

## IP<sub>3</sub> receptors, stress and apoptosis

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**Abstract.** Inositol 1,4,5-trisphosphate (IP<sub>3</sub>) receptors are intracellular calcium channels that are able to release calcium from intracellular stores upon activation by IP<sub>3</sub> and modulation by calcium. IP<sub>3</sub> receptors are involved in variety of processes during physiological, but also in the pathophysiological states. Unraveling their regulation and function, especially under the pathological situations can result in a development of new therapeutic strategies based on the IP<sub>3</sub> receptor's activation and/or blocking. To the stimuli that can modulate IP<sub>3</sub> receptors belong several stress factors (e.g. immobilization stress, oxidative stress and hypoxia) and also apoptosis. Depending on the length and strength of the stress stimulus, expression of IP<sub>3</sub> receptors can be increased, or decreased. Therefore, in this minireview modulation of IP<sub>3</sub> receptors by some stressors is discussed. Since it was already shown that strong hypoxia might lead to the apoptosis induction, special focus will be given to the hypoxic stress and induction of apoptosis.

**Key words:** Inositol-1,4,5-trisphosphate receptors — Stress — Hypoxia — Apoptosis

### Inositol 1,4,5-trisphosphate (IP<sub>3</sub>) receptors

Inositol 1,4,5-trisphosphate (IP<sub>3</sub>) receptors are intracellular membrane calcium channels essential for the release of calcium from intracellular stores, mainly from the sarcoplasmic/endoplasmic reticulum (ER). However, IP<sub>3</sub> receptors are able to stimulate calcium release not only from the ER, but also from the Golgi apparatus, from within a nucleus and also from secretory vesicles (for review see Taylor and Tovey 2010). Up to now, three types of IP<sub>3</sub> receptor (IP<sub>3</sub> receptor type 1, 2 and 3), originating from three different genes were cloned and characterized (for review see Krizanova and Ondrias 2003; Foskett et al. 2007). Sequences of three full-length IP<sub>3</sub> receptor's isoforms are 60–80% homologous. The IP<sub>3</sub> channel consists of homotetramers or heterotetramers of IP<sub>3</sub> receptor's subunits with approximately 2,700 amino acids *per* subunit, and approximate molecular mass of 300 kDa. Each subunit contains three functionally distinct regions: the transmembrane ion channel pore domain at the C-terminal end, the IP<sub>3</sub>-binding domain at the N-terminal region and the modulatory (transduction) domain between them (for review see Krizanova and Ondrias 2003). The trans-

membrane domain contains 6 transmembrane segments, with a pore-forming region between segments 5 and 6. Majority of calcium binding sites occur in the modulatory domain, where also putative binding sites for calmodulin and adenosine triphosphate (ATP) are localized. Binding of the IP<sub>3</sub> together with calcium (two principal co-activators of the receptor) is prerequisite for the activation of all three types of IP<sub>3</sub> receptors. The isoforms have distinct and overlapping patterns of expression with most cells expressing more than one, and expression levels can be modified during differentiation and by use-dependent degradation (Foskett et al. 2007). This impressive diversity of expression suggests that cells require distinct IP<sub>3</sub> receptors to regulate specific functions.

IP<sub>3</sub> receptors have been shown to modulate variety of different processes in distinct tissues. IP<sub>3</sub> receptors play an important role in mitochondrial bioenergetics (Cardenas et al. 2010), induction and modulation of apoptotic process (Hanson et al. 2004; Joseph and Hajnóczky 2007), induction of neuronal plasticity (Kim et al. 2008), etc.

Individual types of IP<sub>3</sub> receptors are tissue- and cell-dependent. In the brain, neuronal form of the type 1 IP<sub>3</sub> receptor predominates, while in the brain stem the non-neuronal spliced variant is more abundant (Kocan et al. 2002). Although this type is the most abundant isoform in the brain, other isoforms are also present (Sudhof et al. 1991). Besides regional and cellular variations, IP<sub>3</sub> receptor isoforms may

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differ in neuronal compartmentalization. Type 1 IP<sub>3</sub> receptor occurs in dendrites, dendritic spines, cell bodies, axons and axonal terminals of cerebellar Purkinje cells, but appears to be more compartmentalized in most other neurons with highest levels generally in cell bodies and proximal dendrites (Nakanishi et al. 1991; Dent et al. 1996). Type 2 IP<sub>3</sub> receptor was only detected in glia (Sharp et al. 1999). Type 3 IP<sub>3</sub> receptor is abundantly expressed in several basal forebrain and limbic system-associated regions including olfactory tubercle, bed nucleus of the *stria terminalis* and central nucleus of the amygdala (Sharp et al. 1999). Variations in regional localizations of types 1 and 3 IP<sub>3</sub> receptors may reflect different roles in control of behavior.

In cardiomyocytes, expression of the IP<sub>3</sub> receptors has been shown by several groups (Kijima et al. 1993; Moschella and Marks 1993). In these cells, type 2 IP<sub>3</sub> receptor predominates, while the type 1 IP<sub>3</sub> receptor is the most abundant in intrinsic neuronal ganglia of cardiac atria (Lencesova et al. 2002; Slavikova et al. 2006).

All three types of IP<sub>3</sub> receptor were found in kidney (Fujino et al. 1995). Type 1 was detected in glomerular mesangial cells and vascular smooth muscle cells (Monkawa et al. 1998). Interestingly, the mRNA and protein levels of IP<sub>3</sub> receptor type 1 were found in renal medulla, but not in renal cortex of rats (Zacikova et al. 2000). Type 2 was expressed exclusively in intercalated cells of collecting ducts from the cortex to the inner medulla. Type 3 was expressed in vascular smooth muscle cells, glomerular mesangial cells, and some cells of cortical collecting ducts, probably principal cells (Monkawa et al. 1998). The IP<sub>3</sub> receptor type 3 is expressed in adult pancreatic islets, kidney, gastrointestinal tract (Blondel et al. 1993).

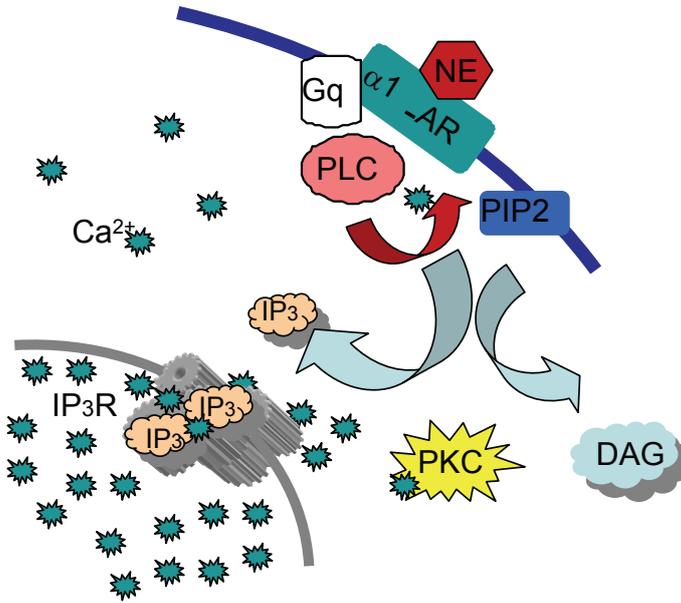
Because of its ubiquitous expression and a role in regulating diverse cell physiological processes in so many cell types, it is perhaps not surprising that the IP<sub>3</sub> receptors has been implicated in a number of disease states, including polycystic kidney disease (Li et al. 2005), cardiac arrhythmias (Kockskamper et al. 2008), spinocerebellar ataxias 15 and 16 (Foskett et al. 2010; Bezprozvanny 2011) and inflammation (Park et al. 2008). Together with ryanodine receptors, IP<sub>3</sub> receptors are also involved in neuronal excitotoxicity (Ruiz et al. 2009). Recently, it was shown that individual types of IP<sub>3</sub> receptors could be involved in development of some pathophysiological states. For example, colon cancer cells expressed type 3 IP<sub>3</sub> receptors *de novo* and increased expression of this isoform is associated with increased aggressiveness of the tumor and decreased long-term survival (Shibao et al. 2010). The relation may be causal one, because knockdown of this receptor isoform enhances susceptibility to apoptosis. Thus, based on these observations it seems to be truth that functional implications of IP<sub>3</sub> receptor's diversity, at the single channel, cellular and organ level, remain largely unappreciable (Foskett et al. 2007).

### IP<sub>3</sub> receptors and stress

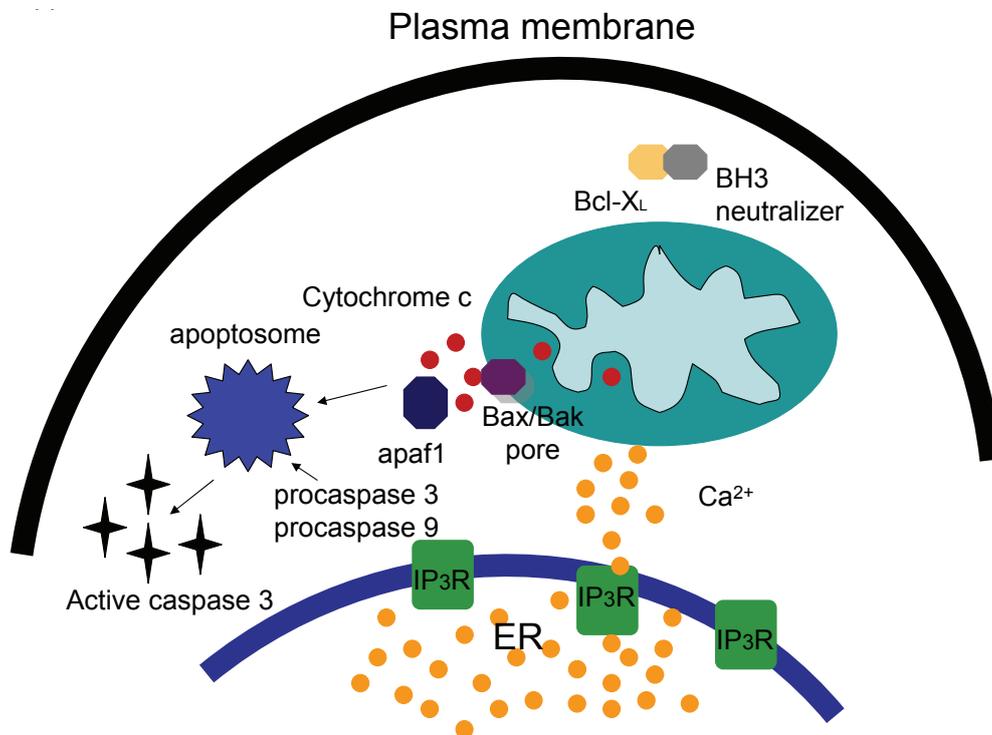
Stress as a modern civilization factor is deeply incorporated in modern life style. Repeated stress may be detrimental, since it can result in the development of the pathological state in some organs. Variety of stressors is currently used as a model of stress and each stressor has a neurochemical "signature" with quantitatively, if not qualitatively distinct central and peripheral mechanisms (Goldstein et al. 1996).

Involvement of IP<sub>3</sub> receptors in the stress response could be predicted, because of coupling adrenergic system (particularly through the  $\alpha$ 1-adrenoceptors) to the regulation of calcium homeostasis. Particularly, the  $\alpha$ 1-adrenoceptors act by binding to G $\alpha$ q subunits of the G proteins, causing activation of phospholipase C (PLC). PLC converts phosphatidylinositol 4,5-bisphosphate into the IP<sub>3</sub> and diacylglycerol (DAG), which have downstream effects on cytosolic Ca<sup>2+</sup> concentration (Fig. 1). Some (but not all) stressors are known to affect the gene expression of the IP<sub>3</sub> receptors, depending on their duration and on the affected tissue (Zacikova et al. 2000; Lencesova et al. 2002; Micutkova et al. 2003).

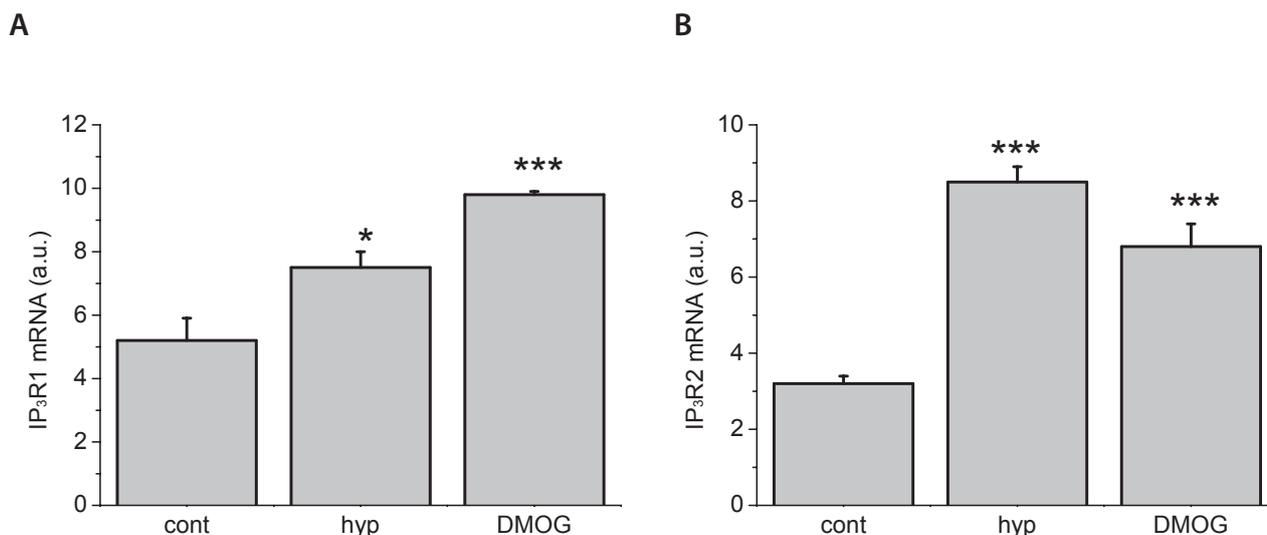
Immobilization stress is one of the strong stressors, since it activates both components of the sympathoadrenal system – adrenomedullary and sympathoneural (Kvetnansky and Mikulaj 1970). The ability to activate both these pathways ranks the immobilization stress to the most potent experimentally used stressors in rats and mice (Goldstein et al. 1996). Immobilization stress affects variety of systems, among them also calcium transport systems, like Na<sup>+</sup>/Ca<sup>2+</sup> exchanger in a rat heart, or  $\alpha$  subunit of calcium channels (Zacikova et al. 1999). When rats were exposed to a single exposure of the immobilization stress, mRNA levels of type 1 and 2 IP<sub>3</sub> receptor were upregulated in rat cardiac atria (Lencesova et al. 2002). Nevertheless, in the rat heart, type 1 IP<sub>3</sub> receptors were down-regulated after the exposure to repeated immobilization (Krepsova et al. 2004). Decrease in the expression of IP<sub>3</sub> receptors is consistent with observations of reduced amount of these types IP<sub>3</sub> receptors in rat renal medulla (Zacikova et al. 2000) and stellate ganglion (Micutkova et al. 2003) after repeated immobilization stress. Physiological relevance of this decrease was not studied yet, nevertheless, it might be proposed that it should have protective or compensatory effect, e.g. against calcium overload. Physiological role is strengthened by similar decrease in IP<sub>3</sub> mRNA and IP<sub>3</sub> receptor's protein levels observed after development of pathophysiological state due to an ischemia (Farwell et al. 1998; Uhm et al. 1998), or in brains of patients suffering from Alzheimer's disease (Haug et al. 1996). It was already suggested that down-regulation of the IP<sub>3</sub> receptor's function may thus be a common denominator for neurodegenerative diseases (Dahl et al. 2000). Therefore, physiological significance of decreased levels of IP<sub>3</sub> receptors after prolonged stress or development of pathological



**Figure 1.** Modulation of IP<sub>3</sub> receptors by α1-adrenergic receptors (α1-AR). After binding noradrenaline (NE) to the α1-AR, phospholipase C (PLC) is activated and cleaves phosphatidylinositol 4,5-diphosphate (PIP2) to IP<sub>3</sub> and diacylglycerol (DAG). Subsequently, IP<sub>3</sub> binds in the presence of calcium to the IP<sub>3</sub> receptor (IP<sub>3</sub>R), calcium is released through this receptor and activation of several pathways (e.g. through protein kinase C; PKC) occurs.



**Figure 3.** Role of IP<sub>3</sub> receptors in the activation of mitochondrial pathway of apoptosis. Calcium released through IP<sub>3</sub> receptors (IP<sub>3</sub>R), is sequestered to mitochondria and induces release of the cytochrome c through the Bax/Bak pore. This process further results in the activation of pro-apoptotic protein apaf1 and generation of the apoptosomes, with subsequent activation of the executive enzyme caspase 3. During this process, anti-apoptotic protein Bcl-X<sub>L</sub> binds to BH3 neutralizer and becomes inactive. ER, endoplasmic reticulum.



**Figure 2.** Effect of 4% hypoxia (hyp) and DMOG (as a model of chemical hypoxia) on mRNA levels of the type 1 IP<sub>3</sub> receptors (IP<sub>3</sub>R1; **A**) and the type 2 IP<sub>3</sub> receptors (IP<sub>3</sub>R2; **B**) in PC12 cells. Both these stimuli increased significantly IP<sub>3</sub>R1 and IP<sub>3</sub>R2 and prove the effect of hypoxia on these receptors. Results are displayed as mean  $\pm$  S.E.M. and each value represents an average of at least 9 parallels from three independent cultivations. \*  $p < 0.05$ , \*\*\*  $p < 0.001$  vs. control. DMOG, dimethyl oxallyl glycine.

state is of a special importance and remains to be further elucidated.

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. It is generally presumed that metabolic activity generates oxidative stress *via* an increase in the production of reactive oxygen species (Richter 1997). Enhanced neuroprotection to the oxidative stress through purinergic receptor (P2Y-R) was blocked by oligomycin and by Xestospongine C, inhibitors of the ATP synthase and of IP<sub>3</sub> binding to the IP<sub>3</sub> receptor, respectively (Wu et al. 2007). Thus, mitochondrial Ca<sup>2+</sup> uptake *via* Ca<sup>2+</sup> release from the IP<sub>3</sub> receptor protected astrocytes from the oxidative stress. The mitochondrial theory of aging proposes that degeneration of physiological processes over the course of a lifetime is fundamentally a result of the accumulation of oxidative damage (Sohal and Dubey 1994; Warner 1994; Gadaleta et al. 1998; Cortopassi and Wong 1999). These data also reveal a signaling pathway in which IP<sub>3</sub> receptor is involved that can rapidly respond to central energy requirements throughout the aging process (Wu et al. 2007). Kaja and coworkers (2011) described a novel mechanism for increased intracellular Ca<sup>2+</sup> release following oxidative stress in a neuronal cell line. These authors have shown that sub-lethal tert-butyl hydroperoxide-mediated oxidative stress result in a selective up-regulation of type 2 IP<sub>3</sub> receptors. This oxidative stress functionally resulted in increased Ca<sup>2+</sup> release into the

nucleoplasm from the membranes of the nuclear envelope at a given receptor-specific stimulus.

Deranged Ca<sup>2+</sup> signaling and an accumulation of aberrant proteins cause ER stress, which is a hallmark of cell death implicated in many neurodegenerative diseases (Higo et al. 2010). ER stress is defined as "an imbalance between the cellular demand for ER function and ER capacity" (Ni and Lee 2007), and results from accumulation of unfolded/mis-folded protein (Kim et al. 2006). ER stress response appears after ischemia and is attenuated by preconditioning paradigm (Lehotsky et al. 2009). An ER chaperone, GRP78, acts as the master regulator of unfolded protein response signaling to improve biogenetic processes and has a cytoprotective function against ER stress (Hendershot 2004; Mimura et al. 2007; Wang et al. 2010). During ER stress, ER chaperone GRP78 dislocates from IP<sub>3</sub> receptors, thereby affecting subunit assembly and reducing the number of functional IP<sub>3</sub> receptors. This underlies ER-stress-induced apoptosis (Higo et al. 2010). On contrary, Li et al. (2009) have shown that ER-stress-induced apoptosis involves the activation of IP<sub>3</sub> receptors *via* C/EBP homologous protein-induced ERO1- $\alpha$ .

Hypoxia is another type of stressor manifested with a decreased oxygen supply. Hypoxia participates in a development of various consequential diseases, e.g. cardiovascular, neuronal and also tumorigenesis. The switch to glycolytic metabolism represents a significant response to hypoxia which is mediated by hypoxia-inducible factor 1 (HIF-1) (Semenza 2010). There is an evidence

that prolonged hypoxia can result in the apoptosis (Yao et al. 2010).

Among tissues, cardiac and neuronal tissue is the most affected, because of the high demand of oxygen required for their proper function. During hypoxic insults, intracellular free Ca<sup>2+</sup> concentration rises, but the magnitude of change depends on the severity of insult (Budd 1998). Hypoxia activates the L-type calcium channels in pulmonary vasculature (Weir and Olschewski 2006). Also, hypoxia significantly increases gene expression and also protein levels of the sodium-calcium exchanger type 1 (NCX1) in the rat cardiomyocytes (Hudecova et al. 2007) and in human embryonic kidney (HEK 293) cell line (Hudecova et al. 2011), probably through the HIF-1 $\alpha$ . NCX1 plays a key role in inducing apoptosis after hypoxia in cultured guinea pig ventricular myocytes (Eigel et al. 2004).

Prominent functional modifications induced by hypoxia consist of substantial membrane depolarization and rapid failure of synaptic transmission (Nieber et al. 1999). Enhancing the capacity of neurons to adapt to hypoxic stress has implications for improving the survival of neurons during lethal insults from diseases such as stroke and hypoxic encephalopathy. Changes in cellular oxidation/reduction balance during hypoxia increase cytosolic NAD(P)H, and this triggers the release of Ca<sup>2+</sup> from the ER *via* activation of IP<sub>3</sub> receptors. This Ca<sup>2+</sup> release, in turn, activates pathways involved in survival signaling. Ca<sup>2+</sup> release from the ER is therefore a key determinant of the adaptive or neuroprotective response of neurons to hypoxic stress (Bickler et al. 2009). Thus, hypoxic stress seems to be attractive model for studies of the mechanism of such serious diseases, like ischemia, tumor progression, etc. Therefore, besides hypoxic models with decreased oxygen, the model of chemical hypoxia was established. Among variety of chemical compounds, dimethyl oxallyl glycine (DMOG), an iron chelator, which stabilizes HIF-1 $\alpha$  and acts as a prolyl hydroxylase inhibitor, is commonly used. Both, the effect of 4% hypoxia or DMOG induce expression of type 1 and 2 IP<sub>3</sub> receptors (Fig. 2).

Number of IP<sub>3</sub> receptors increased in the cerebral cortex of the guinea pig fetus due to the hypoxic stimuli (Zanelli et al. 2005). Kaplin and coworkers (1996) published that chemical hypoxia mobilizes calcium from IP<sub>3</sub>-sensitive intracellular stores via nicotinamide adenine dinucleotide production. Also, hypoxia significantly increases gene expression and protein levels of the type 1 and 2 IP<sub>3</sub> receptors in neuronal cells, without affecting gene expression of SERCA2 and ryanodine receptors (Jurkovicova et al. 2007). Among several physiological consequences suggested, remodeling of neuronal cells is very probable. Increased transcription of the type 1 IP<sub>3</sub> receptor might be probably mediated by heterodimeric HIF-1, which is activated during the hypoxic conditions, or through the transcription factor NF- $\kappa$ B. The

responsive element for this transcription factor is present in the promoter region of gene for IP<sub>3</sub> receptor type 1 and also type 2 (Morikawa et al. 1997; Deelman et al. 1998). NF- $\kappa$ B transcription factor is probably activated by reactive oxygen species, which are released from mitochondria during hypoxic conditions (Bickler and Donohoe 2002). This suggestion was verified by adding antioxidant quercetin to cells before their exposure to hypoxia. Quercetin completely prevented hypoxia-induced increase of IP<sub>3</sub> receptors in cerebellar granular cells (Jurkovicova et al. 2007), thus supporting the role of reactive oxygen species in hypoxia.

The question remains, whether strong and prolonged hypoxia can induce apoptosis in certain types of the cells. It was already showed that hypoxia-induced apoptosis of retinal ganglion cells (RGCs) is the major cause of progressive vision loss in numerous retinal diseases, including glaucoma and diabetic retinopathy (Chen et al. 2009; Nakayama et al. 2011). Hypoxia-induced apoptosis was also found in human hepatoma cells (Park et al. 2006). Nevertheless, mechanism by which apoptosis in hypoxia is induced remains to be elucidated.

### IP<sub>3</sub> receptors and apoptosis

Apoptosis, or programmed cell death is a process of the orderly disposal of unwanted cells without development of an inflammatory response, which is often associated with necrotic cell death (Joseph and Hajnóczky 2007). There is substantial evidence that calcium fluxes occur during most forms of apoptosis, and that inhibiting such fluxes protects cells from death (Hanson et al. 2004). Calcium efflux from ER and calcium accumulation into the mitochondria is linked to the effects of various apoptotic stimuli. IP<sub>3</sub> receptors may be critical regulators of apoptosis triggered by stimuli that engage ER and/or mitochondrial mechanisms (Fig. 3). The positive feed-forward between IP<sub>3</sub> receptor-mediated Ca<sup>2+</sup> release and mitochondria underlies the generation of Ca<sup>2+</sup> signals that accelerate the rate of cell death. The apoptosis-inducing cycle of Ca<sup>2+</sup> between IP<sub>3</sub> receptors and mitochondria can be initiated by a variety of mechanisms, including non-specific entry of Ca<sup>2+</sup> following membrane damage (Hanson et al. 2004).

Two different pathways for apoptosis, extrinsic and intrinsic, were described involving distinct upstream apical caspases: the extrinsic pathway involves activation of death receptors (e.g. FAS), while the intrinsic pathway involves the release of cytochrome c from the mitochondria. IP<sub>3</sub> receptors are involved in the intrinsic pathway for apoptosis (Joseph and Hajnóczky 2007).

Involvement of IP<sub>3</sub> receptors in apoptosis was demonstrated by reports that identified IP<sub>3</sub> receptor type 1 as a substrate of a caspase 3 during apoptosis (Hirota et al.

1999). One of the cleavage sites of IP<sub>3</sub> receptor type 1 is the DEVD consensus sequence for caspase 3, thereby generating a truncated 95-kDa protein containing all of the transmembrane domains and the channel pore (Hirota et al. 1999). The expression of the caspase-cleaved C-terminus of IP<sub>3</sub> receptor type 1 increased the rate of thapsigargin-mediated Ca<sup>2+</sup> leak and decreased the rate of Ca<sup>2+</sup> uptake into the ER, although it was not sufficient by itself to deplete intracellular Ca<sup>2+</sup> stores (Verbert et al. 2008). Cleavage of IP<sub>3</sub> receptor type 1 by caspase 3 resulted in inhibition of IP<sub>3</sub>-induced Ca<sup>2+</sup> release activity, in a digestion-dependent manner, an event that may possibly interfere with the IP<sub>3</sub>/Ca<sup>2+</sup> signaling pathway and intracellular Ca<sup>2+</sup> homeostasis within cells undergoing apoptosis. IP<sub>3</sub> receptor-knockout cells are less sensitive to apoptotic stimuli (Sugawara et al. 1997; Assefa et al. 2004), and anti-sense knockout of IP<sub>3</sub> receptor type 1 blocks cell death in Jurkat T-lymphoma cells (Jayaraman and Marks 1997). Boehning and coworkers (2003) showed that early in apoptosis, cytochrome c translocates to ER where it selectively binds IP<sub>3</sub> receptor, resulting in sustained, oscillatory cytosolic calcium increases. Early cytochrome c release increases IP<sub>3</sub> receptor function, resulting in augmented cytochrome c release that amplifies the apoptotic signal. Cytochrome c binding to IP<sub>3</sub> receptor depends on a cluster of glutamic acid residues within the C terminus of the channel. A cell permeant peptide derived from this sequence displaces cytochrome c from IP<sub>3</sub> receptor and abrogates cell death induced by staurosporine treatment of HeLa cells and Fas ligand stimulation of Jurkat cells (Boehning et al. 2005).

To allow transfer of calcium ions from the ER to the mitochondria, it is important that IP<sub>3</sub> receptors are localized very close to the mitochondrial uptake sites. As different IP<sub>3</sub> receptor isoforms exist, an important point to take into consideration is whether interaction with the mitochondria represents an isoform-specific behavior (for review see Decuyper et al. 2011a).

Type 1 IP<sub>3</sub> receptors are significantly involved in the apoptosis. Although playing crucial role in ER, after induction of apoptotic process they translocate also to the nucleus, where they form clusters, which most likely results from fusion of the nucleoplasmic reticulum and/or type 1 IP<sub>3</sub> receptor translocation to the nucleus (Ondrias et al. 2011). Inhibitors of the IP<sub>3</sub> receptor's calcium release – 2-aminoethoxydiphenyl borate (2-APB) and Xestospongine C – completely prevented Bax and caspase-3 mRNA increase after treatment with the apoptosis inducer set, and this reinforces the importance of the type 1 IP<sub>3</sub> receptor in the apoptosis of PC12 cells. From these results it has been proposed that after the apoptosis induction the amount of intranuclear calcium decreased dramatically due to the increase of calcium permeability of the nuclear calcium store vesicles. Therefore, increase of the calcium permeability may result from IP<sub>3</sub> receptors translocation to nuclei that can boost the calcium

transport through IP<sub>3</sub> receptors (Ondrias et al. 2011). It was already suggested that regulation of the IP<sub>3</sub> receptor clustering retunes IP<sub>3</sub> receptor sensitivity to IP<sub>3</sub> and calcium (Taufig et al. 2009).

Type 2 IP<sub>3</sub> receptors probably participate in the uranylacetate-induced nephrotoxicity. Uranylacetate increases gene expression and protein levels of the type 2 IP<sub>3</sub> receptors, which might, at least partially, contribute to the process of apoptosis (Kopacek et al. 2009).

The deregulation of apoptosome function has been reported for several types of cancer in which it can play a role in carcinogenesis and also in chemoresistance. Whether apoptosis or survival occurs, depends in part on the balance of pro-apoptotic or anti-apoptotic Bcl-2 proteins primarily at the mitochondrial membrane (Wenner 2012). In response to apoptotic stimuli, activation of the pro-apoptotic Bax and Bak causes increased mitochondrial membrane permeability and release of cytochrome c, a critical mediator in the commitment to cell death (Korsmeyer et al. 2000; Wei et al. 2000). The ability of pro-apoptotic Bcl-2 proteins to disrupt mitochondrial membrane integrity is held in check through heterodimeric interactions with anti-apoptotic members, including Bcl-2, Bcl-X<sub>L</sub>, and Mcl-1 (Decaudin et al. 1997; Yang et al. 1997). It was already shown that the anti-apoptotic protein Bcl-2 inhibits Ca<sup>2+</sup> release from the ER (Chen et al. 2004). One proposed mechanism involves an interaction of Bcl-2 with the IP<sub>3</sub> receptor (Rong et al. 2008). White and coworkers (2005) provide evidence that Bcl-2 proteins regulate the IP<sub>3</sub> receptor ER Ca<sup>2+</sup> release channel resulting in increased cellular apoptotic resistance. Anti-apoptotic Bcl-X<sub>L</sub> interacts with the carboxy-terminus of the IP<sub>3</sub> receptor and sensitizes single IP<sub>3</sub> receptor channel activity *in vitro* and *in vivo*, reducing ER Ca<sup>2+</sup> content and stimulating mitochondrial energetics. Bcl-2, Bcl-X<sub>L</sub> and Mcl-1, three structurally related anti-apoptotic proteins with differential expression and responses to different stimuli, bind with similar affinity to the carboxy-terminus of all three mammalian isoforms of the IP<sub>3</sub> receptor (Eckenrode et al. 2010). Bcl-2 and Mcl-1 mediated protection from apoptosis induced by staurosporine or etoposide was enhanced in cells expressing IP<sub>3</sub> receptors, demonstrating that their interactions with IP<sub>3</sub> receptor enable Bcl-2 and Mcl-1 to be fully efficacious antiapoptotic mediators. Overexpression of the antiapoptotic family member Bcl-X<sub>L</sub> has been reported to decrease the IP<sub>3</sub>-releasable Ca<sup>2+</sup> pool and reduce expression of IP<sub>3</sub> receptor type 1 in lymphocytes (Li et al. 2002). Oakes et al. (2005) found that Bcl-2 and IP<sub>3</sub> receptor type 1 physically interact at the ER membrane, and their binding is enhanced in DKO cells that lack Bax and Bak. The Bcl-2 interaction domain was identified in the central, modulatory region of the IP<sub>3</sub> receptor (amino acid 1389-1408) (Rong et al. 2008). Antiapoptotic Bcl-2 targets IP<sub>3</sub> receptor *via* its BH4 domain, thereby suppressing IP<sub>3</sub> receptor Ca<sup>2+</sup>-flux

properties and protecting against Ca<sup>2+</sup>-dependent apoptosis (Rong et al. 2009; Monaco et al. 2012). The recent findings mention that BH4-Bcl-2 and BH4-Bcl-X<sub>L</sub>, although very similar in primary sequence and secondary structure, act differentially on IP<sub>3</sub> receptors, IP<sub>3</sub>-induced Ca<sup>2+</sup> release and Ca<sup>2+</sup>-dependent apoptosis (Monaco et al. 2012). The regulation of IP<sub>3</sub> receptor can be mediated by the prosurvival kinase PKB, which protects cells from apoptosis-inducing stimuli that engage the IP<sub>3</sub> receptor/Ca<sup>2+</sup>-dependent cell death pathway (Szado et al. 2008). Under normal growth conditions with physiological PKB activity, IP<sub>3</sub> receptors are basally phosphorylated, transfer of Ca<sup>2+</sup> to the mitochondria is minimal, and ATP synthesis is promoted. In the absence of growth factors, PKB activity is reduced, and the level of IP<sub>3</sub> receptor is diminished. Thus, upon toxin application, Ca<sup>2+</sup> flux from the ER to the mitochondria is enhanced, causing permeability transition and cell death. In situations with enhanced PKB activity, such as during cancer, the level of IP<sub>3</sub> receptor phosphorylation is increased, thereby decreasing the flux of Ca<sup>2+</sup> from the ER to the mitochondria in response to an apoptotic stimulus (Szado et al. 2008).

It is of interest that pharmacological inhibitors of the IP<sub>3</sub> receptor, such as caffeine, Xestospongins C and 2-APB inhibited the growth of estrogen-dependent human breast cancer cell lines (MCF-7) stimulated by 5% fetal calf serum (Szatkowski et al. 2010). This discrepancy between the positive role of the IP<sub>3</sub> receptors in the process of apoptosis and inhibitory effect of IP<sub>3</sub> receptor's antagonist on the cancer cells still remains to be clarified.

### IP<sub>3</sub> receptors and autophagy

Apart from apoptosis and senescence, there are alternative outcomes in response to drug-induced DNA damage: cells may still be induced to die by nonapoptotic mechanisms, such as necrosis, autophagy and mitotic catastrophe. Autophagy is a tightly regulated pathway involving the lysosomal degradation of cytoplasmic organelles or cytosolic components. This pathway can be stimulated by multiple forms of cellular stress, including nutrient or growth factor deprivation, hypoxia, reactive oxygen species, DNA damage, protein aggregates, damaged organelles or intracellular pathogens (Kroemer et al. 2010). In stress situation, this process offers the cell a fresh pool of building blocks and thus has a prosurvival function (Klionski 2007).

The ER is not only involved in protein synthesis and maturation (including correct folding), but may also constitute a major source/scaffold of the autophagic isolation membrane (Hayashi-Nishino et al. 2009; Yla-Anttila et al. 2009). The unfolded protein response, the major ER stress pathway (Buchberger et al. 2010), is a potent stimulus of autophagy.

Hypoxia and anoxia (with oxygen concentrations < 3% and < 0.1%, respectively) both cause autophagy through a variety of different mechanisms. Hypoxia-induced autophagy depends on hypoxia-inducible factor, HIF, while anoxia-induced autophagy is HIF-independent (Majmundar et al. 2010; Mazure and Pouyssegur 2010).

It was already proposed that IP<sub>3</sub> receptors might be involved in the process of autophagy (Decuyper et al. 2011b). The IP<sub>3</sub> receptor interacts with Beclin 1 (an essential autophagy protein) indirectly, *via* Bcl-2. Beclin interacts with Bcl-2 and therefore may inhibit Bcl-2 anti-apoptotic function (Liang et al. 1999). Also, IP<sub>3</sub> receptors are suggested as key players in starvation-induced autophagy, since its sensitivity toward IP<sub>3</sub> is directly regulated by Beclin 1 during this process (Decuyper et al. 2011b). During starvation and upon cellular reduction of IP<sub>3</sub> levels (or antagonist binding), IP<sub>3</sub> receptor – Beclin 1 interaction is disrupted (Vicencio et al. 2009). It was already proved that knockdown or chemical inhibition of IP<sub>3</sub> receptors or depletion of IP<sub>3</sub> induces autophagy (Criollo et al. 2007; Khan and Joseph 2010). Beclin 1 binds to both, type 1 and type 3 IP<sub>3</sub> receptors, but the molecular determinants are slightly different (Decuyper et al. 2011b).

### Conclusion

Interest in the IP<sub>3</sub> receptors as an intracellular calcium channels rose during the last few years. Although these receptors are known to play a role in physiological processes, their importance is boosted in either development, or progression of several civilization diseases. Stress as an important civilization factor contributes to the development of several civilization diseases. From the current knowledge about the IP<sub>3</sub> receptors it is clear that modulation of these receptors can result to the development of pathological state, which strengthens the evidence of an involvement of IP<sub>3</sub> receptors in a stress pathology. Thus, further experiments should be focused on unraveling the mechanism of their involvement in development of these diseases as well as a potential therapeutic application in the treatment of neuronal, cardiovascular, oncological and other diseases.

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