Glutamate-Gated Calcium Fluxes in Arabidopsis

Kirsten L. Dennison and Edgar P. Spalding

Department of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, Wisconsin 53706

It is well accepted that endogenous and environmental signals can influence cellular activities by changing \([Ca^{2+}]_{\text{cyt}}\) (Malhó et al., 1998; Sanders et al., 1999). Despite the importance of this mechanism for coupling stimuli to responses (Malhó et al., 1998), the molecules responsible for generating increases in \([Ca^{2+}]_{\text{cyt}}\) during cell signaling in plants are not known at the genetic level. The results presented here raise the possibility that ligand-gated ion channels in plants such as those predicted by the discovery of ionotropic glutamate receptor (iGluR)-like genes in Arabidopsis (Lam et al., 1998) are key components of a \(Ca^{2+}\) influx mechanism important to signal transduction.

In animal brains iGluR channels mediate fast chemical transmission across synapses by increasing the permeability of the post-synaptic cell membrane to \(K^+\), \(Na^+\), and \(Ca^{2+}\) after binding Glu released by the presynaptic cell (Hille, 1992; Hollmann and Heinemann, 1994; Dingledine et al., 1999). The resulting \(Ca^{2+}\) entry in particular has been associated with long-term potentiation of the synapse, a physiological adaptation important to the learning process (Baudry and Lynch, 1993; Bliss and Collinridge, 1993). The Glu receptor homologs recently identified in plants (Lam et al., 1998) are too divergent from animal iGluRs to know with any certainty what ligand(s) gate them, what ions are conducted in the open state, and in which membrane(s) of the cell they function (Chiu et al., 1999). Thus the identification of iGluR sequences in the Arabidopsis genome raises intriguing questions about the physiological functions of neurotransmitter-gated channels in plant cells.

The possibility that Glu gates \(Ca^{2+}\)-permeable channels at the plasma membrane of plant cells was explored by measuring \([Ca^{2+}]_{\text{cyt}}\) in transgenic seedlings expressing aequorin, a \(Ca^{2+}\)-sensitive luminescent protein (Knight et al., 1991). As shown in Figure 1A, Glu application immediately triggered a very large, transient spike in \([Ca^{2+}]_{\text{cyt}}\). In separate experiments the effect of Glu on membrane potential \(V_m\) was measured with intracellular microelectrodes inserted into root apices of intact seedlings. Figure 1A also shows that switching the bathing medium from 1 mM KCl to 1 mM K-Glu induced a large and rapid depolarization of the membrane, as would be expected if the abrupt increase in \([Ca^{2+}]_{\text{cyt}}\) was due to Glu opening \(Ca^{2+}\)-permeable channels at the plasma membrane. The average peak change in \(V_m\) induced by 1 mM Glu was \(55 \pm 7\) mV \((n = 6\) seedlings). This positive shift in \(V_m\), though consistent with Glu gating an inward electrogenic \(Ca^{2+}\) current across the plasma membrane, may also be due to secondary effects of the increased \([Ca^{2+}]_{\text{cyt}}\) on other ion transporters. Another scenario to consider is that Glu directly gates channels permeable to ions such as Cl\(^-\) (Cully et al., 1994) in addition to \(Ca^{2+}\)-permeable channels to cause the depolarization. And last, an electrogenic Glu-uptake mechanism (Boorer et al., 1996) may also contribute to the electrical response. Because these and perhaps other scenarios are not mutually exclusive, more electrophysiological studies of the connection between the large Glu-gated changes in \([Ca^{2+}]_{\text{cyt}}\) and the effect on \(V_m\) are warranted.

Figure 1B shows that the magnitude and time course of the rapid increase in \([Ca^{2+}]_{\text{cyt}}\) was similar to the well-studied response to cold shock, i.e. in the micromolar concentration range and completed within several seconds (Knight et al., 1991, 1996; Lewis et al., 1997). Treatment with 1 mM Glu induced a response that was typically hundreds of fold higher than the control injection of equimolar KCl, which produced a touch response that may reflect \(Ca^{2+}\) entering the cytoplasm from internal stores (Haley et al., 1995; Leguè et al., 1997). The post-peak shoulder apparent in the selected response to Glu was often, but not always observed. Activation of iGluRs by Glu causes very similar \(Ca^{2+}\) changes in cells of the mammalian nervous system (Kirischuk et al., 1999; Obrist et and van den Pol, 1999).

If the increase in \([Ca^{2+}]_{\text{cyt}}\) triggered by Glu resulted at least in part from flux across the plasma membrane from the apoplasm, impermeent channel blockers and external chelators of \(Ca^{2+}\) should reduce the response. The results in Figure 2A demonstrate that pretreatment with La\(^3+\), a frequently used blocker of plasma membrane \(Ca^{2+}\) channels, inhibited the \(Ca^{2+}\) spike to the low level induced by the control treatment. Chelating extracellular \(Ca^{2+}\) by pre-treating seedlings with EGTA was similarly inhibitory (Fig. 2B). The combined evidence support our suggestion that Glu triggers an influx of \(Ca^{2+}\) across the plasma membrane and this leads to a dramatic change in \([Ca^{2+}]_{\text{cyt}}\).

\(^1\) This work was supported by the National Science Foundation (career award no. IBN-9734478 to E.P.S.).
nal stores such as the vacuole may also contribute (Allen et al., 1995).

La$^{3+}$ also blocked the depolarization triggered by Glu without affecting the resting $V_m$ (Fig. 2C), indicating that the depolarization is a consequence of the inward Ca$^{2+}$ movement. However, La$^{3+}$ is not a specific Ca$^{2+}$-channel blocker (Lewis and Spalding, 1998) and it may prevent the depolarization by blocking a separate Glu-gated conductance in addition to the Ca$^{2+}$ pathway. Thus despite the fact that La$^{3+}$ blocks the Ca$^{2+}$ flux and the membrane depolarization, the exact relationship between the two Glu-
Glu-gated Ca
tate evolved in Glu receptors after the divergence of methylisoxazole-4-propionate and N-methyl-

Plant Physiol. Vol. 124, 2000 1513

much more effective than other potential agonists, including D-Glu

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It may be that affinity for α-amino-3-hydroxy-5-methylisoxazole-4-propionate and N-methyl-d-aspartate, potent agonists of animal iGluRs, did not induce a response above the control treatment (approximately 2% of the L-Glu response). These non-native agonists of animal iGluRs also did not activate the Synechocystis GLU0 (Chen et al., 1999).

If Glu or some other related small organic acid is the primary endogenous ligand, then it is important to consider how and when the external ligand-binding site would experience 0.3 to 3 mM concentrations. Anion channels at the plasma membrane of plant cells are known to function in the transduction of several signals important to plant growth and development (Ward et al., 1995). These channels are relatively non-selective among anions and may conduct significant efflux of dicarboxylic anions such as malate (Hedrich, 1994; Schmidt and Schroeder, 1994), and therefore perhaps Glu, as well. When environmental or endogenous signals activate such anion channels, apoplastic Glu concentration may rise into the effective range, causing a transient change in [Ca\(^{2+}\)]\(_{cyt}\) that serves to couple a stimulus to downstream responses. This hypothetical scenario may be most plausible in roots, where anion-channel mediated release of dicarboxylic acids has been proposed as a mechanism for combating Al\(^{3+}\) stress (Delhaize and Ryan, 1995; Ryan et al., 1997). Perhaps it is no coincidence that dissection experiments revealed most of the Ca\(^{2+}\) signal recorded from intact Arabidopsis seedlings was contributed by the root; leaves and cotyledons of young plants displayed smaller Glu responses (data not shown).

The results presented here form the basis of our proposition that a key element of a mechanism for altering [Ca\(^{2+}\)]\(_{cyt}\) in plant cells during signaling is similar to that responsible for neurotransmitter action in the central nervous system of animals. The evidence would be bolstered considerably if mutational studies revealed a link between specific iGluR-like genes and Glu-gated Ca\(^{2+}\) fluxes. Plant biologists interested in Ca\(^{2+}\) signaling are presently particularly well equipped to test this connection because there is a wealth of published details about iGluR-mediated Ca\(^{2+}\) signaling in neurons, the Arabidopsis genome is essentially sequenced and searchable, so-

The fact that the response to D-Glu was less than 10% of the L-Glu response indicates high stereospecificity of the binding site(s) on whatever molecules are responsible. Although Glu is clearly an effective ligand in a plausible concentration range, other ligands may be more physiologically important. The Arabidopsis genome contains several iGluR-like genes (Chiu et al., 1999) and that diversity may be matched by a similar diversity of agonists.

Information on the effective concentration range of Glu would help to establish a physiological context for this ligand-gated response in plants. The change in [Ca\(^{2+}\)]\(_{cyt}\) induced by Glu increased between 0.3 and 3 mM, with the concentration for half-maximal response (EC\(_{50}\)) being approximately 1 mM (data not shown). This value is approximately 10-fold greater than the typical value for prokaryotic and animal iGluRs, but very similar to the EC\(_{50}\) of Cl\(^{-}\)-permeable iGluRs from nematodes (Cully et al., 1994).
phisticated reverse-genetic strategies are very practical, and electrophysiological techniques can measure function with high resolution. The stage for exciting developments in Ca\(^{2+}\) signaling is set.

ACKNOWLEDGMENT

We thank Jamie Verheyden for technical assistance.

Received September 11, 2000; accepted September 25, 2000.

LITERATURE CITED