Contents lists available at ScienceDirect

Journal of Plant Physiology

Physiology

Non-selective cation channels in plasma and vacuolar membranes and their contribution to K⁺ transport☆

Igor Pottosin*, Oxana Dobrovinskaya

Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de julio 965, Villa de San Sebastián, 28045 Colima, Mexico

ARTICLE INFO

SUMMARY

Both in vacuolar and plasma membranes, in addition to truly K⁺-selective channels there is a variety of non-selective channels, which conduct K⁺ and other ions with little preference. Many non-selective channels in the plasma membrane are active at depolarized potentials, thus, contributing to K⁺ efflux rather than to K⁺ uptake. They may play important roles in xylem loading or contribute to a K⁺ leak, induced by salt or oxidative stress. Here, three currents, expressed in root cells, are considered: voltage-insensitive cation current, non-selective outwardly rectifying current, and low-selective conductance, activated by reactive oxygen species. The latter two do not only poorly discriminate between different cations (like K⁺ vs Na⁺), but also conduct anions. Such solute channels may mediate massive electroneutral transport of salts and might be involved in osmotic adjustment or volume decrease, associated with cell death. In the tonoplast two major currents are mediated by SV (slow) and FV (fast) vacuolar channels, respectively, which are virtually impermeable for anions. SV channels conduct mono- and divalent cations indiscriminately and are activated by high cytosolic Ca^{2+} and depolarized voltages. FV channels are inhibited by micromolar cytosolic Ca^{2+}, Mg^{2+}, and polyamines, and conduct a variety of monovalent cations, including K⁺. Strikingly, both SV and FV channels sense the K⁺ content of vacuoles, which modulates their voltage dependence, and in case of SV, also alleviates channel's inhibition by luminal Ca^{2+}. Therefore, SV and FV channels may operate as K⁺-sensing valves, controlling K⁺ distribution between the vacuole and the cytosol.

Introduction

There are two fundamental conditions, which underlie K⁺ transport in living cells. First, all living cells, including those of plants, accumulate high K⁺ in the cytosol *vs* relatively low extracellular K⁺, and the resting potential across the plasma membrane is negative and close to E_K, the equilibrium potential for K⁺. At physiological ionic conditions at both sides of the cell membrane, the value of the equilibrium potential for K⁺, when compared to that of other ions, is the lowest one. Second, K⁺ is not only transported via

K⁺-selective channels, but also by *any* cation-permeable channel. From these conditions it follows that when K⁺ is transported via a non-selective channel, it takes place in parallel with a concurrent transport of other ions, which will tend to increase the membrane potential above E_K, providing the activity of the H⁺-ATPase is not sufficient to cope with this depolarization. So, non-selective channels will mediate net K⁺ efflux from cells in this case. Within a cell, large K⁺ concentration gradients hardly exist between intracellular compartments and the cytosol, except in special situations, like salt stress or severe K⁺ deficiency. Among the intracellular compartments, the central lytic vacuole, occupying up to 90% of the cell volume, is especially important for the control of turgor and ion homeostasis. At repleting K⁺ conditions, the vacuolar K⁺ concentration is higher than the cytosolic one, as directly measured in barley roots and leaves (Walker et al., 1996; Cuin et al., 2003). Similar values for the vacuolar K⁺ concentrations are reported for roots, shoots, and leaves of many other species (Tester and Leigh, 2001; Conn and Gilliham, 2010), whereas cytosolic K⁺ appears to be constant in different tissues (Leigh, 2001). Taken together, with a small (∼−20 mV), cytosol-negative tonoplast electrical potential difference, channel-mediated K⁺ transport will be directed from the cytosol to the vacuole (Leigh, 1997; Pottosin and Muñiz, 2002). However, at K⁺ starving conditions, high cytosolic K⁺ is maintained at the expense of the vacuolar K⁺ pool, and the driving force for K⁺

across the tonoplast reverses its direction (Walker et al., 1996; see also Anschütz et al., 2014, in this issue for more details on the role of potassium homoeostasis as a common denominator of plant adaptive responses to the environment). Thus, tonoplast K^+-permeable channels potentially can mediate K^+ transport in both ways, from the vacuole to the cytosol and *vice versa*. Yet, depending on physiological conditions, this transport may be beneficiary or detrimental for plants.

Non-selective cation channels (NSCC) form a group with a cryptic molecular identity and diverse functional characteristics (Demidchik and Maathuis, 2007). Per definition, these channels can weakly differentiate between cations, in particular, between K^+ and Na^+, with the P_K/P_{Na} ratio ranging between 0.3 and 3. Further on, some of these channels also conduct divalent cations, and, eventually, may conduct even anions (Wegner and de Boer, 1997; Zhang et al., 2002). They may be activated by positive and negative going voltages, or may be relatively voltage-independent. Further functional classification is based on whether NSCC are constitutively active or gated by ligands, membrane stretch or reactive oxygen species (ROS) (Demidchik and Maathuis, 2007; for recent reviews see Dietrich et al., 2010; Jammes et al., 2011; see also Demidchik, 2014, in this special issue). When it comes to NSCC, expressed in the plasma membrane, there is a large gap between electrophysiological and molecular biology data. Recently, extracellular purine nucleotides, ATP and ADP, were shown to activate the NSCC, mediating Ca^{2+} influx and, in case of ADP, also K^+ efflux in *Arabidopsis* root epidermis cells (Demidchik et al., 2011). A rapid response to ADP was reminiscent of responses, mediated by the ionotropic purinceptors in animal cells, but respective sequences are lacking in *Arabidopsis* genome. On the other hand, two candidate receptor channel gene families in *Arabidopsis*, cyclic nucleotide gated channel (CNGC), supposedly gated by internal cyclic nucleotides, and glutamate receptors (GLRs), gated by external glutamate and some other amino acids, are considered to form cation channels in the plasma membrane (Dietrich et al., 2010). At least some plant GLRs (AtGLR1.1 or AtGLR1.4) contain functional pore domains, conducting Na^+, K^+, and Ca^{2+} (Tapken and Hollmann, 2008). However, there is a single *in planta* study, showing glutamate-activated voltage-independent currents, carrying monovalent cations, Na^+, K^+, and Cs^+ indiscriminately and being also permeable for Ca^{2+} (Demidchik et al., 2004), although GLR-mediated Ca^{2+} influx and signalling is well documented in *in vivo* studies on plants (Dennison and Spalding, 2000; Dubos et al., 2003; Krol et al., 2007; Stephens et al., 2008; Michard et al., 2011). With respect to CNGCs there are conflicting reports, on whether respective currents are activated (Balagué et al., 2003; Christopher et al., 2007) or inhibited by cyclic nucleotides (Maathuis and Sanders, 2001; Essah et al., 2003). Whereas mostly CNGCs appear to form non-selective channels, there are also reports on highly selective for K^+ over Na^+ ones (Hua et al., 2003). Also kinetic patterns, reported by different authors are quite dissimilar, from inward-rectifying time-dependent currents to leak-like instantaneous ones. Therefore, we will restrict ourselves to the discussion of those NSCCs, which are at least well established on the basis of electrophysiological data, without any reference to their possible molecular identity.

Contrasting to a variety of non-selective cation currents, found in the plasma membrane, the tonoplast likely expresses only two major non-selective cation currents of fast vacuolar (FV) and slow vacuolar (SV) types (Pottosin and Muñiz, 2002; Pottosin and Schönknecht, 2007; Hedrich and Marten, 2011). Early studies in the 90s suggested the presence of additional cation channels, permeable for Ca^{2+}, in the vacuolar membrane, including hyperpolarization-activated ones and those gated by inositol 1,4,5-trisphosphate (IP_3) and cyclic ADP ribose (cADPR) (reviewed by Sanders et al., 2002; Isayenkov et al., 2010). However, the vacuolar

hyperpolarization-activated Ca^{2+}-permeable channel was proved to be an artefact, resulting from recording of SV channels the other way around (Pottosin and Schönknecht, 2007). No genes, encoding IP_3- or ryanodine (cADPR-activated)-receptor channels have been found in plant genomes (Nagata et al., 2004; Ward et al., 2009); nor there were detectable Ca^{2+} fluxes from intact vacuoles, induced either by IP_3 or cADPR (Pottosin et al., 2009). Therefore, passive exchange of K^+ across the tonoplast is mediated by a combination of vacuolar K^+-selective channels, vacuolar K^+ channel (VK) (in *Arabidopsis* AtTPK-1 and likely also AtTPK-2, -3, and -5) and cation channels, unable to differentiate between K^+ and Na^+, SV (AtTPC-1 in *Arabidopsis*) and FV (unknown gene). K^+ uptake into vacuole against electrochemical gradient is mediated by Na^+ (K^+)/H^+ exchangers (in *Arabidopsis* AtNHX-1 and -4; Martinoia et al., 2012; see also Hamamoto and Uozumi, 2014, in this special issue).

In the following we will discuss in more detail the function of non-selective ion conductances of plasma and vacuolar membranes and their role in K^+ transport, in relation to ionic selectivity and gating of contributing channels and in the context of variable concentrations for K^+ and for concurrently transported ions, Na^+ and/or Cl^-.

Voltage-insensitive cation current and K^+/Na^+ exchange upon salt stress

Non-selective instantaneously activated currents with a weak voltage-dependence (VI-NSCC) are common components of the plasma membrane in roots and leaves of different plant species (Tyerman et al., 1997; Demidchik and Tester, 2002; Demidchik et al., 2002; Shabala et al., 2006, 2007; Zhao et al., 2007, 2011; Velarde-Buendía et al., 2012). Prototypic and best studied VI-NSCC was reported for *Arabidopsis* root protoplasts. It weakly differentiates among different cations $K^+ > NH^+_4 > Rb^+ \sim Cs^+ \sim Na^+ > Li^+ >$ tetraethylammonium (TEA^+), but has quite substantial permeability, 1/3 of that for K^+, even for such large cations as TEA^+. This current is efficiently suppressed by di- (Ca^{2+}, Ba^{2+}, Zn^{2+}) and tri- (lantanides) valent cations (Demidchik and Tester, 2002) at submillimolar concentrations. At the same time, the current displays a substantial permeability for Ca^{2+}, Ba^{2+}, and Zn^{2+}, with an average conductance for these cations about 10–20% of that for K^+ (Demidchik et al., 2002). Possessing a broad selectivity spectrum and being active within a wide range of physiologically attainable membrane potentials, VI-NSCC may serve as an important route for the uptake of multiple cation species. As high-selective Ca^{2+} channels are not found in plant cells, Ca^{2+} influx through the plasma membrane and Ca^{2+} signalling is mediated by some available NSCC, and VI-NSCC is one of the candidate currents. Additionally, NSCC can mediate uptake of some important cations, like NH^+_4, which normally cannot be taken up by K^+-selective transporters (concerning the molecular basis of channel selectivity see e.g. Benito et al., 2014). On the other hand, Na^+ uptake via NSCC is important for osmotic adjustment during salt stress. Yet, Na^+ uptake needs to be under control, to avoid toxic Na^+ accumulation in the cytosol. Partial inhibition of VI-NSCC by Ca^{2+} is thought to be part of such control. Interestingly, although VI-NSCC and K^+-efflux channels in *Arabidopsis* leaves and roots differ, both tissues respond to salt by K^+ efflux, which can be ameliorated by external Ca^{2+} (Shabala et al., 2006; see Demidchik, 2014, for more details on the phenomenon of K^+ efflux from root cells). Both Na^+ influx and membrane depolarization are reduced due to partial inhibition of VI-NSCC by Ca^{2+}. Thus, salt-induced K^+ efflux is "just the other side of the medal" in relation to Na^+ influx, which depolarizes the membrane, increasing the driving force for K^+ efflux and causing the activation of outward-rectifying K^+ channels. In cereals and *Arabidopsis* roots most of Na^+-induced K^+