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Calcium signalling and calcium channels: Evolution and general principles

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Abstract

Calcium as a divalent ion was selected early in evolution as a signaling molecule to be used by both prokaryotes and eukaryotes. Its low cytosolic concentration likely reflects the initial concentration of this ion in the primordial soup/ocean as unicellular organisms were formed. As the concentration of calcium in the ocean subsequently increased, so did the diversity of homeostatic molecules. This includes the plasma membrane channels that allowed the calcium entry, as well as extrusion mechanisms, i.e., exchangers and pumps. Further diversification occurred with the evolution of intracellular organelles, in particular the endoplasmic reticulum and mitochondria, which also contain channels, exchanger(s) and pumps to handle the homeostasis of calcium ions. Calcium signalling system, based around coordinated interactions of the above molecular entities, can be activated by the opening of voltage-gated channels, by neurotransmitters, by second messengers and/or mechanical stimulation, and as such is all-pervading pathway in physiology and pathophysiology of organisms.

Keywords

calcium; evolution; prokaryotes; eukaryotes; channels; transporters

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1. Introduction

Calcium ion represents an important cytosolic signalling molecule as it can affect almost all cellular processes. The calcium signalling evolved around variations in the concentration of calcium within the cytosol, with calcium being sourced from the extracellular space and/or the intracellular calcium-storing organelles. The flux of calcium across the plasma membrane and endomembranes, i.e. membranes demarcating internal organelles, critically relies on the operation of various calcium channels within the membranes. Here, we briefly outlined the evolution and general principles of calcium signalling as an introduction to the papers that follow discussing calcium channels, in the namesake special issue of *European Journal of Pharmacology*.

2. Early evolution of Ca^{2+} signalling

Controlled environment is the essence of life. The very first cells appeared only after they were able to fence their entrails against the world by the means of a cellular membrane. This membrane in the animal kingdom is made of lipids, so that it is poorly, if at all, permeable to the majority of biologically relevant hydrophilic molecules and ions; the exceptions are hydrophobic compounds, which can be dissolved in lipids. This cellular separation from the surround was the first step in the long lasting story of biological evolution, which pretty much builds around a simple and effective principle of d*ivide et impera*, i.e., divide the world into external environment and internal space and govern everything which goes into or out of the living cell/organism.

Some of the first cells appeared in the primordial ocean in which the main elements were ions derived from the salts enriching the Earth's crust, the most abundant ions being Na^{+}, K^{+}, Cl^{-}, Ca^{2+} and Mg^{2+}. Out of the two divalent cations which can bind to the same sites in the cell, Ca^{2+} emerged with binding reactions that are ~ 100 times faster than Mg^{2+} (Williams, 2007). The concentrations of these ions in the primeval ocean are not precisely known. However, some paleontologists suggest that Ca^{2+} concentration was very low, somewhere in the range of 100 nM (Kazmierczak et al., 2013). Hence, the very first cells had acquired a very low Ca^{2+} content in their cytoplasms and lived in a low Ca^{2+} environment. Indeed, even today, some organisms like the cyanobacteria (which are probably the most ancient organisms that still live today) have a low Ca^{2+} requirement and are alkalophilic (Brock, 1973; Gerloff and Fishbeck, 1969; Kazmierczak et al., 2013). Low Ca^{2+} in the cytosol of primeval cells is also compatible with energetics based around ATP and the usage of DNA/RNA for genetic encoding, because both cannot tolerate high Ca^{2+} concentrations; at the levels above 10 μM of Ca^{2+}, this ion induces the precipitation of phosphates, causes aggregation of proteins and nucleic acids and disrupts lipid membranes (Case et al., 2007; Jaiswal, 2001; Williams, 2007).

Washout of Ca^{2+} ions from the Earth's crust, in combination with a decreased alkalinisation of the ancient ocean, led to a continuous increase in Ca^{2+} concentration in the sea water, which in turn initiated the evolution of a Ca^{2+} homeostatic system that kept cytosolic Ca^{2+} at a low level. The molecules governing such homeostasis seem to evolve rather early in the genealogical tree as the most primitive bacteria were already in possession of Ca^{2+} pumps and Ca^{2+} exchangers. An increase in environmental Ca^{2+} concentration in combination with an evolving Ca^{2+} homeostatic system assured the build-up of a transmembrane Ca^{2+} gradient, which lies at the very base of Ca^{2+} signalling. This gradient soon was utilised by prokaryotes to develop Ca^{2+} permeable channels, which formed a pathway for a transmembrane Ca^{2+} influx and, thus, made Ca^{2+} signalling possible. In this respect, an increase in the ocean Ca^{2+} concentration could be regarded as a trigger of evolution of complex homeostatic and signalling systems.

3. Ca^{2+} homeostasis and signalling in prokaryotes

All prokaryotic organisms living today have a low (80 – 100 nM) cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) - (Gandola and Rosen, 1987; Watkins et al., 1995) and several systems for Ca^{2+} extrusion that include plasmalemmal Ca^{2+} pumps (which are structurally similar to eukaryotic P-type Ca^{2+} pumps), as well as Ca^{2+}/H^+ and Na^+/Ca^{2+} exchangers (Berkelman et al., 1994; Case et al., 2007; Ivey et al., 1993; Kanamaru et al., 1993; Shemarova and Nesterov, 2005). The prokaryotic cells also have intracellular Ca^{2+} signals, reflecting the activation of transmembrane Ca^{2+} fluxes through Ca^{2+} selective channels. These channels are, indeed, widespread in prokaryotic organisms, being arguably the most ancient ion channels (Shemarova and Nesterov, 2005).

There is evidence about a non-proteinaceous nature of ancient proto-Ca^{2+} channels. These Ca^{2+} channels could have been constructed from large (molecular weight of 60 to 1000 kDa) polymers of poly-3-hydroxybutyrate and smaller (12 kDa) polymers of Ca^{2+} polyphosphate (Reusch, 1999; Reusch et al., 1995). These two polymers were reported to form a transmembrane complex that behaves very much like a Ca^{2+} channel, displaying characteristic selectivity for divalent cations and being inhibited by transition metal cations like La^{3+}, Co^{2+} and Cd^{2+}. Furthermore, these channels show elementary voltage-dependent openings when studied under patch-clamp (Reusch, 1999; Reusch et al., 1995).

Prokaryotic organisms are also in possession of Ca^{2+} channels constructed from protein helices (Durell and Guy, 2001; Matsushita et al., 1989; Tisa et al., 2000). Bacterial voltage-dependent Ca^{2+} channels contain a single domain assembled from six transmembrane α-helix segments S1-S6 (Durell and Guy, 2001), being therefore different from eukaryotes where Ca^{2+} channels have a four-domain structure. Bacterial Ca^{2+} channels are, however, morphologically and functionally similar to eukaryotic analogues, having respectively six transmembrane α-helices, and similar voltage-dependence and pharmacological properties, i.e. sensitivity to phenylalkylamines, dihydropyridines and La^{3+} (Matsushita et al., 1989). For instance, the voltage-dependence of Ca^{2+} channels in *Escherichia coli* resembles that of low-voltage-activated (T) Ca^{2+} channels in eukaryotes (Tisa et al., 2000).

4. Diversification of Ca^{2+} channels in prokaryotes

In eukaryotes, Ca^{2+} signalling systems became more complex; this is primarily associated with the development of intracellular organelles with their specific Ca^{2+} signalling mechanisms. Complexity of Ca^{2+} signalling in eukaryotes is also linked to the appearance of several types of Ca^{2+} permeable channels with distinct gating characteristics and differential Ca^{2+} permeability. In eukaryotes, Ca^{2+} fluxes through the plasma membrane are controlled by two highly Ca^{2+} selective channels, the voltage-gated Ca^{2+} channels and the store-operated Orai channels. In addition, the plasma membrane contains numerous cationic channels that include ligand-gated channels, numerous channels of the transient receptor potential (TRP) family, cyclic-nucleotide-sensitive cationic channels, mechanically-sensitive cationic channels and sperm-associated cation channels. All this remarkable diversity of Ca^{2+} permeable channels occurred very early in the evolution of unicellular organisms (Cai and Clapham, 2012), although some of their precursors have appeared even earlier in bacteria and fungi.

The ligand-gated cationic channels have very early evolutionary roots. The pentameric receptors (which in vertebrates mediate acetylcholinergic, GABAergic, glycinergic and serotoninergic transmissions) are present in cyanobacteria and proteobacteria as orthologous proton-activated channels (Corringer et al., 2012). Similarly, an early analogue of ionotropic glutamate receptors, the glutamatergic receptor GluR0, is also present in bacteria (Traynelis et al., 2010). Functional ancestral ionotropic purinoceptors of P2X class are found in protozoa, such as social amoeba *Dictyostelium discoideum* and in algae *Ostreococcus tauri*, whereas P2X protein homologues were identified in three basal fungi *Allomyces macrogynus*, *Spizellomyces punctatus*, and *Batrachochytrium dendrobatidis* (Burnstock and Verkhratsky, 2009; Cai, 2012).

The first true homologue of voltage-gated Ca^{2+} channels appeared in fungi, represented by Cch1. This fungal protein is similar to the vertebrate channels in its overall structure, being constructed from four repeats of six-transmembrane domains with P-loop selectivity filters (Cai and Clapham, 2012; Zelter et al., 2004). Similarly, proteins homologous to sperm-associated cation channels, generally believed to be associated with animal reproduction, were identified in the basal fungus *Allomyces macrogynus* (Cai and Clapham, 2012).

The Ca^{2+} permeable channels of the TRP family appeared in yeasts, which are in possession of the specific TRPY1 channel that is localised in the vacuolar membrane and arguably is involved in Ca^{2+} release from this organelle (Palmer et al., 2001). More closer relatives to animals, the choanoflagellates, already have several TRP proteins homologous to mammalian TRPC, TRPV, TRPM, TRPML and TRPA channels (Cai and Clapham, 2012). Similarly, choanoflagellates *Monosiga brevicollis*, *Salpingoeca rosetta* and amoeboid animal *Capsaspora owczarzaki* already have proteins for Orai-stromal interaction molecule (STIM) store-operated Ca^{2+} influx complex; these proteins, however, are absent in fungi, indicating that they appeared in ancestral animals (Cai, 2008; Cai and Clapham, 2012).

The origin and development of intracellular Ca^{2+} channels is also associated with early animals and is rather complex. The intracellular Ca^{2+} channels are represented by two types of endoplasmic reticulum channels, the ryanodine and inositol 1,4,5 trisphosphate receptors, as well as by the mitochondrial Ca^{2+} channels, also known as mitochondrial Ca^{2+} uniporters (Baughman et al., 2011; De Stefani et al., 2011; Kirichok et al., 2004; Verkhratsky, 2005). The evolution of endoplasmic reticulum channels begun in protists, which develop quite an extended family of these molecules represented by 36 members of 6 families that share certain properties with mammalian ryanodine and inositol 1,4,5 trisphosphate receptors

(Plattner and Verkhratsky, 2013). Subsequent animal evolution led to a tuning down of this extended number of ancestral forms.

5. Conclusion

Calcium signalling system is based around coordinated interactions of Ca^{2+} channels (that provide for the diffusional Ca^{2+} transport along electro-chemical gradients) and Ca^{2+} transporters (that move Ca^{2+} across membranes against electro-chemical gradients consuming energy). Evolution of Ca^{2+} channels resulted in the appearance of remarkably diversified classes of Ca^{2+} permeable channels, regulated by various physiological stimuli. These Ca^{2+} channels include highly selective voltage-gated and store-operated (Orai) channels and much less selective cationic channels that can be activated by neurotransmitters, second messengers or mechanical stimulation. Properties of these channels and their roles in physiology and pathophysiology form the subject of the special collection of papers that appear in this issue of *European Journal of Pharmacology*.

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