Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Biochemistry and Pharmacology of Plasma-Membrane Calcium Channels in Plants

A. M. Hetherington, A. Graziana, C. Mazars, P. Thuleau and R. Ranjeva

Phil. Trans. R. Soc. Lond. B 1992 **338**, 91-96
doi: 10.1098/rstb.1992.0132

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1992 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

The biochemistry and pharmacology of plasma-membrane calcium channels in plants

A. M. HETHERINGTON[1], A. GRAZIANA[2], C. MAZARS[2], P. THULEAU[2] AND R. RANJEVA[2]†

[1] *Division of Biological Sciences, Lancaster University, Lancaster LA1 4YQ, U.K.*
[2] *Centre de Biologie et Physiologie Vegetales, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse, Cedex, France*

SUMMARY

A review of plasma-membrane calcium channels in higher plants is presented. Data from pharmacological and biochemical studies are used to assess the current state of our knowledge concerning the occurrence of these structures in higher plants. Recent results demonstrate that after purification and reconstitution a phenylalkylamine binding protein will form calcium permeable channels. This result suggests that plants contain structures with some analogy to animal calcium channels. We also suggest that a degree of caution should be observed in the interpretation of results gained in pharmacological studies employing calcium channel active drugs, as it is now clear that even at low doses these compounds have non-specific effects.

1. INTRODUCTION

In plants, there is good circumstantial evidence that the calcium ion controls a number of biological processes (Hepler & Wayne 1985). Thus the influence of calcium on gene expression, enzyme activities, protein secretion and the control of turgor are documented. However, there have been comparatively few direct demonstrations of a stimulus-induced increase in the concentration of calcium in the cytosol of higher plants. Use of the ratiometric fluorescent indicators fura-2 and indo-1 has revealed that in stomata the application of abscisic acid results in an increase in guard cell cytosolic free calcium concentration. The nature of the increase was found to be variable, but importantly, when it occurred it preceded stomatal closure (McAinsh *et al.* 1990; Schroeder & Hagiwara 1990; Gilroy *et al.* 1991; Hetherington & Quatrano 1991). These results are important because the detection of a stimulus-induced increase in free calcium is the central pillar upon which the calcium second messenger hypothesis rests. Recently, additional evidence to support this hypothesis has been obtained using a novel approach. Using *Nicotiana plumbaginifolia* transformed with the apoaequorin gene, Knight *et al.* (1991) have been able to reconstitute aequorin in seedlings. As this calcium-sensitive luminescent protein is localized to the cytosol it has been possible to demonstrate that external stimuli such as touch or fungal elicitors induce increases in the concentration of free calcium in the cytosol of the transformed seedlings. All these experiments are technically demanding and illustrate the difficulties which must be overcome by cell biologists before the central tenet of the calcium messenger hypothesis can be accepted in plant cells.

The study of plant ion channels is also technically demanding. This is highlighted by the fact that although voltage or ligand-gated ion channels have been shown to exist in plants, to the best of our knowledge, there is no example of an ion channel that has been characterized both at the molecular and electrophysiological levels. Both potassium and chloride channels have been functionally identified and some have been shown to be calcium modulated but their molecular structure and the regulation of their gene expression remain to be studied (Hedrich & Schroeder 1989; Tester 1990; Blatt 1991). In the specific case of calcium channels, both inositol trisphosphate-sensitive and insensitive channels are known to exist at the tonoplast. These structures are most probably involved in the mobilization of calcium from internal stores and possibly act in concert with other intracellular release mechanisms but are not believed to participate directly in the entry of calcium from the external milieu (Alexandre *et al.* 1990; Johannes *et al.* 1992). It is the purpose of this paper to review the progress which has been made in our understanding of the biochemistry and physiology of the calcium channel in the plant plasma-membrane. Other recent reviews of plant calcium channels are Schroeder & Thuleau (1991) & Johannes *et al.* (1991). However, before discussing plant plasma-membrane calcium channels a brief overview of these stuctures in animals is appropriate.

2. CALCIUM CHANNELS IN ANIMALS

In animals, plasma-membrane voltage-dependent calcium channels respond to changes in membrane

† To whom correspondence should be sent.

Phil. Trans. R. Soc. Lond. B (1992) **338,** 91–96
Printed in Great Britain

© 1992 The Royal Society and the authors

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions: Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

potential and allow calcium to flow into the cell down its electrochemical gradient. Animal calcium channels have been the subject of a number of recent reviews (Campbell *et al.* 1988; Tsien *et al.* 1988; Bean 1989; Glossman & Striessnig 1990; Porzig 1990; Tsien & Tsien 1990) and the reader is directed to these papers for a full discussion of this topic.

The classification of calcium channels in animal cells is based upon the mechanism by which the channel is regulated. Accordingly, in vertebrate cells Tsien & Tsien (1990) and Glossman & Striessnig (1990) recognize voltage-operated channels (VOCS), receptor-operated channels (ROCS), second messenger-operated channels (SMOCS), mechanically operated channels (MOCS) and tonically operated channels. Of these the VOCS are the most extensively studied and can be further divided into a number of sub-types. L-type VOCS are long lasting, activated at high voltages and blocked by calcium-channel antagonists belonging to the dihydropyridine, phenylalkylamine and benzothiazepine classes and have a 25 pS single-channel conductance. T-type channels are characterized by rapid inactivation, low voltage activation and are largely resistant to the dihydropyridines but are sensitive to the phenylalkylamines and low concentrations of Ni^{+}. A typical single channel conductance (100 mM Ba^{2+}) would be 8–9 pS. The third sub-class of VOC (N-type) is found in most neurons and is activated at high voltages (like the L-type) but inactivates like the T-type. Additionally, in common with the T-type channel it is insensitive to the dihydropyridines. However it is sensitive to omega conotoxins such as GVIA and it exhibits a 13 pS single-channel conductance. In summary, the various calcium channels can be distinguished according to their voltage characteristics, single-channel properties, ionic selectivity and pharmacological properties. As pharmaceuticals have also been used in studies of plant calcium channels they will be discussed in greater detail.

In animals, interest in calcium-channel antagonists stems from their use as pharmaceuticals, where, clinically, they have importance in the treatment of conditions such as coronary heart disease (Naylor 1988). However, these compounds have also proved useful in the pharmacological characterization of calcium-channel subtypes and, through the availability of radiolabelled derivatives have been used in the purification of calcium-channel subunits. On the basis of their structure it is possible to divide the calcium-channel antagonists into a number of different classes. These include the 1,4 dihydropyridines (for example nifedipine); the phenylalkylamines (for example verapamil); the benzothiazepines (for example diltiazem); the diphenylbutylpiperidines (for example, fluspiriline) and bepridil. Each class exhibits a discrete pharmacology with respect to animal calcium channels which has made them useful tools in the differentiation of subtypes within the calcium-channel family. The use of these drugs in the characterization of calcium channels from animal cell membranes has been the subject of a number of recent reviews (Bean 1989; Campbell *et al.* 1988; Hosey & Lazdunski 1988; Tsien *et al.* 1988; Glossman & Striessnig 1990; Porzig 1990).

In animals, one of the most extensively studied calcium channels is the L-type VOC. The use of complementary approaches including pharmacology, electrophysiology, protein biochemistry and molecular biology has allowed the elucidation of the basic properties of L-type calcium channels and their pivotal role in excitation-contraction coupling (Glossman & Striessnig 1990). The channel structure contains five distinct subunits that co-purify and co-immunoprecipitate. It is known that the α_1-subunit contains both the binding sites for calcium channel effectors and the calcium conducting pore and that it possesses discrete receptor domains for each class of antagonist (i.e. the phenylalkylamines, dihydropyridines and benzothiazepines). The corresponding cDNAs direct expression of functional calcium channels in *Xenopus* oocytes and restore channel activity and excitation–contraction coupling in cultured cells from mice with muscular dysgenesis. The α_1-subunit shares structural homology with sodium and potassium channels and belongs to the superfamily of voltage-operated channels. The other subunits have clearly been shown to be encoded by separate genes; co-expression of the genes for the other subunits either enhances the amplitude of the current or modulates the properties of the channel such as its kinetics or sensitivity to agonists. In addition, it has been shown that gene expression is highly tissue specific and may result from alternative splicing of gene transcripts. The physiology, biochemistry and molecular biology of the animal L-type calcium channel have been the subject of a number of recent reviews (Bean 1989; Campbell *et al.* 1988; Tsien *et al.* 1988; Glossman & Striessnig 1990, Porzig 1990).

3. PLANT PLASMA-MEMBRANE CALCIUM CHANNELS

(*a*) *Effects of calcium channel modulators on plant processes*

There is now a considerable body of evidence which suggests that representatives from the various classes of calcium-channel antagonist influence a wide variety of plant processes. In this section, we will assess the applicability of these compounds to studies of calcium homeostasis in plant cells. Rather than provide an exhaustive list of such experiments we will highlight one example from our own laboratories in which calcium-channel antagonists and an agonist have been used in attempts to gain information on the abscisic acid signal transduction pathway in stomatal guard cells (Mansfield *et al.* 1990; Hetherington & Quatrano 1991).

Preliminary evidence supporting the involvement of calcium ions in ABA-mediated stomatal closure was provided by De Silva *et al.* (1985) who reported that both verapamil and nifedipine interfered with this process. This work was recently extended by McAinsh *et al.* (1991) in an attempt to determine whether ABA-stimulated calcium influx from the apoplast or brought about calcium release from internal stores.

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

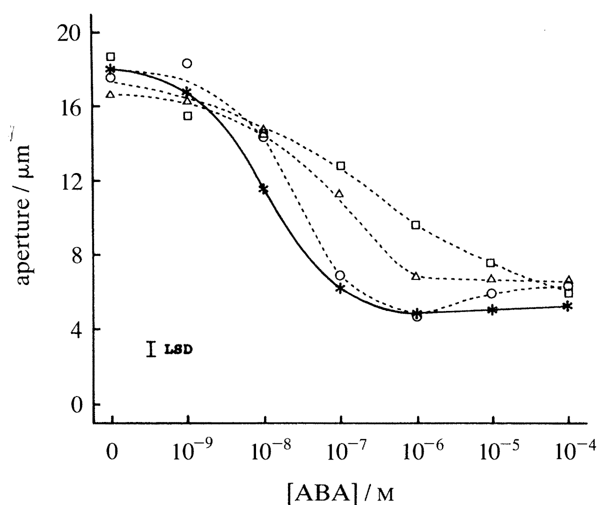

Figure 1. The effects of Ca^{2+}-channel blockers on ABA-induced stomatal closure: ABA alone (filled circles); ABA + 19 µM diltiazem (open circles); ABA + 10 µM nifedipine (triangles); and ABA + 10 µM (+/−) verapamil (squares). Values are expressed as the means of measurements made on 90 randomly selected stomata. The least significant difference, LSD ($p < 0.05$) is indicated. From McAinsh *et al.* (1991); used with permission.

Although none of the compounds used had any effect on stomatal aperture alone, they all interfered with the ability of ABA to induce closure (figure 1). In terms of effectiveness verapamil was greater than nifedipine while diltiazem had only a minimal effect. When the stereoisomers of verapamil were tested it was found that R (+) verapamil was a slightly more potent antagonist of ABA than S (−) verapamil. Interestingly, wash-out experiments demonstrated that inhibition of ABA induced stomatal closure by verapamil was reversible. Alone, the dihydropyridine calcium channel agonist BAY K 8644 had no effect on the turgor relations of the guard cell across a range of concentrations, whereas some slight effects were observed in the presence of 500 µM free calcium. However, compared with the limited data available from binding studies in isolated carrot microsomal membranes where the determined K_ds for the phenylalkylamines are of the order of 80 nM (table 1) (Graziana *et al.* 1988), the concentration of verapamil at which the reduction in stomatal aperture elicited by 100 nM ABA was inhibited by 50% was approximately 10 µM (figure 1). This in turn agrees with the half maximal inhibition ($K_{0.5}$) for the phenylalkylamine inhibition of ^{45}Ca flux in carrot protoplasts (table 1) (Graziana *et al.* 1988). In animals, similar discrepancies exist between data obtained from binding studies (performed on isolated membrane fragments), and the results from *in vivo* investigations. The explanation for this inconsistency may lie in the fact that two somewhat dissimilar systems are being compared. In one, binding is studied in membrane fragments with collapsed membrane potentials in which calcium channels are (presumably) all in the inactivated state, while in the other, intact membranes (with a normal resting potential) are used in which only a proportion of the channels are in the inactive configuration. Data supporting the suggestion that experimentally these systems are not comparable comes from experiments on animal cells in which the blocking effects of the 1,4 dihydropyridines were studied in membranes held at potentials less negative than the normal resting potential. Under these conditions it was found that blockade was dramatically enhanced at the less negative potentials. Bean (1984) used these data to calculate that the apparent binding affinity of resting and inactivated channels for nitrendipine differed by a factor of more than 1000. Additional factors contributing to the discrepancy between *in vivo* and *in vitro* data may be associated with factors such as pH and ionic composition which, although optimized for binding studies may not reflect *in vivo* conditions.

One of the most striking results from the guard cell experiments was the observation that none of the compounds was able to block completely the ABA-induced reduction in guard cell turgor. A possible explanation for this result is that in addition to influx

Table 1. *$K_{0.5}$ values (in nM) of Ca^{2+}-channel inhibitors for half-inhibition of specific (−) [3H]888 to carrot microsomes (and to T-tubule membranes) in comparison with $K_{0.5}$ values for half-inhibition of $^{45}Ca^{2+}$ influx into carrot protoplast*

(From Graziana *et al.* (1988); used with permission.)

Ca^{2+}-channel inhibitor	binding to carrot microsomes	binding to carrot microsomes	$^{45}Ca^{2+}$ flux into protoplasts	binding to T-tubules
(−)D888	90	500	3000	2
(+)D888	80	2000	15000	3
(−)-verapamil	40	200	3000	40
(+)-verapamil	300	4000	15000	10
(−)D6000	66	9000	8000	20
(+)D600	165	3000	15000	40
(−)-bedpridil	60	3000	2000	20
(+)-bedpridil	130	900	5000	15
D-*cis*-diltiazem	400	d	d	60
I-*cis*-diltiazem	400	d	d	900
fluspirilene	900	900	3000	0.4
R 66204	100	100	500	

Downloaded from rstb.royalsocietypublishing.org

from the apoplast ABA also induced calcium release from internal stores. However, it is also possible that such a result reflects a diversity of calcium channels at the guard cell plasmalemma some of which are insensitive to the antagonists. Within the animal literature there are ample precedents for such a suggestion. For example, in a number of cardiac cell types both T- and L-type channels are found (Bean 1989), while in hypocampal pyramidal neurons all three (T, L and N) calcium-channel types are found (Tsien *et al.* 1988). Superimposed upon these observations are problems associated with either secondary or non-specific effects. It is possible that at high concentrations these drugs could produce non-specific effects on other channel types. Some of the data reported by Tester & MacRobbie (1990) are consistent with a direct effect of the calcium-channel modulators on the Chara potassium channel. It would seem likely that the incidence of such non-specific effects would increase with increasing concentrations of the drug. Further, unpublished results from patch clamp investigations by B. R. Terry, S. D. Tyerman and G. P. Findlay indicate that at concentrations as low as 1 μM verapamil, bepridil and nifedipine all inhibit the *Amaranthus* plasma membrane cation outward rectifier. As it is most likely that this is not a calcium-dependent potassium channel (Dr. B. Terry, personal communication), it is clear that the calcium-channel antagonists are bringing about their effect through non-specific interactions. These data highlight some of the problems which can be encountered when compounds of a comparatively undefined (in plants) pharmacology are used in physiological studies. Only when single-channel records from plant calcium channels prove to be routine will it be possible to assess the degree of specificity of each of the calcium-channel modulators. Until a full characterization has been achieved some degree of caution must be observed in the interpretation of data gained from physiological experiments based on the use of calcium-channel antagonists, especially when these compounds are employed at concentrations greater than 25 μM. However, at least in the case of the phenylalkylamines there are now data which suggest that these compounds do inhibit plant calcium-channel activity (Thuleau *et al.* 1992).

(*b*) *Approaches to the purification of calcium channels from plant plasma-membrane*

A popular purification strategy has been based on the capacity of plant membranes to bind specifically calcium channel effectors (Hetherington & Trewavas 1984; Andrejauskas *et al.* 1985; Graziana *et al.* 1988). However, the results differ between lower and higher plants. Tritiated dihydropyridine derivatives (and diltiazem) bind with high specificity to plasma-membrane and endomembranes, derived from algae (Dolle 1988; Dolle & Nultsch 1988*b*). Initial studies suggested that binding sites for dihydropyridines may exist in higher plants (Hetherington & Trewavas 1984); however, when compared to animals the amount of specific binding is low. More data are available concerning binding sites for verapamil derivatives in lower and higher plants (Dolle & Nultsch 1988*a*; Graziana *et al.* 1988).

In all cases, the density of receptor is as high as in animal membranes and compares favourably with skeletal muscle which is considered to be the richest source (Hosey & Lazdunski 1988). However, the relative affinity is very low in plants (compared to animals) and hence raises questions about the specificity of the ligands. For example, although there are data which suggest that the verapamil class of calcium-channel antagonist binds specifically to isolated plant membranes, there are no obvious binding sites for dihydropyridines in zucchini, corn and carrot membranes (Andrejauskas *et al.* 1985; Graziana *et al.* 1988), whereas both sites are allosterically coupled in skeletal and cardiac muscles (Glossman & Striessnig 1990). Moreover, it is apparent that antibodies raised against animal channels fail to cross-react with plant membrane preparations (Graziana *et al.* 1988). Convincing evidence for the presence of calcium channels comes from studies performed on tonoplast-free Characean cells. Measurements of inward current under voltage-clamp have shown that the current was carried by calcium and was specifically blocked by calcium channels antagonists (Okazaki & Tazawa 1990). Similarly, dihydropyridine derivatives inhibit calcium influx into *Chara corallina* (MacRobbie & Banfield 1988) but interestingly not into carrot protoplasts (Graziana *et al.* 1988). By the whole-cell tight-seal voltage-clamp technique it was possible to demonstrate that both verapamil and dihydropyridine derivatives inhibit a calcium transport system that may resemble the L-type calcium channel in corn protoplasts. Interestingly, the sensitivity to dihydropyridines disappears when the cells become older (Ketchum & Poole 1991*a*, *b*). However, it should be noted that these data were obtained using an indirect approach (whole-cell K^+ currents) and further direct studies are required in order to confirm these findings. Nevertheless this might explain why binding sites for dihydropyridine derivatives are difficult to characterize in higher plants (Hetherington & Trewavas 1984; Graziana *et al.* 1988). Data from our laboratory indicate that verapamil derivatives inhibit calcium uptake and modulate cytoplasmic calcium as a function of their affinity for membranes derived from carrot cell-suspension cultures (Graziana *et al.* 1988; Ranjeva *et al.* 1992). In all cases, the channel inhibitors are effective on depolarized protoplasts (Ranjeva *et al.* 1992). Biochemical studies on putative L-type calcium channels in plants are still rare. Detergent-solubilized proteins from corn plasma membrane retain the ability to bind calcium-channel inhibitors of the verapamil series (Harvey *et al.* 1989). After purification the sample was found to be enriched in four main polypeptides of M_r ranging from 30 to 169 kDa. When inserted into lipid planar bilayers, the preparation was able to conduct ion currents and exhibited a good specificity for calcium over anions and other cations (Tester & Harvey 1989). This suggests that functional calcium channels have been isolated.

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A complementary approach has been developed with carrot cell suspension cultures where the phenylalkylamine photoactivitable calcium channel blocker (LU 49888) has been used to generate covalent linkage with its potential receptors (Thuleau *et al.* 1990). In these studies, most of the specific binding component was located at the plasma membrane and to a lesser extent at the tonoplast. The label was borne by a 75 kDa glycopeptide which has been partially purified. When inserted into giant liposomes, single channel recordings by patch-clamp exhibit a calcium conductance that is inhibited by channel antagonists (Thuleau *et al.* 1991). All these experimental results provide strong support for the existence of calcium conducting structures at the plasma membrane of plants.

However, there remain some rather enigmatic observations for which at present there is no immediate explanation. For example, although the number of potential calcium-channel antagonist binding sites at the plasma-membrane can be regarded as high there has yet only been a single direct measurement of (stretch activated) calcium-channel activity using the patch-clamp technique (Cosgrove & Hedrich 1991). As patch-clamp investigations (in higher plants) have been restricted to protoplasts it is conceivable that during protoplast production calcium channels are highly labile. Additionally, it is now apparent that some of the antagonists are not absolutely specific for calcium channels and calcium influx may occur through non specific channels or even through potassium channels (Schroeder & Hagiwara 1990); S. Assmann, personal communication.

(*c*) *Conclusions and future prospects*

In this review we have focused upon the results gained from adopting biochemical and pharmacological approaches towards the characterization of the plant plasma-membrane calcium channel. Currently, on the basis of results obtained from the use of these strategies it would be premature to make definitive statements about the occurrence of plasma-membrane calcium channels in plants. In addition to the problems associated with the specificity of the various calcium channel ligands which we have discussed it is also apparent from animal work that only 5 to 10% of the total ligand binding sites represent bona fide calcium channels (Hosey & Lazdunski 1988). This of course makes it essential that proteins purified on the basis of ligand binding are assesed for channel activity following reconstitution using electrophysiological procedures.

Nevertheless, with the recent demonstration of a stretch-activated calcium channel using the patch-clamp technique (Cosgrove & Hedrich 1992) and our results which demonstrate that solubilized phenylalkylamine binding proteins from plants will exhibit calcium channel activity when reconstituted (Thuleau *et al.* 1992) there is now strong evidence for the existence of such structures. However, it seems likely that in order to provide rigorous data on the occurrence of plasma-membrane calcium channels in plants, their mechanisms of regulation and their relationship to animal calcium channels that it will be necessary to adopt a multidisplinary approach using electrophysiological, biochemical and molecular biological methods. Towards this end our understanding of plant plasma-membrane calcium channels will be greatly facilitated by the cloning and sequencing of the corresponding channel gene. Once this is achieved it will be possible to study both the regulation of gene activity and through expression in model cell systems accurately describe the properties of the channel at the molecular level.

We are grateful to the Gatsby Charitable Foundation, AFRC of the U.K., the French CNRS, Ministère de l'Education Nationale and the EC for providing research support during the period of writing this review.

REFERENCES

Alexandre, J., Lassalles, J.P. & Kado, R.T. 1990 Opening of calcium channels in isolated red beet vacuole membranes by inositol, 1,4,5-trisphosphate. *Nature, Lond.* **343**, 567–570.

Andrejauskas, E., Hertel, R. & Marmé, D. 1985 Specific binding of the calcium antagonist [^{3}H] verapamil to membrane fractions from plants. *J. biol. Chem.* **260**, 5411–5414.

Bean, B.P. 1984 Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6388–6392.

Bean, B.P. 1989 Classes of calcium channels in vertebrate cells. *A. Rev. Physiol.* **51**, 363–384.

Blatt, M.R. 1991 Ion channel gating in plants: physiological implications and integration for stomatal function. *J. Membr. Biol.* **124**, 95–112.

Campbell, K.P., Leung, A.T. & Sharp, A.H. 1988 The biochemistry and molecular biology of the dihydropyridine-sensitive calcium channel. *Trends Neurosci.* **11**, 425–430.

Cosgrove, D.J. & Hedrich, R. 1991 Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba*. *Planta* **186**, 143–153.

De Silva, D.L.R., Cox, R.C., Hetherington, A.M. & Mansfield, T.A. 1985 Suggested involvement of calcium and calmodulin in the response of stomata to abscisic acid. *New Phytol.* **101**, 555–563.

Dolle, R. 1988 Isolation of plasma membrane and binding of the calcium antagonist nimodipine in *Chlamydomonas reinhardtii*. *Physiologia Pl.* **73**, 7–14.

Dolle, R. & Nultsch, W. 1988*a* Specific binding of the calcium channel blocker [^{3}H] verapamil to membrane fractions of *Chlamydomonas reinhardtii*. *Arch. Microbiol.* **149**, 451–458.

Dolle, R. & Nultsch, W. 1988*b* Characterization of D-[^{3}H] cis-diltiazem binding to membrane fractions and specific binding of calcium channel blockers to isolated flagellar membranes of *Chlamydomonas reinhardtii*. *J. Cell Sci.* **90**, 457–463.

Gilroy, S., Fricker, M.D., Read, N.D. & Trewavas, A.J. 1991 Signal transduction through calcium in guard cells of *Commelina communis*. *Pl. Cell* **3**, 333–344.

Glossman, H. & Striessnig, J. 1990 Molecular properties of calcium channels. *Rev. Physiol. Biochem. Pharmacol.* **114**, 1–105.

Graziana, A., Fosset, M., Ranjeva, R., Hetherington, A. & Lazdunski, M. 1988 Calcium channel inhibitors that

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

bind to plant cell membranes block calcium entry into protoplasts. *Biochemistry* **27**, 764–768.

Harvey, H.J., Venis, M.A. & Trewavas, A.J. 1989 Partial purification of a protein from maize (Zea mays) coleoptile membranes binding the calcium channel antagonist verapamil. *Biochem J.*, **257**, 95–100.

Hedrich, R. & Schroeder, J.I. 1989 The physiology of ion channels and electrogenic pumps in higher plant cells. *A. Rev. Pl. Physiol. Pl. molec. Biol.* **40**, 539–569.

Hepler, P.K. & Wayne, R.O. 1985 Calcium and plant development. *A. Rev. Pl. Physiol.* **36**, 397–439.

Hetherington, A.M. & Trewavas, A.J. 1984 Binding of nitrendipine, a calcium channel blocker, to pea shoot membranes. *Pl. Sci. Lett.* **35**, 109–113.

Hetherington, A.M. & Quatrano, R.S. 1991 Tansley Review No. 31. Mechanism of action of abscisic acid at the cellular level. *New Phytol.* **119**, 9–32.

Hosey, M.M. & Lazdunski, M. 1988 Calcium channels: molecular pharmacology, structure and regulation. *J. Membr. Biol.* **104**, 81–105.

Johannes, E., Brosnan, J.H. & Sanders, D. 1991 Calcium channels and signal transduction in plant cells. *BioEssays*, **13**, 331–336.

Johannes, E., Brosnan, J.H. & Sanders, D. 1992 Parallel pathways for intracellular Ca^{2+} release from the vacuole of higher plants. *Pl. J.* **2**, 97–102.

Ketchum, K.A. & Poole, R.J. 1991*a* Cytosolic calcium regulates a potassium current in corn (*Zea mays*) protoplasts. *J. Membr. Biol.* **119**, 277–288.

Ketchum, K.A. & Poole, R.J. 1991*b* Dihydropyridine-sensitive calcium transport in protoplasts from corn suspension cells. *Pl. Physiol.* **96** (S), 193.

Knight, M.R., Campbell, A.K., Smith, S.M. & Trewavas, A.J. 1991 Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors of cytoplasmic calcium. *Nature, Lond.* **352**, 524–526.

McAinsh, M.R., Brownlee, C. & Hetherington, A.M. 1990 Abscisic acid induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. *Nature, Lond.* **343**, 186–188.

McAinsh, M.R., Brownlee, C. & Hetherington, A.M. 1991 Partial inhibition of ABA induced stomatal closure by calcium channel blockers. *Proc. R. Soc. Lond.* B **243**, 195–201.

MacRobbie, E.A.C. & Banfield, J. 1988 Calcium influx at the plasmalemma of *Chara corallina*. Planta **176**, 98–108.

Mansfield, T.A., Hetherington, A.M. & Atkinson, C.J. 1990 Some current aspects of stomatal physiology. *A. R. Pl. Physiol. Pl. molec. Biol.* **41**, 55–75.

Nayler, W.G. 1988 *Calcium antagonists*. New York: Academic Press.

Okazaki, Y. & Tazawa, M. 1990 Calcium ion and turgor regulation in plant cells. *J. Membr. Biol.* **114**, 189–194.

Porzig, H. 1990 Pharmacological modulation of voltage-dependent calcium channels in intact cells. *Rev. Physiol. Biochem. Pharmacol.* **114,** 209–262.

Ranjeva, R., Graziana, A., Mazars, C. & Thuleau, P. 1992 Putative L-type calcium channels in plants: biochemical properties and subcellular localization. In *Transport and receptor proteins of plant membranes* (ed. D. T. Clarkson & D. T. Cooke), pp. 145–153. Plenum Press.

Schroeder, J.I. & Hagiwara, S. 1990 Repetitive increases in cytosolic Ca^{2+} of guard cells by abscisic acid activation of nonselective Ca^{2+} permeable channels. *Proc. natn. Acad. Sci. U.S.A.* **87**, 9305–9309.

Schroeder, J.I. & Thuleau, P. 1991 Calcium channels in higher plant cells. *Pl. Cell* **3**, 555–559.

Tester, M. 1990 Plant ion channels: whole cell and single channel studies. *New Phytol.*, **114**, 305–340.

Tester, M. & Harvey, H.J. 1989 Verapamil-binding fractions form calcium channels in planar lipids bilayers. In *Plant membrane transport: the current position* (ed. J. Dainty, M. I. De Michelis, E. Marre & F. Rasi-Cadolgno), pp. 277–78. Amsterdam: Elsevier.

Tester, M. & MacRobbie, E.A.C. 1990 Cytoplasmic calcium affects the gating of potassium channels in the plasma membrane of *Chara corallina*: a whole-cell study using calcium-channel effectors. *Planta* **180**, 569–581.

Thuleau, P., Graziana, A., Canut, H. & Ranjeva, R. 1990 A 75-KDa polypeptide, located primarily at the plasma membrane of carrot cell-suspension cultures, in photoaffinity labelled by the calcium channel blocker LU49888. *Proc. natn. Acad. Sci. U.S.A.* **87**, 10000–10004.

Thuleau, P., Graziana, A., Ranjeva, R. & Schroeder, J.I. 1991 Purified calcium channel blocker binding protein from carrot cells forms calcium-permeable ion channels. *International Symposium of Plant Molecular Biology*, *Tuscon.* (In the press.)

Tsien, R.W., Lipscombe, D., Madison, D.V., Bleg, K.R. & Fox, A.P. 1988 Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci.* **11**, 431–438.

Tsien, R.W. & Tsien, R.Y. 1990 Calcium channels, stores, and oscillations. *A. Rev. Cell Biol.* **6**, 715–760.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES