European Journal of Pharmacology 739 (2014) 4–18

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Intracellular calcium channels in protozoa

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ARTICLE INFO

Article history:
Accepted 21 November 2013
Available online 28 November 2013

Keywords:
Calcium channels
Protozoa
Paramecium
Toxoplasma
Trypanosoma
Mitochondrial calcium uniporter

ABSTRACT

Ca^{2+}-signaling pathways and intracellular Ca^{2+} channels are present in protozoa. Ancient origin of inositol 1,4,5-trisphosphate receptors (IP_3Rs) and other intracellular channels predates the divergence of animals and fungi as evidenced by their presence in the choanoflagellate *Monosiga brevicollis*, the closest known relative to metazoans. The first protozoan IP_3R cloned, from the ciliate *Paramecium*, displays strong sequence similarity to the rat type 3 IP_3R. This ciliate has a large number of IP_3- and ryanodine (Ry)-like receptors in six subfamilies suggesting the evolutionary adaptation to local requirements for an expanding diversification of vesicle trafficking. IP_3Rs have also been functionally characterized in trypanosomatids, where they are essential for growth, differentiation, and establishment of infection. The presence of the mitochondrial calcium uniporter (MCU) in a number of protozoa indicates that mitochondrial regulation of Ca^{2+} signaling is also an early appearance in evolution, and contributed to the discovery of the molecular nature of this channel in mammalian cells. There is only sequence evidence for the occurrence of two-pore channels (TPCs), transient receptor potential Ca^{2+} channels (TRPCs) and intracellular mechanosensitive Ca^{2+}-channels in *Paramecium* and in parasitic protozoa.

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1. Introduction

Calcium ion (Ca^{2+}) controls a variety of cellular functions in protozoa. As occurs with mammalian cells, the cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$ of protozoa is maintained at very low levels (of the order of 10^{-7} M). The cytosolic Ca^{2+} level is responsible for

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http://dx.doi.org/10.1016/j.ejphar.2013.11.015

the regulation of Ca^{2+}-dependent and Ca^{2+}-controlled proteins. Although the total calcium inside protozoan cells is much higher than 10^{-7} M, the bulk of this calcium is either bound to proteins, polyphosphate, membranes or other cellular constituents, or is sequestered inside intracellular organelles through the activity of pumps, channels, and exchangers, and released when needed by a variety or intracellular Ca^{2+} channels.

Recent genomic studies (King et al., 2008) have revealed that many ion channels including Ca^{2+} channels previously thought to be restricted to animals, can be traced back to one of the unicellular ancestors of animals, *Monosiga brevicollis*, a choanoflagellate protozoan belonging the supergroup Opisthokonta, which also includes animals, and fungi. Genes encoding homologs to various types of plasma membrane Ca^{2+} channels are present: store-operated channel (Orai) and the endoplasmic reticulum sensor protein stromal interaction molecule (Stim); voltage-operated channel (similar to dihydropyridine-sensitive L-type Ca^{2+} channel); ligand-operated channels (nicotinic acetylcholine receptor and P2X purinergic receptor); transient receptor potential (TRP) channels; and second messenger-operated channel (cyclic nucleotide-gated channel) (Cai, 2008). This protozoan appears to possess all five modes of regulated Ca^{2+} entry across the plasma membrane identified in animals (Parekh and Putney, 2005), although their physiological validation is needed (Cai, 2008). *Monosiga brevicollis* has also four homologs of the inositol 1,4,5-trisphosphate receptor (IP_3R), and a homolog to the mitochondrial calcium uniporter (XP_001749044), but no homologs to ryanodine receptors (RyR) (Cai, 2008). However, no functional studies have been reported with any of these channels.

Evidently the evolution of eukaryotic cells is characterized by increasing genomic information that allows for increasing complexity of intracellular structure, dynamics and signaling mechanisms. Target-oriented vesicle trafficking requires not only an inventory of membrane-specific proteins, such as SNAREs (Malsam et al., 2008) and small GTPases (Zerial and McBride, 2001), but also provisions for Ca^{2+} signaling in a very local area where membranes have to interact (Neher, 1998). Ca^{2+} may come from the external medium or be locally released from stores via Ca^{2+}-release channels (CRC) so that Ca^{2+} can locally drive docking, priming and eventual fusion of membranes (Rizo et al., 2006). Cell contraction is another example. Ca^{2+} is most appropriate for such functions because of its specific, reversible binding to Ca^{2+}-binding proteins, CaBP, which in the end transmit the signal by a conformational change in effector protein molecules (Klee et al., 1980; Rizo et al., 2006). On the one hand global regulation of intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, is mandatory to avoid the overall toxic effect of Ca^{2+} (Case et al., 2007). On the other hand, local $[Ca^{2+}]_i$ regulation also has to account for diffusional spread by a square function, whereas most molecular effects of Ca^{2+} depend on a higher power-function of $[Ca^{2+}]_i$ (Neher, 1998). Binding to CaBPs, sequestration into organelles and extrusion from the cell antagonize the occurrence of too high and diffuse $[Ca^{2+}]_i$ values after stimulation. Remarkably, the phenomena described in this review, as well as the CRC types mentioned, are all found already in protozoa. Nevertheless, with these cells stringent analyses of Ca^{2+} signaling and the subsiding intracellular CRCs have remained elusive until quite recently.

The protozoan organisms whose Ca^{2+} signaling and subsiding CRCs are currently investigated in our labs include ciliates (*Paramecium*), their close relatives, Apicomplexa (including pathogenic species of *Plasmodium* [malaria causing agent] and *Toxoplasma*) as well as some pathogenic flagellates (trypanosomatids). With these organisms, CRCs have been characterized at a molecular level, in conjunction with functional studies. There is a wide gap between evolutionary levels: ciliates close to recent forms have emerged ~800 to 850 million years ago, non-parasitic Apicomplexa ~500 million years (Douzery et al., 2004) and mammalian apicomplexan parasites ~13 million years ago (Ricklefs and Outlaw, 2010). There is also some information available on the Ca^{2+} dynamics in social ameba of the myxomycete *Dictyostelium,* which clearly possesses Ca^{2+} signaling pathways (Allan and Fisher, 2009), but information about CRCs in these cells is scant.

A *Paramecium tetraurelia* cell is up to ~100 μm in size and exhibits distinct intracellular vesicle trafficking pathways (Allen and Fok, 2000), essentially including all those known from metazoan cells. The pathogenic forms discussed are ~10 times smaller, but also contain specific vesicle-trafficking pathways, such as endocytosis vesicles and organelles for intracellular digestion (trypanosomatids, Apicomplexa). Apicomplexa also possess secretory organelles for exocytosis. Due to their small size and their complicated lifestyle the parasites are much more difficult to study than their free-living relatives. Using fluorescent dyes in both ciliates and Apicomplexa, a considerable Ca^{2+} signal could be recorded during exocytosis of secretory organelles, such as trichocysts (Klauke and Plattner, 1997) and during motility (Lovett and Sibley, 2003), respectively.

Values for steady state $[Ca^{2+}]_i$ in widely different cells, from protozoa to mammals, are of the order of 50–100 nM at rest and stimulation generally causes an increase by a factor of 10–100 (Bootman and Berridge, 1995). This frame also applies to ciliates (Klauke and Plattner, 1997) and to parasitic protozoa (Vieira and Moreno, 2000; Moreno et al., 1994). $[Ca^{2+}]_i$ determined in *Paramecium* under steady state conditions yields values between 60 and 100 nM. It has to be stressed that measurements performed with fluorescent dyes, even when calibrated, systematically underestimate the real local $[Ca^{2+}]_i$ increase during activation because of its considerable local restriction. More realistic local, functionally relevant values are obtained by probing the threshold inhibitory effect of Ca^{2+} chelators with appropriate binding properties (Neher, 1995). For instance, during exocytosis stimulation $[Ca^{2+}]_i$ in the cell cortex peaked at ~400 nM with fluorescent dyes measurements, whereas chelator application during stimulation indicated the increase in $[Ca^{2+}]_i$ to the micromolar range (Klauke and Plattner, 1997).

2. Calcium stores

The paradigm of a Ca^{2+} store in all eukaryotic cells is the endoplasmic reticulum (ER), together with the sarcoplasmic reticulum (SR) in muscle cells (Berridge et al., 2000, 2003; Clapham, 2007; Cai, 2008). Since Ca^{2+} is stored in many more organelles such stores and their CRCs deserve special attention also in protozoa, including ciliates and parasitic protozoa (Plattner et al., 2012).

Subsequent to stimulation Ca^{2+} is sequestered into different organelles and then may be available later on for release via CRCs in a constitutive manner or in the context of signaling processes. Ca^{2+} can, thus, regulate exocytosis, endocytosis, phagocytosis, fusion of endosomes of different stages with phagosomes, phagosome formation, membrane recycling, phago-lysosome fusion etc. (Hay, 2007; Zampese and Pizzo, 2012). In mammalian cells, many of these organelles, specifically early endosomes (Luzio et al., 2010) and lysosomes (Christensen et al., 2002), are known to store Ca^{2+} (Hay, 2007; Sherwood et al., 2007) and the membranes of many of them contain CRCs (Zampese and Pizzo, 2012). The main types of CRCs found in metazoans up to mammalian cells are IP_3R (Taylor et al., 2004; Bezprozvanny, 2005), RyR (Hamilton, 2005; Mackrill, 2012), transient receptor potential Ca^{2+} channels, TRPC (Patel and Docampo, 2009), and two pore channels, TPC, occurring mainly in acidic compartments (Galione et al., 2009; Galione et al., 2010; Patel and Docampo, 2010). All these channel types also occur in protozoa.