

## Is ATP a Signaling Agent in Plants?<sup>1</sup>

Vadim Demidchik, Christopher Nichols, Markiyani Oliynyk, Adeeba Dark, Beverley J. Glover, and Julia M. Davies\*

Department of Plant Sciences, University of Cambridge, CB2 3EA, Cambridge, United Kingdom (V.D., C.N., A.D., B.J.G., J.M.D.); and Department of Biochemistry, University of Cambridge, CB2 1GA, Cambridge, United Kingdom (M.O.)

Physiological processes in plant cells are regulated by intrinsic and extrinsic signals. Numerous signaling molecules have been identified, including hormones, elicitors, and secondary metabolites. Cognate receptors and receptor genes have been reported in some cases (Hua and Meyerowitz, 1998; Inoue et al., 2001; Nam and Li, 2002). Several second messengers (responsible for signal amplification and encoding specificity) have been demonstrated in plants; for example, cytosolic  $\text{Ca}^{2+}$ , cyclic nucleotides, and lipid derivatives. Calcium is a common second messenger in plant cell signaling; the amplitude and duration of transient elevations of  $[\text{Ca}^{2+}]_{\text{cyt}}$  may play an encoding role (Allen et al., 2001). Plants have evolved many plant-specific signaling molecules. Nevertheless they also "share" some signaling moieties with animals: nitric oxide, reactive oxygen species, and other regulators function in both Kingdoms. Glu (previously considered an exclusively animal signaling agent, a neurotransmitter) is now regarded as a likely plant signaling compound (Dennison and Spalding, 2000), and genes encoding putative Glu receptor subunits have been identified in the Arabidopsis genome (Lacombe et al., 2001). The possible conservation of Glu signaling between plants and animals suggests that other neurotransmitters might function in plants as signaling agents. One such candidate is extracellular ATP (eATP).

### ATP AND SIGNALING

Although ATP is an ubiquitous energy source, it also acts extracellularly as a neurotransmitter. The concept of ATP and purine derivatives as extracellular signaling molecules was born in the late 1920s and early 1930s when physiological effects of adenine derivatives were discovered (for review, see Ralevic and Burnstock, 1998). Further studies culminated in the proposal in the 1970s of purinergic neurotransmission mediated by specific plasma membrane receptors: "purinoceptors" (Burnstock,

1978). Two main families of purinoceptors (P1 and P2) have now been confirmed in animal cells (for review, see Ralevic and Burnstock, 1998); members exhibit varying sensitivity to eATP, purines, and pyrimidines. P1 receptors are activated by adenosine (P2 are not) and couple with heterotrimeric G proteins. P2 receptors divide into two classes; ligand-gated nonselective cation channels (P2X receptors) and G-protein-coupled receptors (P2Y receptors). P2X receptors activate at higher eATP concentrations (micromoles) than P2Y (nanomoles; Khakh, 2001). Purinoceptors may contribute to  $\text{Ca}^{2+}$  signal transduction cascades directly (through  $\text{Ca}^{2+}$  permeability of P2X channels) or indirectly (via downstream effects of the P2Y-coupled G-protein), and they are now implicated in a range of physiological functions ranging from neurotransmission to cell death (for review, see Ralevic and Burnstock, 1998; Burnstock and Williams, 2000).

In contrast, eATP has received little attention from plant biologists. In the late 1960s and early 1970s, eATP effects on plants were largely being interpreted in terms of supplementation of cellular energy or chelation of divalent cations rather than signaling activity. Plant mechanical movements (e.g. Jaffe, 1973),  $\text{K}^+$  uptake (e.g. Lüttge et al., 1974), and endonuclease activity (Udvardy and Farkas, 1973) were found to be stimulated by eATP. Most studies tested eATP in the millimolar range, but it is notable that Venus fly trap closure was accelerated by 100  $\mu\text{M}$  eATP (Jaffe, 1973), a concentration consistent with receptor activity (animal P2X receptors may exhibit one-half maximal activation by over 300  $\mu\text{M}$  ligand; Humphrey et al., 1998). Since then, eATP effects have been rarely studied and mostly in the context of an exogenous energy supply. For example, stomatal aperture of *Commelina communis* was enhanced by eATP supplied at 5 to 20 mM, and tracer experiments indicated that eATP at such high levels could be taken up by epidermal tissue, possibly to act as an energy source for stomatal movement (Nejdat et al., 1983; Shaish et al., 1989). Only recently has experimentation begun to reveal possible purine signaling activity. Lew and Dearnaley (2000) found that eATP and extracellular ADP (eADP) depolarized the plasma membrane of Arabidopsis root hairs (one-half maximal effect produced by 0.4 mM eATP and 10  $\mu\text{M}$  eADP). This would be consistent with ion channel

<sup>1</sup> This work was supported by the Biotechnology and Biological Sciences Research Council and by the Leverhulme Trust.

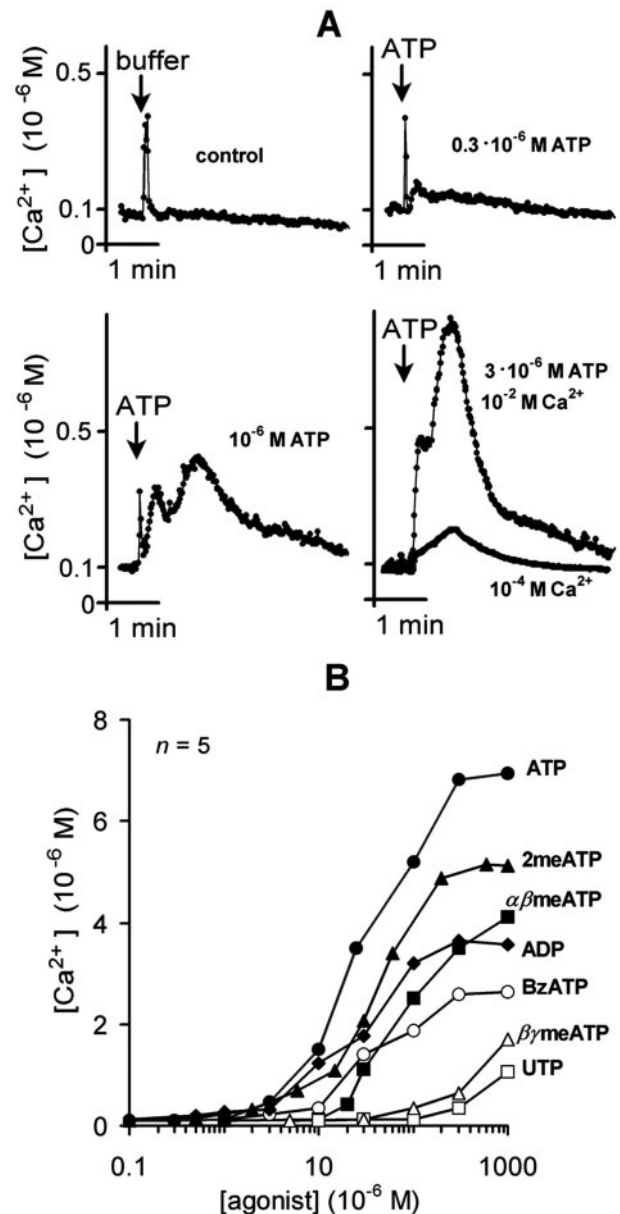
\* Corresponding author; e-mail jmd32@cam.ac.uk; fax 44-1223-333-953.

Article, publication date, and citation information can be found at [www.plantphysiol.org/cgi/doi/10.1104/pp.103.024091](http://www.plantphysiol.org/cgi/doi/10.1104/pp.103.024091).

opening. The effect of eATP on auxin distribution, gene expression, and gravitropism in *Arabidopsis* roots has been recently reported (Tang et al., 2003) and the authors considered one possible explanation to be eATP acting as a regulatory signal. Therefore, previous data suggest the existence of plant eATP signaling. The objective of the present work was to test for possible eATP-induced signaling events. As  $\text{Ca}^{2+}$  is a key, quantifiable second messenger in plant cells and is downstream of eATP in animal cells, we tested the null hypothesis that eATP has no effect on  $[\text{Ca}^{2+}]_{\text{cyt}}$ .

#### ATP ELEVATES ROOT $[\text{Ca}^{2+}]_{\text{cyt}}$

To test for changes in  $[\text{Ca}^{2+}]_{\text{cyt}}$  *Arabidopsis* plants constitutively expressing aequorin in the cytosol (35S promoter-driven expression; Knight et al., 1996) were used. Roots (excised) were tested because roots are naturally directly exposed to the soil solution where eATP occurs due to release from microorganisms (Thomas et al., 1999) and perhaps damaged or sloughed off root cells, and root hairs have been shown to be purine responsive (Lew and Dearnaley, 2000). An eATP range from 100 nM to 1 mM was tested. Application of assay solution alone caused a "touch response" (about 5 s long) and such transients, caused by injection of solution, were also observed in the case of eATP addition (Fig. 1A). After the touch response, eATP caused a dose-dependent transient elevation in  $[\text{Ca}^{2+}]_{\text{cyt}}$  (Fig. 1A), with even 300 nM causing a 2-fold increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$ . At  $[\text{eATP}] < 3 \mu\text{M}$ , the  $[\text{Ca}^{2+}]_{\text{cyt}}$  elevation comprised two distinguishable peaks. The first peak reached its maximum in 10 to 20s, whereas the second maximum was attained 1 to 2 min after application. Above 3  $\mu\text{M}$  [eATP], the first peak was not so clearly distinguishable (merging with the second peak). As a general trend, both peaks increased as [eATP] increased, but the ratio between the amplitudes of first and second peaks tended to decrease.  $[\text{Ca}^{2+}]_{\text{cyt}}$  transients lasted 5 to 10 min in total, and basal  $[\text{Ca}^{2+}]_{\text{cyt}}$  was fully recovered. eATP was added as the disodium salt, therefore, control measurements with equimolar NaCl were carried out. At 0.1 mM, NaCl caused no  $[\text{Ca}^{2+}]_{\text{cyt}}$  elevation ( $n = 3$ ; data not shown). At 2 mM, NaCl caused a transient  $[\text{Ca}^{2+}]_{\text{cyt}}$  elevation that lasted approximately 1 min with a mean  $\pm$  SEM peak value of  $0.32 \pm 0.06 \mu\text{M}$ ,  $n = 4$  (compare with basal value before addition;  $0.11 \pm 0.01 \mu\text{M}$ ). In contrast, 1 mM  $\text{Na}_2\text{ATP}$  caused a mean peak value of  $6.97 \pm 0.82 \mu\text{M}$ ,  $n = 5$  (compare with basal value before addition;  $0.10 \pm 0.01 \mu\text{M}$ ). Thus,  $[\text{Ca}^{2+}]_{\text{cyt}}$  transients were due to eATP rather than  $\text{Na}^+$ . Extracellular  $\text{Ca}^{2+}$  depletion (10–0.1 mM) abolished 85% of the  $[\text{Ca}^{2+}]_{\text{cyt}}$  increase induced by 3  $\mu\text{M}$  eATP, demonstrating eATP-activated  $\text{Ca}^{2+}$  entry from the extracellular space (Fig. 1A). Maximal peak  $[\text{Ca}^{2+}]_{\text{cyt}}$  values were plotted against [eATP] (Fig. 1B). The dose-dependence



**Figure 1.** Effect of externally applied purines and UTP on *Arabidopsis* root  $[\text{Ca}^{2+}]_{\text{cyt}}$ . A, Typical  $[\text{Ca}^{2+}]_{\text{cyt}}$  transients induced by different eATP concentrations. External  $\text{Ca}^{2+}$  was 10 mM unless indicated. B, Mean dose-response curves for effects of different agonists on  $[\text{Ca}^{2+}]_{\text{cyt}}$ . SEM did not exceed 15% of mean (not shown). Experimental procedures are described in detail by Demidchik et al. (2003). *Arabidopsis* constitutively expressing cytosolic aequorin driven by the cauliflower mosaic 35S promoter (Knight et al., 1996), were grown vertically at 22°C (16-h daylength;  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  irradiance) on full-strength Murashige and Skoog medium (Duchefa, Harlem) with 1% (w/v) Suc and 0.35% (w/v) Phytigel (Sigma, St. Louis). Recording solution contained 10 mM  $\text{CaCl}_2$  (or 0.1 mM), 2 mM MES, pH 5.7 (Tris), and  $4 \mu\text{g mL}^{-1}$  coelenterazine (free base; NanoLight Technology, Prolume, Pittsburgh). Luminescence was recorded at 1 Hz.

curve reached saturation at 0.3 mM eATP with  $\text{EC}_{50}$  at 2.6  $\mu\text{M}$  (analyzed by SigmaPlot 4.01 software). Overall, the null hypothesis was disproved; in root

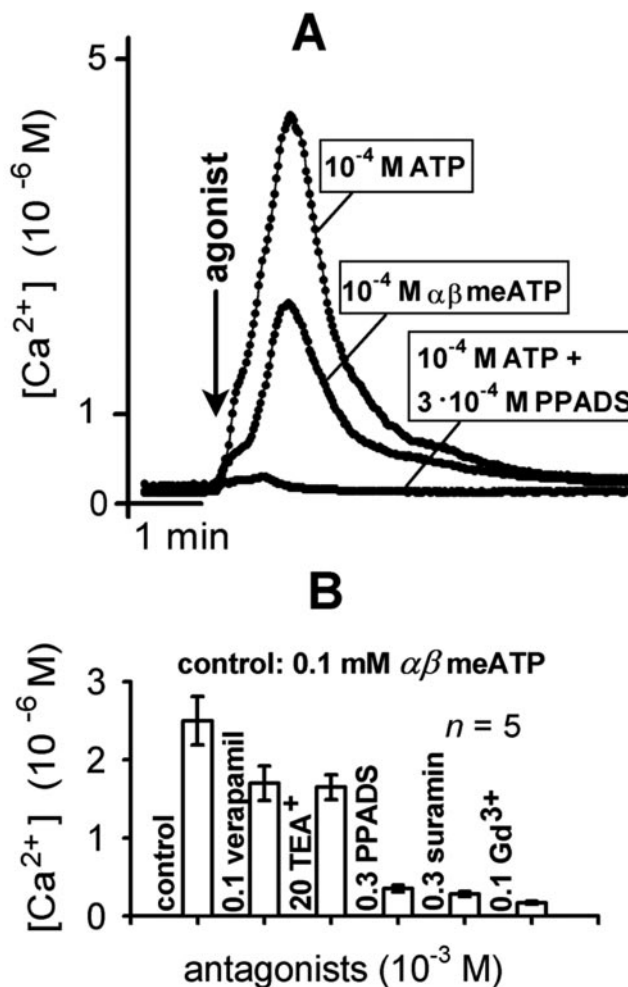
cells at least, eATP can elevate  $[Ca^{2+}]_{cyt}$ . The specificity of this response was then tested.

### PURINE VERSUS PYRIMIDINE

Duration and amplitude of purine-induced  $Ca^{2+}$  transients in animal cells are determined by the structure of the purine derivative (White et al., 2001). The effects of ATP derivatives (see Ralevic and Burnstock, 1998), ADP, and the pyrimidine UTP are shown in Figure 1B. All compounds tested elicited a double peak  $[Ca^{2+}]_{cyt}$  transient response and maximal peak values are reported. ADP was a potent activator; with up to 10  $\mu M$  ADP, the magnitude of response was lower but still comparable with ATP (e.g. mean response to 3  $\mu M$  addition: eATP, 480 nM  $Ca^{2+}$ ; eADP, 310 nM  $Ca^{2+}$ ). 2-Methylthio ATP (an ATP analog with a modified purine ring) was, at lower concentrations, as effective as ATP and ADP. BzATP (ATP analog with modified Rib moiety) produced a weaker effect over the concentration range tested. Critically, the nonhydrolyzable ATP analogs  $\alpha\beta meATP$  (methylene insert between first and second phosphates) and  $\beta\gamma meATP$  (methylene insert between second and third phosphates) were effective (Figs. 1B and 2A). This clearly demonstrates that hydrolysis is not required for the activation of  $[Ca^{2+}]_{cyt}$  transients by eATP.  $\alpha\beta meATP$  was the more potent of the two. Even though ADP was an effective agonist, AMP and Pi (as other ATP breakdown products; tested at 0.1–1 mM) did not elicit statistically significant elevations of  $[Ca^{2+}]_{cyt}$  (measured 1 min after addition). For AMP experiments, mean  $\pm$  SEM  $[Ca^{2+}]_{cyt}$ : basal,  $0.096 \pm 0.004 \mu M$ ,  $n = 10$ ; 1 mM AMP,  $0.138 \pm 0.017 \mu M$ ,  $n = 4$ . For Pi (as  $H_3PO_4$ , buffered with Tris), mean  $\pm$  SEM  $[Ca^{2+}]_{cyt}$ : basal,  $0.112 \pm 0.006 \mu M$ ,  $n = 4$ ; 1 mM Pi  $0.119 \pm 0.007 \mu M$ ,  $n = 4$ . The pyrimidine UTP (which can also elicit a  $[Ca^{2+}]_{cyt}$  response in animal cells) was only effective at higher concentrations ( $>100 \mu M$ ), thus demonstrating not only the significance of purine structure, but also that the ATP effect was unlikely to have been due to a surface charge effect mediated by the phosphate groups of the agonist. Adenosine did not cause  $[Ca^{2+}]_{cyt}$  transients (mean  $\pm$  SEM  $[Ca^{2+}]_{cyt}$ : basal,  $0.107 \pm 0.007 \mu M$ ; 1 mM adenosine 1 min postaddition,  $0.106 \pm 0.006 \mu M$ ,  $n = 5$ ); the simplest structure, adenine, was also ineffective (mean  $\pm$  SEM  $[Ca^{2+}]_{cyt}$ : basal,  $0.119 \pm 0.008 \mu M$ ; 1 mM adenine 1 min postaddition,  $0.122 \pm 0.009 \mu M$ ,  $n = 5$ ).

### INVOLVEMENT OF CHANNELS

Here, the  $[Ca^{2+}]_{cyt}$  transients manifested from a multicellular system comprising various cell types in different developmental and physiological states, all with potentially different capacities to respond to purine derivatives. Therefore, quantitative comparison of the data shown in Figure 1B would have little



**Figure 2.** Effect of antagonists on eATP-induced  $[Ca^{2+}]_{cyt}$  elevation. A, Typical  $[Ca^{2+}]_{cyt}$  response to  $10^{-4}$  M ATP or  $\alpha\beta meATP$  and the effect of 0.3 mM PPADS. B, Reduction of  $\alpha\beta meATP$ -induced  $[Ca^{2+}]_{cyt}$  response (mean  $\pm$  SEM). Antagonists were added 10 min before addition of  $\alpha\beta meATP$ .

meaning. Having demonstrated that changes in ligand structure evoked measurably different responses, the mechanism of  $[Ca^{2+}]_{cyt}$  elevation was explored. Reducing external  $Ca^{2+}$  diminished the eATP-induced transient (see above), suggesting that  $Ca^{2+}$  influx contributed to the response. To confirm the operation of a plasma membrane influx pathway, the effect of cation channel blockers was investigated. In this pharmacological analysis, the nonhydrolyzable ATP analog  $\alpha\beta meATP$  (0.1 mM) was used to prevent possible effects caused by phosphorylation or ATP breakdown. Of the conventional cation channel blockers (Fig. 2B), application of the  $K^+$  channel blocker tetraethylammonium<sup>+</sup> (20 mM) or  $Ca^{2+}$  channel blocker verapamil (0.1 mM) resulted in mean maximum  $[Ca^{2+}]_{cyt}$  increases that were 65% and 68% of the control value, respectively ( $n = 5$ ; Fig. 2B). Whatever the mechanism of initiating the  $[Ca^{2+}]_{cyt}$  increase, it was resistant to these channel antagonists. The 35% and 32% decreases in peak  $[Ca^{2+}]_{cyt}$  re-

sponse by tetraethylammonium and verapamil, respectively, may reflect the sensitivity of contributing transport systems downstream of the initiating mechanism in this multicellular test system. The  $[Ca^{2+}]_{\text{cyt}}$  increase was most strongly affected by  $Gd^{3+}$  (6% of mean control response at 0.1 mM, Fig. 2B;  $n = 5$ ).  $Gd^{3+}$  is a nonspecific blocker of plant cation channels (including Arabidopsis root nonselective cation channels; Demidchik et al., 2002a, b) and this result clearly demonstrates that plasma membrane channel-mediated  $Ca^{2+}$  influx is an initial step in eATP-induced  $[Ca^{2+}]_{\text{cyt}}$  elevation. The role of  $Ca^{2+}$  intracellular stores (if any) in generating the transients requires greater resolution than aequorin affords.

### PURINOCEPTORS?

The mechanistic basis of eATP-induced  $[Ca^{2+}]_{\text{cyt}}$  elevation in plants could be absolutely different from that found in animals or could show some degree of conservation. If the mechanism of activating the  $Ca^{2+}$ -permeable channels that underlie eATP-induced  $Ca^{2+}$  elevation were similar in plants and animals, it is reasonable to predict a conserved pharmacological profile. Animal purinoceptor-mediated signaling is highly sensitive to pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) and suramin (see Ralevic and Burnstock, 1998). In animal preparations, PPADS has been shown to specifically block P2 receptors (see Ralevic and Burnstock, 1998). Suramin, apart from blocking animal P2 receptors, may also affect other receptors and enzymes in animal and plant cells (Ralevic and Burnstock, 1998; Stratmann et al., 2000), but it remains a key diagnostic tool for P2 delineation. We have tested these antagonists (0.3 mM) on excised roots. The eATP and  $\alpha\beta\text{meATP}$  effects on  $[Ca^{2+}]_{\text{cyt}}$  were strongly attenuated to a degree comparable with application of  $Gd^{3+}$  (Fig. 2;  $n = 5$ ).

If, as the data suggest, eATP were to activate  $Ca^{2+}$ -permeable plasma membrane channels, what is the most likely mechanism of activation? If eATP were to permeate, cation channel phosphorylation at the cytosolic face could activate  $Ca^{2+}$  influx. Hyperpolarization-activated  $Ca^{2+}$  channels (which can contribute to  $Ca^{2+}$  influx across the plant cell plasma membrane; Véry and Davies, 2000; Demidchik et al., 2002a) perhaps activate at cytosolic  $[ATP] > 0.5$  mM (Köhler and Blatt, 2002). Cytosolic ADP or nonhydrolyzable ATP do not increase the activity of these channels, therefore, the ATP effect is due to phosphorylation (Köhler and Blatt, 2002). Additionally, if eATP were to permeate, it could conceivably activate ABC transporters by binding to their cytoplasmic nucleotide-binding domains. ABC transporters are increasingly recognized as being capable of regulating ion channel activity and have a role in plant cell signaling (Schmid-Antomarchi et al., 1987;

Bryan and Aguilar-Bryan, 1999; Klein et al., 2003). In the present study, it is difficult to make a case for the ATP influx necessary for cytosolic phosphorylation of channels or ABC activation. The negative membrane voltage of plant root cells (about  $-160$  mV in the conditions used here; Demidchik et al., 2002a) and the estimated species concentrations of cytosolic ATP (Davies et al., 1993) strongly support anion channel-mediated ATP efflux over the range of eATP tested. Even if ATP influx were feasible, phosphorylation at the cytosolic or extracellular face is clearly not essential for generation of the  $[Ca^{2+}]_{\text{cyt}}$  transients, as ADP and  $\alpha\beta\text{meATP}$  (nonhydrolyzable ATP analog) were potent agonists acting at micromolar concentrations and exhibiting saturation. Thus, it is also unlikely that  $Ca^{2+}$  influx was due simply to a stimulation of the plasma membrane  $H^+$ -ATPase and hence membrane hyperpolarization. However, it is important to note that the  $\alpha\beta\text{meATP}$  response does not rule out the possibility of the involvement of a regulatory ABC transporter. If  $\alpha\beta\text{meATP}$  were to permeate (although this appears unlikely), it could feasibly change the conformational state of an ABC transporter, as this can be achieved by ATP binding alone (Rosenberg et al., 2001).

The antagonistic effect of PPADS and suramin raises the possibility of P2 equivalents in plant cells. It is feasible that eATP-activated P2Y equivalent(s), sensitive to PPADS and suramin, couple to  $Gd^{3+}$ -sensitive  $Ca^{2+}$ -permeable channels. It is feasible that there is an eATP-binding and PPADS/suramin-sensitive periplasmic or membrane moiety that regulates  $Gd^{3+}$ -sensitive  $Ca^{2+}$ -permeable channels. It is equally feasible that the eATP-induced  $[Ca^{2+}]_{\text{cyt}}$  elevation is mediated by a class of ionotropic receptors, the eATP-gated  $Ca^{2+}$ -permeable cation channel. In all cases, other plasma membrane  $Ca^{2+}$ -permeable channels could lie downstream of the initial eATP response and could contribute to the observed  $[Ca^{2+}]_{\text{cyt}}$  elevation. These possibilities must now be addressed for individual root cell types using electrophysiological techniques. This would enable direct testing of at least ionotropic versus metabotropic activity and permit comparison of the pharmacological response of ion channels with that of the excised roots reported here. The Arabidopsis genome has been reported to contain no sequences with obvious similarities to animal P2X genes (Tang et al., 2003), but extensive *in silico* analysis has revealed sequences predicted to encode polypeptides with two membrane-spanning domains linked by a Cys-rich extracellular loop bearing a putative ATP-binding motif, reminiscent of a P2X subunit (V. Demidchik, M. Oliynyk, B. J. Glover, and J. M. Davies unpublished data).

### ATP RELEASE AND BREAKDOWN

The ability of eATP (and to some extent eADP) to elevate  $[Ca^{2+}]_{\text{cyt}}$  in plants implies further possible

conservation with animal purine signaling. The mechanism of eATP/eADP signaling in plants (by analogy with animals) should include systems for rapid release from cells and rapid removal from the extracellular space. In animal cells, ATP is released via anion channels, ABC transporters, or exocytosis (Bodin and Burnstock, 2001; Dutta et al., 2002); ATP and ADP are quickly hydrolyzed by extracellular apyrases, which attack the  $\beta$ - and  $\gamma$ -phosphate bonds (Todorov et al., 1997). To date, in plants, the ABC transporter PGP1 is thought to mediate ATP release from *Arabidopsis* (Thomas et al., 2000), and extracellular apyrases (which are found in roots) are known to hydrolyze ATP more rapidly than any other extracellular phosphatase (Handa and Guidotti, 1996; Thomas et al., 1999). Therefore, plant cells do have the potential "machinery" to use eATP as a signaling agent. It is notable that if eATP (or perhaps eADP) were a signal hydrolyzed by apyrase, then (as shown in this study) the hydrolysis products AMP and Pi would be without effect on  $[Ca^{2+}]_{\text{cyt}}$ ; the signal would be terminated.

#### FUTURE DIRECTIONS

This study has demonstrated that eATP could be a signaling agent in plants. The physiological responses downstream of an eATP-induced  $[Ca^{2+}]_{\text{cyt}}$  transient remain to be elucidated, but in light of the present data, a re-examination of previously reported eATP effects on plant cells is now warranted. For example, inhibition of the *Arabidopsis* root gravitropic response by eATP (at millimolar levels incorporated into the growth medium) has been reported recently (Tang et al., 2003). Here, a lower ATP concentration that can elicit a root  $[Ca^{2+}]_{\text{cyt}}$  response also inhibited gravitropism. Buffered solutions (0.5 mM  $CaCl_2$ , 0.5 mM Tris, and 1 mM MES, pH 5.7) were applied to 4-d-old *Arabidopsis* roots before a 90° gravitropic stimulus in the dark. After 3 h, the mean  $\pm$  SEM curvature response of the Na-buffer control was  $47^\circ \pm 1^\circ$  ( $n = 37$  plants in three independent trials), and the mean 100  $\mu$ M ATP response was  $34^\circ \pm 1^\circ$  ( $n = 68$ ); this is a statistically significant difference at the 99.9% level (Student's  $t$  test). Preliminary experiments indicate that 100  $\mu$ M ADP is also inhibitory, but AMP and UTP are not. Clearly, to link eATP securely to gravitropism via  $[Ca^{2+}]_{\text{cyt}}$  now requires a thorough study of the sensing and responding cells.

Release of ATP could be from damaged cells or as a result of cell death. In this respect, a role in defense signaling could be envisaged. In contrast to the animal paradigm, certain plant cells could also be exposed to eATP released by other organisms. Because microbes can release ATP and other purines, plant-microbe interaction at the epidermis may perhaps involve eATP as a signaling agent. ATP release by

plant cells may be mediated by ABC transporters (Thomas et al., 2000) and it is interesting to note the possible connections between this type of transporter and the eATP-activated  $Ca^{2+}$  influx reported here. eATP turnover is thought to regulate ABC transporters in *Arabidopsis* (Thomas et al., 2000), and disruption of  $Ca^{2+}$  homeostasis (by limiting  $Ca^{2+}$  influx) has been found to increase expression of an ABC transporter in wheat (*Triticum aestivum*) root (Sasaki et al., 2002). Perhaps, increasing ATP release by increasing ABC expression compensates for lowered  $[Ca^{2+}]_{\text{cyt}}$  through eATP activation of  $Ca^{2+}$  influx. In animals, neurotransmitters establish and maintain a communication network between neurons. eATP could play a similar role in plants, perhaps providing intercellular communication to coordinate developmental programs or responses to environmental stimuli. Further studies are now required to identify the plant ATP-activated receptor(s) and delineate physiological significance.

#### ACKNOWLEDGMENTS

We thank Mark Knight for providing seeds and Baljit Khakh for advice on animal purinoceptors.

Received March 24, 2003; returned for revision April 27, 2003; accepted July 16, 2003.

#### LITERATURE CITED

- Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffman T, Tang YY, Grill E, Schroeder JI (2001) A defined range of guard cell calcium oscillation parameters encodes stomatal movements. *Nature* **411**: 1053–1057
- Bodin P, Burnstock G (2001) Purinergic signaling: ATP release. *Neurochem Res* **26**: 959–969
- Bryan J, Aguilar-Bryan L (1999) Sulfonylurea receptors: ABC transporters that regulate ATP-sensitive  $K^+$  channels. *Biochim Biophys Acta* **1461**: 285–303
- Burnstock G (1978) A basis for distinguishing two types of purinergic receptors. In L Bolis and RW Straub, eds, *Cell Membrane Receptors for Drugs and Hormones*. Raven Press, New York, pp 107–118
- Burnstock G, Williams M (2000) P2 purinergic receptors: modulation of cell function and therapeutic potential. *J Pharm Exp Therap* **295**: 862–869
- Davies JM, Poole RJ, Sanders D (1993) The computed free energy change of hydrolysis of inorganic pyrophosphate and ATP: apparent significance for inorganic-pyrophosphate-driven reactions of intermediary metabolism. *Biochim Biophys Acta* **1141**: 29–36
- Demidchik V, Bowen HC, Maathuis FJM, Shabala SN, Tester MA, White PJ, Davies JM (2002a) *Arabidopsis thaliana* root non-selective cation channels mediate calcium uptake and are involved in growth. *Plant J* **32**: 799–808
- Demidchik V, Davenport RJ, Tester MA (2002b) Nonselective cation channels in plants. *Annu Rev Plant Biol* **53**: 67–107
- Demidchik V, Shabala SN, Coutts KB, Tester MA, Davies JM (2003) Free oxygen radicals regulate plasma membrane  $Ca^{2+}$  and  $K^+$ -permeable channels in plant root cells. *J Cell Sci* **116**: 81–88
- Dennison KL, Spalding EP (2000) Glutamate-gated calcium fluxes in *Arabidopsis*. *Plant Physiol* **124**: 1511–1514
- Dutta AK, Okada Y, Sabirov RZ (2002) Regulation of an ATP-conductive large-conductance anion channel and swelling-induced ATP release by arachidonic acid. *J Physiol* **542**: 803–816
- Handa M, Guidotti G (1996) Purification and cloning of a soluble ATP-diphosphohydrolase (apyrase) from potato tubers (*Solanum tuberosum*). *Biophys Biochem Res Commun* **218**: 916–923

- Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell* **94**: 261–271
- Humphrey PPA, Khakh BS, Kennedy C, King BF, Burnstock G (1998) The IUPHAR Compendium of Receptor Characterization and Classification. Pub. IUPHAR Media, London, UK, pp 195–217
- Inoue T, Higuchi Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature* **409**: 1060–1063
- Jaffe MJ (1973) The role of ATP in mechanically stimulated rapid closure of the Venus's-Flytrap. *Plant Physiol* **51**: 17–18
- Khakh BS (2001) Molecular physiology of P2X receptors and ATP signalling at synapses. *Nature Rev Neurosci* **2**: 165–174
- Klein M, Perfus-Barbeoch L, Frelet A, Gaedecke N, Reinhardt D, Mueller-Roeber B, Martinoia E, Forestier C (2003) The plant multidrug resistance ABC transporter AtMRP5 is involved in guard cell hormonal signaling and water use. *Plant J* **33**: 119–129
- Knight H, Trewavas AJ, Knight MR (1996) Cold calcium signaling involves two cellular pools and a change in calcium signature after acclimation. *Plant Cell* **8**: 489–503
- Köhler B, Blatt MR (2002) Protein phosphorylation activates the guard cell  $Ca^{2+}$  channel and is a prerequisite for gating by abscisic acid. *Plant J* **32**: 185–194
- Lacombe B, Becker D, Hedrich R, DeSalle R, Hollmann M, Kwak JM, Schroeder JL, Le Novere N, Nam HG, Spalding EP, Tester M, Turano FJ, Chiu J, Coruzzi G (2001) The identity of plant glutamate receptors. *Science* **292**: 1486–1487
- Lew RR, Dearnaley JDW (2000) Extracellular nucleotide effects on electrical properties of growing *Arabidopsis thaliana* root hairs. *Plant Sci* **153**: 1–6
- Lüttge U, Schöch EV, Ball E (1974) Can externally applied ATP supply energy to active ion uptake mechanisms of intact plant cells? *Aust J Plant Physiol* **1**: 211–220
- Nam KH, Li JM (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signalling. *Cell* **110**: 203–212
- Nejidat A, Itai C, Roth-Bejerano N (1983) Stomatal response to ATP mediated by phytochrome. *Physiol Plant* **57**: 367–370
- Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. *Pharmacol Rev* **50**: 413–492
- Rosenberg MF, Verlarde G, Ford RC, Martin C, Berridge G, Kerr ID, Callaghan R, Schidlin A, Wooding C, Linton KJ, Higgins CF (2001) Repacking of the transmembrane domains of P-glycoprotein during the transport ATPase cycle. *EMBO J* **20**: 5615–5625
- Sasaki T, Ezaki B, Matsumoto H (2002) A gene encoding multidrug resistance (MDR)-like protein is induced by aluminium and inhibitors of calcium flux in wheat. *Plant Cell Physiol* **43**: 177–185
- Shaish A, Roth-Bejerano N, Itai C (1989) The response of stomata to  $CO_2$  relates to its effect on respiration and ATP level. *Physiol Plant* **76**: 107–111
- Schmid-Antomarchi H, De Weille J, Fosset M, Lazdunski M (1987) The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated  $K^+$  channel in insulin-secreting cells. *J Biol Chem* **262**: 15840–15844
- Stratmann J, Scheer J, Ryan CA (2000) Suramin inhibits initiation of defense signalling by systemin, chitosan, and a  $\beta$ -glucan elicitor in suspension-cultured *Lycopersicon peruvianum* cells. *Proc Natl Acad Sci USA* **97**: 8862–8867
- Tang W, Brady SR, Sun Y, Muday GK, Roux SJ (2003) Extracellular ATP inhibits root gravitropism at concentrations that inhibit polar auxin transport. *Plant Physiol* **131**: 147–154
- Thomas C, Sun Y, Naus K, Lloyd A, Roux SJ (1999) Apyrase functions in plant phosphate nutrition and mobilizes phosphate from extracellular ATP. *Plant Physiol* **119**: 543–551
- Thomas C, Rajagopal A, Windosr B, Dudler R, Lloyd A, Roux SJ (2000) A role for ectophosphatase in xenobiotic resistance. *Plant Cell* **12**: 519–533
- Todorov L, Mihaylova-Todorova S, Westfall T, Sneddon P, Kennedy C, Bjur R, Westfall D (1997) Neuronal release of soluble nucleotidases and their role in neurotransmitter inactivation. *Nature* **387**: 76–79
- Udvardy J, Farkas GL (1973) ATP stimulates the formation of nucleases in excised *Avena* leaves. *Z Pflanzenphysiol* **69**: 394–401
- Véry A-A, Davies JM (2000) Hyperpolarization-activated calcium channels at the tip of *Arabidopsis* root hairs. *Proc Natl Acad Sci USA* **97**: 9801–9806
- White SM, Imig JD, Thu-Thuy K, Hauschild BC, Insko EW (2001) Calcium signaling pathways utilized by P2X receptors in freshly isolated preglomerular MVSMC. *Am J Physiol Renal Physiol* **280**: 1054–1061