In animal brains, ionotropic glutamate receptors (GluRs) function as glutamate-activated ion channels in rapid synaptic transmission. We have now discovered that genes encoding putative ionotropic GluRs exist in plants, and we present preliminary evidence for their involvement in light-signal transduction. It may be that signalling between cells by excitatory amino acids in animal brains evolved from a primordial signalling mechanism that existed before the divergence of plants and animals. Our findings also help to explain why neuroactive compounds made by plants work on receptors in human brains.

We isolated from Arabidopsis two full-length complementary DNAs, GLR1 and GLR2. Each of these cDNAs encodes all of the signature domains of animal ionotropic GluRs, including the ‘three-plus-one’ transmembrane domains (M1 to M4) and the putative ligand-binding domains (GlnH1 and GlnH2), previously shown to be conserved between Escherichia coli glutamine permease (GlnH) and animal ionotropic GluRs (Fig. 1). Hydrophathy plots, transmembrane prediction and protein-sorting programs predict that the Arabidopsis GLRs encode a plasma-membrane signal peptide and four transmembrane domains (M1 to M4), of which M2 is predicted not to span the membrane (Fig. 1b). This membrane topology is analogous to animal ionotropic GluRs in which the putative ligand-binding GlnH domains are exposed to the external side of the membrane.

In addition to their similar secondary structures, the Arabidopsis GLRs also share extensive sequence identity with animal ionotropic GluRs in the signature domains GlnH1 (including the ligand-binding residue R), GlnH2, and M1 to M4, especially within M3 (Fig. 1a). The degree of identity between Arabidopsis GLRs and animal ionotropic GluRs within these domains (63% to 16%) is similar to that between animal ionotropic GluR subtypes that have kainate/AMPA rather than NMDA as agonists (60% to 32%). Arabidopsis, like animals, seems to have a family of expressed genes encoding ionotropic GluRs, as judged by the existence of other GLR genes (Fig. 1a) and northern blot analysis (data not shown). GLR-related genes also seem to exist in a variety of higher plants, including both dicotyledons (tobacco and pea) and monocotyledons (rice and maize), as judged by heterologous Southern blot analysis (not shown).

To investigate the possible in vivo...
function(s) of putative ionotropic GluRs in plants, Arabidopsis seedlings were grown in the presence of DNQX, an antagonist of animal kainate/AMPA ionotropic GluRs. Plants grown on media containing DNQX phenocopy Arabidopsis long-hypocotyl (hy) mutants impaired in light-signal transduction³, as judged by two independent criteria: DNQX at least partly blocks the ability of light to inhibit hypocotyl elongation (Fig. 2a–c) and to induce chlorophyll synthesis (Fig. 2d–f). Plants grown in the light in the presence of DNQX display a significant light- and dose-dependent increase in hypocotyl elongation compared with control untreated plants (Fig. 2b,c). This effect is specific for light, as the same DNQX concentration has no such effect in the dark (Fig. 2a). The increase in hypocotyl length in DNQX-treated plants (1.5- to 2-fold) is similar to that of Arabidopsis hy mutants deficient in the synthesis of the phytochrome photoreceptor chromophore (hy1 and hy2; 1.5- to 2.5-fold)⁴,⁵.

Because light and hormones interact to control hypocotyl elongation, one or both may be responsible for the DNQX effect. We therefore measured the effects of DNQX on a second light-dependent process, chlorophyll synthesis. DNQX treatment impairs the ability of light to induce the synthesis of chlorophyll in plants grown in the dark and exposed to light for 5 hours (Fig. 2d–f). DNQX-treated plants exhibited a 60% reduction in light-induced chlorophyll accumulation (Fig. 2e) compared with controls (Fig. 2d). This effect is light specific as growth and chlorophyll levels are unaffected in DNQX-treated plants grown in the dark (Fig. 2a,f).

Taken together, these findings indicate that ionotropic GluRs may be involved in light-signal transduction in plants. Because Arabidopsis, like animals, seems to possess a family of GLR genes, the in planta effects of DNQX-treated plants may be due to inhibition of one or more ionotropic GluR receptors in vivo.

The existence of putative ionotropic GluRs in plants is surprising, and provides an insight into why plants make chemicals that act on human brains. Several classical ionotropic GluR agonists that activate and define specific ionotropic GluR subtypes in brains are natural plant products. Examples include kainate, synthesized in the seaweed Digenea simplex, and quisqualic acid, found in Quisqualis indica seeds⁶. Other less well known plant-derived ionotropic GluR agonists include β-N-methyl-amino-l-alanine, produced in cycads, and β-N-oxalylamino-l-alanine, from chick-peas, both of which are associated with neurodegenerative diseases in animals.

It is traditionally believed that plants produce such neurotoxins for defence against herbivory. However, the discovery of putative ionotropic GluRs in plants and their possible role in light-signal transduction suggest an alternative theory: that plants may synthesize ionotropic GluR agonists in order to regulate their endogenous ionotropic GluR receptors, and that selective pressure in certain species then led to high-level production for defence against herbivores.

The existence of putative glutamate receptors in plants raises the possibility that other neurologically active compounds made by plants (such as nicotine, cocaine, caffeine), which act on receptors in animal brains, may also act as ligands for endogenous receptors in plants to regulate a variety of as yet unknown cell-to-cell signalling systems in higher plants.

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Figure 2. Effects of ionotropic GluR antagonist DNQX on Arabidopsis seedlings. Seedlings were sown on MS medium (Gibco BRL Murashige and Skoog basal medium containing 3% sucrose and 0.9% Difco bactoagar) in the absence or presence of the ionotropic GluR antagonist DNQX. All plates contained the solvent dimethyl sulfoxide at 16 μl per ml medium. a–c, Effect of DNQX on hypocotyl length. Seedlings were germinated for 7 days either in the dark (a) or in a normal day–night cycle (16 h light: 8 h dark) (b) at 22°C in the presence (400 μM) or absence of DNQX. Hypocotyl length was determined after light-grown seedlings (n, 44–51) germinated on 0, 100, 200 and 400 μM DNQX (c). An unpaired t-test comparing values from control and DNQX treatments: 100 μM DNQX (no significant difference); 200 μM DNQX (P < 0.0001); 400 μM DNQX (P < 0.0001). d–f, Effect of DNQX on light-induced chlorophyll levels. Seedlings were germinated in total darkness in the absence (d) or presence (e) of 400 μM DNQX for 7 days. Plants were transferred to light for 5 h and chlorophyll levels quantified in three separate pools of 30–40 seedlings grown in the dark (columns 1, 2) and subsequently exposed to light for 5 h (columns 3, 4) (f). An unpaired t-test indicates a significant difference (P = 0.0031) in chlorophyll levels between DNQX-treated and untreated controls in light-treated plants, and no difference in dark-grown plants.

**References**