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Review

IP_3, a small molecule with a powerful message ☆

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ABSTRACT

Research conducted over the past two decades has provided convincing evidence that cell death, and more specifically apoptosis, can exceed single cell boundaries and can be strongly influenced by intercellular communication networks. We recently reported that gap junctions (i.e. channels directly connecting the cytoplasm of neighboring cells) composed of connexin43 or connexin26 provide a direct pathway to promote and expand cell death, and that inositol 1,4,5-trisphosphate (IP_3) diffusion via these channels is crucial to provoke apoptosis in adjacent healthy cells. However, IP_3 itself is not sufficient to induce cell death and additional factors appear to be necessary to create conditions in which IP_3 will exert proapoptotic effects. Although IP_3-evoked Ca^{2+} signaling is known to be required for normal cell survival, it is also actively involved in apoptosis induction and progression. As such, it is evident that an accurate fine-tuning of this signaling mechanism is crucial for normal cell physiology, while a malfunction can lead to cell death. Here, we review the role of IP_3 as an *intra*cellular and *inter*cellular cell death messenger, focusing on the endoplasmic reticulum-mitochondrial synapse, followed by a discussion of plausible elements that can convert IP_3 from a physiological molecule to a killer substance. Finally, we highlight several pathological conditions in which anomalous intercellular IP_3/Ca^{2+} signaling might play a role. This article is part of a Special Issue entitled:12th European Symposium on Calcium.

1. Introduction

Several *intra*cellular key events in various cell death modes are triggered/regulated by Ca^{2+} signals [1–3]. However, research from the past 20 years has demonstrated that cell death is not a 'privilege' of one single cell, but that the message can be actively transferred to healthy cells in close contact with the dying cell, a process termed bystander death [4]. Cell-to-cell communication is established by vast arrays of plasma membrane channels called gap junctions (GJs) which directly connect neighboring cells [5]. GJs allow the passage of small molecular weight (MW) molecules (<1–1.5 kDa) including glucose, glutamate, glutathione, cyclic adenosine monophosphate (cAMP), adenosine 5′-triphosphate (ATP), inositol 1,4,5-trisphosphate (IP_3) and ions (e.g. Ca^{2+}, K^+, Na^+, Cl^-) [5,6]. They are composed of 12 transmembrane connexin (Cx) proteins of which more than 20 isoforms have been identified in mammals [7]. Their nomenclature is based on their MW, which ranges from 25 to 62 kDa. Cxs also form hemichannels (HCs), positioned as pores between the intra- and extracellular environment [8]. Of note, pannexins are another family of channel-forming proteins that also constitute HCs, but these are less likely to assemble into GJs [9–12]. Cxs are present in most organs and display a tissue and cellular specificity with Cx43 being the most abundant Cx in mammals [7,13].

Abbreviations: ANT, adenine nucleotide translocator; ATP, adenosine triphosphate; BBB, blood-brain barrier; Bcl-2, B-cell lymphoma-2; BH, Bcl-2-homology domain; Bi-1, Bax-inhibitor-1; $[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CT, C-terminal; Cx, connexin; CytC, Cytochrome C; DAMs, damage-associated molecules; ER, endoplasmic reticulum; GJ, gap junction; GPCR, G-protein-coupled receptor; grp, glucose-regulated protein; HC, hemichannel; IICR, inositol 1,4,5-trisphosphate-induced Ca^{2+} release; IMM, inner mitochondrial membrane; IP_3, inositol 1,4,5-trisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor; KO, knockout; MAM, mitochondria-associated endoplasmic reticulum membrane; MCU, mitochondrial Ca^{2+} uniporter; MEFs, mouse embryonic fibroblasts; MW, molecular weight; NT, N-terminal; OMM, outer mitochondrial membrane; PKA, protein kinase A; PKG, protein kinase G; PLC, phospholipase C; PS, presenilins; PTP, permeability transition pore; ROS, reactive oxygen species; RyR, ryanodine receptor; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; TNF-α, Tumor Necrosis Factor-α; VDAC, voltage-dependent anion channel

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Numerous physiological processes are regulated by Cx channels as well as non-channel functions as demonstrated by Cx knockout (KO) studies in mice and the association of several human diseases with mutations of specific Cx genes [13,14]. However, the very same communication pathways can have deleterious consequences as well, including an active contribution to the intercellular spreading of cell death signals from dying to healthy neighboring cells. The role of GJs in cell death communication -primarily apoptosis- has already been firmly established and an increasing number of reports demonstrate that HCs can kick in by forming toxic pores in the plasma membrane or providing a paracrine cell death communication pathway [4,15,16]. One major caveat in this research area is related to identifying the signal(s) which convey the cell death message. Importantly, we recently demonstrated that the passage of the Ca^{2+} messenger IP_3 through GJs is crucial for intercellular cell death communication using an *in vitro* apoptosis model. This work provided evidence that the physiological messenger IP_3 becomes a toxic intercellular messenger when the cells that are generating IP_3, are undergoing apoptosis [17]. This review furnishes an overview of what is known about the role of IP_3 in cell death on the *intra*cellular and *inter*cellular level, focusing on the intrinsic mitochondria-related pathway of apoptosis. Finally, the importance of targeting this intercellular pathway will be underscored by discussing some pathologies where intercellular IP_3 spreading could contribute to exaggerated cell death.

2. IP_3, a major determinant of intra- and intercellular Ca^{2+} signaling

The discovery of IP_3 as a second messenger about 30 years ago can be regarded as one of the major breakthroughs in understanding how cells can chemically relay stimuli from the plasma membrane to intracellular Ca^{2+} increases [18]. Numerous extracellular stimuli act on plasma membrane receptors and subsequently trigger phospholipase C (PLC) activity. There are 13 different PLC isoforms which are divided in six different classes based on their structure: PLCβ, PLCγ, PLCδ, PLCε, PLCζ and PLCη. They can be activated via different mechanisms including the stimulation of G-protein-coupled receptors (GPCRs), receptor and non-receptor tyrosine kinases, the activation of the small G-protein Ras, or an increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) [19]. PLC catalyzes the cleavage of phosphoinositol-4,5-bisphosphate into diacylglycerol and IP_3. The latter diffuses in the cytoplasm and binds its receptor which is mainly located on the endoplasmic reticulum (ER), the largest and most controllable intracellular Ca^{2+} source [2]. The subsequent cytoplasmic Ca^{2+} rise can take many different forms which are highly organized in both time and space, with oscillations appearing as repetitive and localized Ca^{2+} spikes, and localized Ca^{2+} increases being amplified into spreading waves respectively. This spatiotemporal organization forms the basis for a versatile signaling system used by virtually all cell types—excitable as well as non-excitable cells [20,21].

The Ca^{2+} signaling system needs to be endowed with sufficient reliability and specificity to control various physiological processes as diverse as fertilization, cell proliferation, differentiation, neurotransmitter release, secretion, gene expression, immune responses, muscle contraction, endothelial permeability, apoptosis and many others [22,23]. Repetitive oscillating Ca^{2+} spikes encode information in their amplitude, spike duration and frequency [24]. Single Ca^{2+} transients, on the other hand, tend to last longer and the resulting signal usually spreads out as a cytoplasmic Ca^{2+} wave. These waves are not always restricted to the cytosol of one cell but can propagate toward other cells as *inter*cellular Ca^{2+} waves [20,21]. Ca^{2+} waves can be communicated between cells by GJs as well as paracrine signaling. Since Ca^{2+} passage via GJs is known to be limited due to the presence of cytoplasmic Ca^{2+} binding proteins, IP_3 is considered to be the primary coordinating messenger [25–27]. IP_3 has a much higher effective diffusion constant compared to Ca^{2+} (283 $\mu m^2/s$ versus 13–65 $\mu m^2/s$ respectively), and a 100 times larger GJ permeability over Ca^{2+} [27,28]. ATP is considered to be the primary paracrine messenger, released in the extracellular space, and acting on GPCRs or triggering Ca^{2+} entry via plasma membrane channels in neighboring cells [29–33]. Multiple pathways can be involved in the release of ATP including exocytosis, diffusion through either Cx or pannexin HCs, or P_2X receptor channels [30,34–41]. Intercellular wave propagation is most of the times sustained by both the gap junctional and paracrine pathway [32,42,43], and supported by regenerative steps. These steps include the regeneration of IP_3 by the Ca^{2+}-triggered activation of PLCδ [28], and the Ca^{2+}-dependency of several ATP release mechanisms [34,37,44]. Both mechanisms eventually result in more extensive wave propagation [21]. Intercellular Ca^{2+} wave propagation has been observed in *in vitro* monolayer cultures of diverse cell types (e.g. glial cells, endothelia, hepatocytes), where they generally spread with a velocity of 10–40 μm/s in response to a variety of stimuli [35,43,45,46], as well as in *ex vivo* and *in vivo* conditions [31,47–50]. It appears to be a fundamental mechanism to synchronize the function of a large group of cells.

Both GJs and HCs also influence the occurrence of Ca^{2+} oscillations. GJs are known to modulate the spike frequency, the number of cells displaying Ca^{2+} oscillations and the synchronization of Ca^{2+} oscillations [45,51–53]. HCs seem to be involved in the generation of Ca^{2+} oscillations through the activation of different regenerative signaling loops starting with the Ca^{2+}-induced opening of HCs followed by ATP or nicotinamide adenine dinucleotide release, or the uptake of Ca^{2+} itself via open HCs [54–56].

3. Intracellular Ca^{2+} signaling: a combined effort between IP_3 receptors, the ER and mitochondria

The spatiotemporal organization of Ca^{2+} signals largely depends on (i) the characteristics and (ii) the spatial distribution of the Ca^{2+} release channels involved. The IP_3 receptor (IP_3R) is a tetramer with each subunit consisting of a cytoplasmic N-terminal (NT) domain which is composed of a suppressor domain and an IP_3-binding core, a C-terminal (CT) channel domain (six transmembrane domain), and an intervening regulatory and coupling domain [57]. The latter mediates the transfer of the ligand binding signal from the IP_3-binding domain to the channel domain. This domain also functions to keep the inactivated IP_3R channel closed and contains many target sites for regulators of IP_3R activity [57,58]. IP_3Rs are widely expressed in different types of organs and tissues. The gating of IP_3Rs (i.e. closing and opening of the channel) is influenced by different factors including cytoplasmic IP_3, Ca^{2+} and ATP concentrations, ER luminal Ca^{2+} concentration, redox and phosphorylation status, and protein interactions [57,58]. Of note, the affinity for IP_3 binding and the sensitivity of the IP_3R to the modulatory signals depend on the isoform of which the receptor is constituted (IP_3R1, IP_3R2 or IP_3R3) [57–59]. The best known regulatory factor is $[Ca^{2+}]_i$ which has a biphasic action on the receptor: Ca^{2+} activates the receptor at low concentrations (100–300 nM), but becomes inhibitory at higher concentrations (above 300 nM) [60–65].

The spatial organization of IP_3Rs is related to the arrangement of IP_3Rs as clusters on the ER, as well as their close contact with other Ca^{2+}-sequestering organelles such as mitochondria [66,67]. The role of mitochondria in cellular Ca^{2+} signaling has been underestimated for a long time, but it becomes increasingly recognized that mitochondria actively participate in shaping the Ca^{2+} signaling pattern [68,69]. Mitochondria extrude protons to create an electrochemical gradient that enables ATP synthesis. The same gradient is used to drive mitochondrial Ca^{2+} uptake, with the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane (OMM) and the recently identified mitochondrial Ca^{2+} uniporter (MCU) in the inner mitochondrial membrane (IMM) as major regulators; in addition to other, less well-characterized transport systems [2,3,70–72]. Mitochondria accumulate Ca^{2+} more effectively when they are close to sites of high Ca^{2+} concentration [73,74]. Different reports have demonstrated that IP_3Rs can be concentrated, together with other proteins involved in cellular Ca^{2+} homeostasis like the sarco-endoplasmic reticulum