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Review

Plant Ion Channels as Potential Targets of Agro-Chemicals

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In the past 20 years, plant ion channels have been studied electrophysiologically, genetically and biochemically. However, much less study has been done on plant ion channels than animal and insect ion channels, especially in the field of pharmacology. Plant ion channels can serve as targets of agro-chemicals to control growth, stress tolerance and disease resistance, since they mediate physiological processes, like animal and insect ion channels. In this article, we review the current status of research on plant ion channels, in order to present guiding principles for designing new plant growth regulators and disease controlling agents.

Keywords: agro-chemical, inhibitor, ion channel, plasma membrane.

1. INTRODUCTION

Ion channels are membrane-bound proteins that allow the passage of ions across impermeable cell membranes. In animals, their most obvious role is in inducing the action potentials of neurons and cardiac muscles. They also have other wide-ranging functions in cell volume control, growth and differentiation of cells, and neurotransmitter release.

In plant cells as in animal cells, ion channels are thought to fulfil three main physiological functions: 1) cell osmoregulation because of their ability to accommodate large net ion fluxes over a short period of time, 2) cell signaling by amplification and propagation of electrical signals or transport of secondary messengers, such as Ca^{2+}, and 3) control of the membrane potential.

Studies on different plant species and various cell types have revealed that all subcellular membranes, plasma membrane, tonoplast, plastidial and mitochondrial membranes, are equipped with a variety of channels exhibiting different ion selectivity and specific regulatory mechanisms. Among these, the ion channels in plasma membrane are expected to be effective target sites of agro-chemicals that may have pharmacological and toxicological actions toward plants, because they face the apoplastic space.

This article presents the ion channels in plant plasma membrane as a target of agro-chemicals, in comparison with the ion channels of animals and insects which provide the action sites of many medicinal drugs and agricultural insecticides.

2. PLANT ION CHANNELS

In plants, K^+ is involved in processes such as cell elongation, stomatal movements to regulate gas exchange, and the transduction of various signals. The first ion transport systems in plants were identified in 1992 from *Arabidopsis* to be two plasma membrane K^+ channels, *AKT1*[1)] and *KAT1*.[2)] Comparisons of genome sequences revealed that *AKT1* and *KAT1* were the members of a family of plant channels similar in structure and sequence to the *Shaker* superfamily of animal voltage-dependent K^+ channels. Thereafter, several types of plasma membrane K^+ channels were identified. The integration of molecular biological, electrophysiological and reverse genetical approaches has already revealed the functions of some of these ion channels. Plant voltage-gated channels belonging to the *Shaker* family participate in sustained K^+ transport processes at the cell and whole plant levels, such as K^+ uptake from soil solution, long-distance K^+ transport in the xylem and phloem, and K^+ fluxes in guard cells during stomatal movements.[3)]

Calcium channels are predominantly involved in signal

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Abbreviations: 9-AC, anthracene-9-carboxylic acid; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; GABA, γ-aminobutyric acid; IAA-94, [6,7-dichloro-2-cyclophenyl-2,3-dihydro-2-methyl-1-oxo-1*H*-indan-5-yl oxy]acetic acid; NPPB, 5-nitro-2,3-phenylpropylaminobenzoic acid; TBPS, *t*-butylbicyclophosphorothionate; TEA, tetraethylammonium; PTX, picrotoxinin; CTX, charybdotoxin; DTX, α-dendrotoxin.

transduction. In many cases, the cytoplasmic Ca^{2+} concentration is increased by Ca^{2+} influx across the plasma membrane *via* Ca^{2+} channels. The physiological poise of a plant cell is determined, in part, by its cytoplasmic Ca^{2+} concentration. Some of them are nonselective cation channels permeable to inorganic monovalent cations as well as divalent cations, and are different from the highly selective Ca^{2+} channels in neurons (Fig. 1A).[4–6]

Anion channels are also identified in various tissues, cell types and membranes of higher plants. Current evidence supports their central role in controlling cellular functions, including osmoregulation, stomatal movements, anion transport and signal transduction. Information on the molecular structure of anion channels has been published recently.[7]

3. DISEASES DUE TO ION CHANNEL DEFECTS AND DRUGS

It is well known that defects of ion channels cause a wide variety of diseases in animals. They include cardiac diseases (hereditary cardiac arrhythmia, long QT syndrome, hypertension, *etc.*) and neurological diseases (myasthenia, epilepsy, episodic ataxia, *etc.*). Ion channel activators and blockers have been shown to be as effective as older medications. A lot of drugs and therapies including a genetic therapy have already been developed to treat these ion channel diseases.

In 2001, the top 100 best selling drugs acted on 43 different molecular targets and three of these 43 targets were ion channels (L-type Ca^{2+} channels, *e.g.* amlodipine for hypertension; GABA-A receptor modulators, *e.g.* benzodiazepines as anticonvulsants and anaesthetics; sulphonylureas for diabetes), albeit with sales in the order US$7 billion.[8]

4. ION CHANNELS AS TARGETS OF INSECTICIDES

Most of the commercially provided insecticides act on the sodium channel and the GABA system. The exception is a series of organophosphate and carbamate insecticides, which inhibit acetylcholinesterases.[9,10] Ion channels are also the primary target sites for several classes of natural and synthetic insecticidal compounds. The voltage-sensitive sodium channel is the major target site for DDT and pyrethroids, the veratrum alkaloids and *N*-alkylamides. The GABA-gated chloride channel is the primary site of action for the insecticides, such as lindane, endosulfan, and fipronil. These compounds affect channel gating and ion permeability.

5. STRESS RESISTANCE AND PLANT DEFENSE INVOLVED IN ION CHANNELS IN PLANTS

In plants, no diseases like animal ion channel diseases (channelopathy) have been reported. However, recent reports using ion channel mutants revealed that ion channels are closely associated with stress resistance and plant defense (disease resistance), as follows.

First, dominant negative mutants of *Arabidopsis* with respect to the function of guard cell K^+ channels have shown that it affects the responses to light and water stress. Reduced light-induced stomatal opening and the transpirational water loss from leaves were evident in these mutants, along with the reduced inward-rectifying K^+ currents.[11] A similar deregulation of stomatal opening and the increased drought susceptibility are also shown to be caused by the disruption of the plasma membrane ABCC-type ABC transporter in guard cells.[12]

Second, the implication of the K^+ channel in the growth regulation has been demonstrated. The growth rates of plants supplied with rate-limiting concentrations of K^+ depended on the presence of *AKT1* but not *AKT2*. This indicates that *AKT1* but not *AKT2* mediates the growth-sustaining uptake of K^+ into roots.[13] Tumor development was inhibited in plants lacking a functional *AKT1* or *AKT2/3* that encodes the phloem K^+ channel.[14]

Third, the signaling pathways leading to a hypersensitive response to pathogen attack, which is commonly associated with disease resistance and programmed cell death, were disrupted in *dnd1* and *hlm1* mutants.[15,16] The *DND1* and *HLM1* genes encode a cyclic nucleotide-gated channel identical to the product of *CNGC2* and *CNGC4*, which might be involved in the hypersensitive response to pathogens.

6. EFFECTS OF ION CHANNEL BLOCKERS ON PLANT ION CHANNELS

In the 1980s and 1990s, only a few studies employed toxins as potential tools to characterize plant ion channels. Some ion channels exhibited a typical pharmacology, but other ion channels were atypical.

As in the case of animal cells, both inward and outward rectifying K^+ currents have been found in a variety of plant cells. Plant K^+ channels that are responsible for the generation of K^+ currents have been reported. Some of those channels showed similarity to those in animal cells in terms of sensitivity to chemicals, but other K^+ channels are specific to plants and have no parallel with those of animals (Fig. 1B). For example, Ca^{2+}-dependent K^+ channels in corn cell culture protoplasts were inhibited by charybdotoxin (CTX), a scorpion venom toxin that is a 'hallmark' blocker of Ca^{2+}-dependent K^+ channels in animal cells.[17] By contrast, the outward K^+ currents of *Vicia* guard cells are insensitive to α-dendrotoxin (DTX), a typical inhibitor of outward-rectifying K^+ channels in animal neurons. Interestingly, the inward rectifier current in *Vicia* guard cells is blocked by low concentrations of DTX.[18]

Similar examples are also observed in the case of anion channels. Two distinct types of anion channel currents have been found in *Vicia* guard cells. These two types are significantly different in terms of the voltage- and time-dependent regulation. One is the slow-type (S-type) anion channel current (Fig. 1C) and the other is the rapid-type (R-type) (Fig. 1D). Among the blockers of animal anion channels,

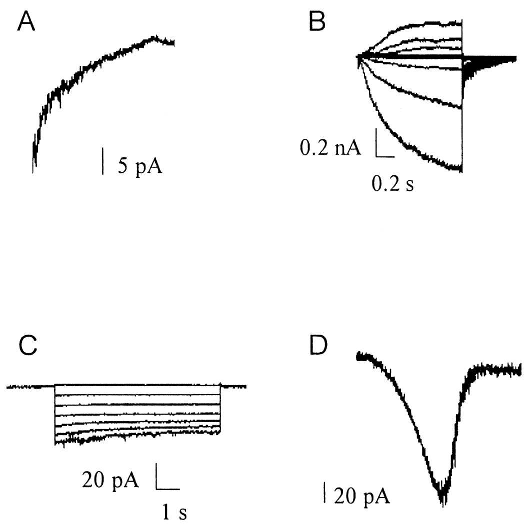

Fig. 1. Plasma membrane ion channel currents of *Arabidopsis* guard cells. (A) Non-selective cation (Ca^{2+}) channel currents activated by hyperpolarization. (B) Inward rectifying K^+ channel currents (downward) and outward rectifying K^+ channel currents (upward). (C) Slow-type (S-type) anion channel currents. (D) Rapid-type (R-type) anion channel currents.

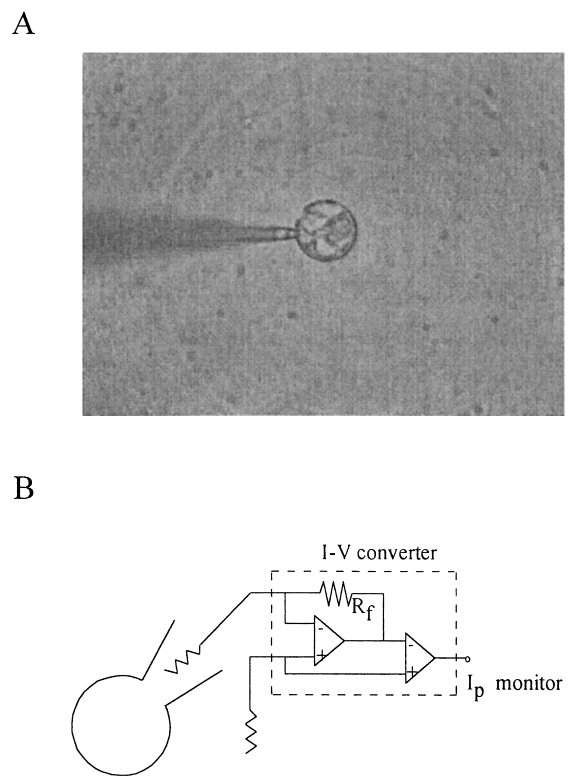

Fig. 2. Patch-clamp technique. (A) Patch-clamping of tobacco suspension cultured cell protoplasts. (B) Circuit layout for the measurement of channel currents.

NPPB, IAA-94, 9-AC and niflumic acid, blocked S-type anion channel currents in plants effectively. On the other hand, DIDS did not block S-type anion currents, but strongly blocked R-type anion currents.[19-21] In some cases, NPPB and IAA-94 strongly inhibited R-type anion currents in plant cells, but the inhibition by 9-AC was partial.[22]

Ca^{2+} channels that mediate rapid Ca^{2+} transport across membranes are predominantly involved in cell signaling in animal cells, and a similar role has been proposed in plants. However, specific inhibitors of particular Ca^{2+} channels are not available at present so the Ca^{2+} channels in plants have not been characterized in detail. Animal Ca^{2+} channel inhibitors, such as TEA^+, La^{3+}, Al^{3+}, Gd^{3+} and verapamil, commonly block a wide variety of plant ion channels at different concentrations. That is, many plant Ca^{2+} channels exhibit no typical pharmacology.[23] This may be because of the presence of several types of Ca^{2+} permeable channels, including nonselective cation channels that also contribute to Ca^{2+} influxes across the plasma membrane.

In spite of active studies in this field, the data for inhibitors or blockers of plant ion channels are insufficient, and they still cannot be classified on the basis of their pharmacology. The unavailability of good chemical agents currently makes it difficult to elucidate the physiological role of ion channels.

7. MEASUREMENT OF PLANT ION CHANNEL ACTIVITIES

The current method of choice for obtaining high-quality data on the functional effects of compounds at ion channels is the patch clamp technique (Fig. 2). However, patch clamping is a technically challenging method with a low throughput that crucially limits its applicability to drug discovery and safety testing.

The patch clamp technique also allows recordings of the ion flow through membranes at a high resolution. In 1984, the patch clamp technique was employed to study ion fluxes across the membrane of plant cells.[24,25] However, difficulty in obtaining gigaseals has limited the application of the patch clamp technique to plant cells.

The use of heterologous expression systems, such as yeast, *Xenopus* oocytes, and insect cells, as well as homologous misexpression has enabled further studies on the selectivity, control and physiological role of channels encoded by a wide range of plant genes. The stable expression of plant ion channels in *Xenopus* oocytes, yeast cells and insect cells has been achieved[26] and the functional expression of plant ion channels in *Escherichia coli* was also established.[27] However, homomultimeric receptors show reasonable pharmacological fidelity to native channels, but heteromultimeric assemblies may be required for faithful reconstruction of the pharmacology of native channels.

8. PERSPECTIVE

The insect and vertebrate channels are pharmcologically distinguishable despite their similarity, which has led to the design of insect-selective toxicants. The structural differences between the ion channels from plants and those from animals (vertebrates) and insects may prove useful to produce new lead structures for the design of new agro-chemicals.

An alanine to serine or glycine substitution at position 302 (A302S or A302G) in a GABA receptor subunit resulted in a decrease in sensitivity to the channel blockers. Ten-fold (TBPS), 100-fold (PTX), or 1000-fold (lindane) resistance has been observed in the neurons expressing the mutated receptor, *in vitro.*[28] This result suggests that plants or crops whose ion channels are mutated may help in the development of new agro-chemicals.

Further studies of native and mutated plant ion channels should help to elucidate the roles of ion channels in plants and the physiological importance of plant ion channels. They should also help us to find a way to control physiological processes such as osmoregulation, plant movements, growth and photosynthesis. Information on plant ion transporters including ion channels is needed to exploit agro-chemicals designed to target these transporters.

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