other tropisms in plants /2, 4, 6, 7/. There is also evidence that, along with the statolith, cytoskeletal elements have an important role in the gravity perception process in ***Chara*** and ***Lepidium*** /8, 9/. Stipes were also treated with cytochalasin B to determine whether the cytoskeleton had a role in gravity perception in fungi, where there is no obvious gravity sensing organelle, such as a statolith.

METHODS

All experiments were performed with post-meiotic fruit bodies of ***Coprinus cinereus*** grown as described previously /5/. Mushrooms were decapitated, the stipes measured and immersed in water (control) or inhibitor for 1-2 hrs in the dark at room temperature (21-23° C) while being kept vertical throughout these procedures. The concentrations of Ca^{2+} antagonists and the calmodulin inhibitor used were as previously described /5/. To determine the role of the cytoskeleton in gravitropism, stipes were treated with 10, 50, and 100 μM cytochalasin B for 1.5 h. After treatment, stipes were secured in a horizontal position and videotaped for 3-6 h. Gravitropism was monitored by analysing digitized images from videotapes and measurements were made of the total stipe length, the distance between the bend and the base, and the angle between the apex and the horizontal (tip angle) as described previously /5, 10/. The tip angle

represents accumulated bending integrated with any bend compensation which has been invoked to constrain the apex towards the vertical /10/. Baseward bend movement is characteristic of the normal response /10/ and allows accumulation of bending potential to accelerate the response of the organ. The inhibitors were considered to have an effect on gravitropism if they significantly delayed the response, caused a decreased final tip angle, or interfered with bend movement without inhibiting stipe extension.

RESULTS AND DISCUSSION

Ca^{2+} Antagonists and Calmodulin Inhibitor

A comparison between control and inhibitor-treated stipes revealed that verapamil, a Ca^{2+} channel blocker, significantly inhibited stipe extension at the concentration used, whereas the Ca^{2+} ionophore A23187 significantly increased stipe extension (Table 1). Stipe extension was not inhibited by treatment with calmidazolium or the Ca^{2+} chelator, BAPTA. Both the chelator and the ionophore decreased the rate of bending of the stipe apex, causing a decrease in the final tip angle (Table 1) while calmidazolium and verapamil had no effect on the final tip angle. Baseward movement of the bend was significantly inhibited by the chelator and the ionophore (Table 1), but verapamil and calmidazolium had no effect.

TABLE 1. Effects of Ca^{2+} and calmodulin inhibitors on extension and bending. Asterisks indicate which values significantly differ (P = 0.05) from the control values using Student's t-test. Czm = calmidazolium; Vera = verapamil; A23187 = ionophore; BAPTA = chelator.

Treatment	% length increase ± SD	Bend shift, mm ± SD	Response time, min ± SD	Bending rate, deg min^{-1} ± SD	Final angle, deg ± SD
Control (1 h)	23.5 ± 7.1	7.2 ± 4.3	54.9 ± 31.1	0.49 ± 0.23	44.1 ± 21.1
Czm, 100 µM	24.4 ± 8.5	6.9 ± 4.5	50.4 ± 42.1	0.45 ± 0.21	35.1 ± 17.5
Vera, 1mM	16.1 ±3.6*	7.9 ± 2.6	58.1 ± 38.7	0.39 ± 0.16	36.4 ± 19.2
Control (2 h)	22.5 ± 7.5	7.9 ± 4.3	50.3 ± 34.8	0.49 ± 0.15	37.7 ± 15.8
A23187, 1µM	28.1 ± 6.0*	3.7 ± 3.6*	73.4 ± 31.9	0.28 ±0.11*	25.5 ±10.8*
BAPTA, 10 mM	21.5 ± 4.7	3.8 ± 3.7*	61.3 ± 36.8	0.21 ±0.19*	18.8 ±17.5*

Most importantly, under these experimental conditions, none of these inhibitors eliminated or delayed the gravitropic response, which suggests that Ca^{2+} is not directly involved in gravity sensing, as has been suggested in plants /1, 2, 4/. Whereas Ca^{2+} may not be involved in the initial steps of gravity sensing, an extracellular Ca^{2+} gradient and intracellular sequestration of Ca^{2+} are required for the bending response in *C. **cinereus***, as suggested by the results with the chelator and the ionophore, respectively.

The chelator also had a long-term effect on the curvature compensation process which brings stipes back to the vertical. Stipes treated with BAPTA before being placed horizontal had curled past the vertical after 18 h whereas control stipes had returned to and remained vertical. Thus, Ca^{2+} may also have a role in the curvature compensation mechanism and/or may be required to maintain the apical dominance which is so clearly expressed by this phenomenon /10/. The fact that both chelator treatment (expected to restrict flow of Ca^{2+} into stipe cells) and ionophore treatment (expected to increase Ca^{2+} concentrations from intra- and intercellular stores) reduce the gravitropic response suggests that gravitropism in *C. **cinereus*** consists of steps dependent on both high and low Ca^{2+} concentrations.

Calmidazolium did not affect the final tip angle but it has been shown to decrease another gravitropic parameter, the contact angle /5/, which suggests that calmodulin is also involved in Ca^{2+} uptake during normal gravitropism. Whether the role of calmodulin-mediated Ca^{2+} uptake

is to remove accumulated Ca^{2+} (resulting from gravity-dependent influx) from the cytoplasm via Ca^{2+}-ATPases, to shuttle Ca^{2+} to another destination in the cytoplasm, such as the endoplasmic reticulum or vacuole, or as a complex with Ca^{2+}, is responsible for initiating a cascade of reactions resulting in asymmetrical extension, has yet to be investigated in *C. **cinereus***.

Verapamil, which blocks Ca^{2+} channels and thus impedes facilitated transport of Ca^{2+} across membranes, had no noticeable effects on gravitropism but significantly reduced stipe extension at the concentration used. Although Ca^{2+} uptake seems to be required for the gravitropic response, uptake through Ca^{2+} ion channels seems to be critical for the mechanisms involved in stipe extension. Our results suggest that regulation of intracellular Ca^{2+} is required for normal gravitropism since bending, bend movement, and bend compensation were all affected by loss of the extracellular Ca^{2+} gradient. However, the role and destiny of Ca^{2+} within cells during the gravitropic response needs to be determined to establish what biochemical processes are involved.

Cytochalasin B

Measurements were extended to 6h since most stipes had not reached the vertical by the end of 3h under the experimental conditions used. A comparison of control stipes with those treated with 3 concentrations revealed that extension was unaffected only at 10 μM cytochalasin B (Table 2). Thus, meaningful comparisons, with respect to the involvement of actin microfilaments in the gravitropism of *C. **cinereus***, could be made only between control and 10 μM-treated stipes. Any deleterious effects observed in stipes treated with higher concentrations of cytochalasin B could not be attributed solely to interference with gravitropic processes.

TABLE 2. Effects of Cytochalasin B on extension and bending after 6 h. Asterisks indicate which values significantly differ ($P = 0.05$) from the control values using Student's t-test.

Treatment	% length increase ± SD	Response time, min ± SD	Bending rate, deg min^{-1} ± SD	Final tip angle, deg ± SD
Control	51.1 ± 2.4	21.3 ± 6.8	0.36 ± 0.10	90.5 ± 9.0
100 μM	14.3 ± 6.4*	161.8 ± 46.2*	0.12 ± 0.06*	61.9 ± 29.0*
50 μM	27.4 ± 4.9*	89.6 ± 22.0*	0.24 ± 0.09*	74.2 ± 18.1*
10 μM	45.4 ± 2.4	51.5 ± 15.5*	0.48 ± 0.15*	89.4 ± 13.1

Gravitropism was not eliminated by treatment with cytochalasin B, but the response was significantly delayed. Most importantly, bending was delayed in stipes treated with 10 •M cytochalasin B, which had no effect on extension, the rate of bending or the final tip angle (Table 2). Bend movement was also unaffected (data not shown). This is in contrast to experiments performed on plants where Ca^{2+} and calmodulin inhibitors caused gravitropic bending in roots to be delayed or eliminated /1, 2, 4/. These results suggest that cytoskeletal elements, specifically actin microfilaments, may be involved in sensing gravity in *C. **cinereus***, just as they seem to be involved in gravity perception in ***Chara*** rhizoids /9/ except that there are no specific gravity-sensing organelles, such as statoliths, in fungal cells to amplify the initial transduction event. This function would have to be fulfilled by cytoplasmic organelles, such as nuclei or vacuoles. There is evidence that actin microfilaments, which have been shown to surround nuclei in stipes of ***Flammulina velutipes***, may function in sensing gravity /11/. Microtubules (MTs) do not seem to have a role in the gravitropic response of *C. **cinereus*** based on preliminary studies using microtubule inhibitors (data not shown), although MTs have been shown to envelope the nuclei in ***Coprinus*** /12/. Further studies using cytochalasin D and phalloidin are in progress to establish the role of actin filaments in the gravitropic response of *C. **cinereus***. The use of clinostats along with these inhibitors would establish whether actin filaments were involved in the initial transduction events in gravity perception or in the initial response events. Clearly, gravitropism in organisms which do not have obvious gravity-sensing organelles is a complex process which requires careful dissection with specific inhibitors.

SUMMARY

Experiments with antagonists to Ca^{2+} transport and inhibitors of calmodulin suggest that Ca^{2+} is not involved in gravity perception but does contribute to transduction of the gravitropic impulse in *Coprinus cinereus*. Regulation of extracellular as well as intracellular Ca^{2+} levels is required for successful gravitropic bending. This suggests that the bending process requires accumulation of Ca^{2+} within cytoplasmic, membrane-bound compartments. Interfering with cytoskeletal actin filaments has no effect on the gravitropic response at concentrations where stipe extension is unaffected, but does significantly delay initiation of bending. This suggests that actin filaments are directly involved in gravity perception by *Coprinus cinereus* and may act as the gravity sensing organelles, since fungi do not have specific structures for this purpose.

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