

Involvement of Ca^{2+} in the Apoptotic Process – “Friends or Foes”

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Over the past two decades, the ubiquitous position of Ca^{2+} signaling in cellular apoptosis has been carefully investigated. The body of work described here highlights the complex role played by Ca^{2+} in generation, regulation and coordination of apoptosis.

The word ‘apoptosis’ is derived from a Greek word meaning ‘falling leaves’ and is used to describe a form of cell death occurring in multicellular organisms. It is defined as **programmed cell death** that involves altruistic suicide of individual cells in favor of the organism as a whole. Apoptosis is a tightly regulated, highly efficient and energy-requiring process which engages multiple cell signaling pathways. The apoptotic network components are genetically encoded and are usually in place in a cell ready to be activated by a death-inducing stimulus.^{1,3}

Apoptotic activity is desirable during organism development and morphological changes especially at the embryonic stage (for example the formation of digits), as well as during the activation of the immune system.⁴ Additionally, this process is essential in the homeostasis of cell number in organs in order to determine the cell numbers of the whole organism.⁵ Defects in apoptosis or apoptotic prevention can result in cancer, autoimmune diseases and spreading of viral infections, while neurodegenerative disorders, AIDS and ischemic diseases are caused or enhanced by excessive apoptosis.⁵

In contrast to the necrotic mode of cell death, apoptosis results in a loss of membrane integrity, swelling and disruption of the cell undergoing apoptosis without causing damage to the surrounding cells. Apoptotic cells can be characterized by stereotypical morphological changes which include cell shrinkage, loss of cell surface shape, chromatin condensation, DNA degradation, protein fragmentation, organelle disassembly and degradation of cells into small apoptotic bodies, which are disposed of by the immune system.¹

The apoptotic process can be driven by various stimuli from outside or inside the cell; in some cases, absence of survival factors is enough to propel a cell into apoptosis, but it can also be

stimulated by DNA damage, oxidative stress, treatment with cytotoxic drugs or irradiation, interruption in cell cycle signaling and death receptor ligands (TNF and Fas ligand). Cell signaling modulators, such as the universal protein kinase inhibitor **Staurosporine**⁶ (Fig. 1) and the tyrosine kinase inhibitor **Genistein**⁷ are usually used to induce apoptosis experimentally. Apoptosis occurs through two types of pathways: the death receptor pathway (extrinsic apoptotic pathways) and the mitochondrial pathway (intrinsic apoptotic pathways). The apoptotic signal involves activation of several powerful enzymes (mainly the Bcl and Caspase families). The Bcl family has a controlling role in maintaining the apoptotic signal, while the Caspase family plays a central role in cell protein degradation.^{8,9}

Ca^{2+} is a fundamental second messenger in cell signaling. Stimulation of cells by Ca^{2+} -linked signaling agents increases Ca^{2+} levels within the cell cytosol and the nucleus. This can modulate numerous Ca^{2+} -regulated enzymes which have

different subcellular localizations and create a wide range of spatial and temporal signals. Cytosolic Ca^{2+} has been implicated in the activity of Ca^{2+} /calmodulin-dependent protein kinase (CaMK), protein kinase A (PKA) and C (PKC), mitogen-activated protein kinase (MAPK), and phosphatidylinositol (PI)-3 kinase.¹⁰⁻¹⁴ Ca^{2+} metabolism plays a crucial role during the propagation of apoptosis. However, the role of Ca^{2+} in generation of apoptosis is controversial, in view of the fact that in different systems, Ca^{2+} was observed as both a survival supporter and apoptosis inducer. For instance, high K^+ depolarization and subsequent Ca^{2+} entry into the cytosol helps to sustain the survival of granule cells.^{15,18} On the other hand, compounds that chronically elevate intracellular Ca^{2+} , such as Ca^{2+} ionophores, have been observed to induce apoptosis in a variety of cell lines.¹⁹⁻²¹

Moreover, processing of the apoptotic signal, involves intracellular Ca^{2+} elevation as an obligatory factor for reactive oxygen generation and mitochondrial cytochrome *c* release, which are both considered as apoptotic triggers.²²

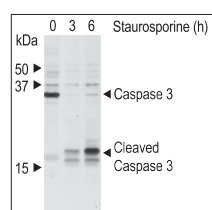
The following paper reviews the divisive role of Ca^{2+} as either a positive or negative generator of the apoptotic processes and its involvement in their progression.

Ca^{2+} Signaling: Suppressor and Promoter

Ca^{2+} /Calmodulin-Dependent Protein Kinase in Apoptosis

Ca^{2+} signaling is the center of multifunctional CaMK family regulation and activation. This family, which phosphorylates a large variety of substrates, has activation properties that allow it to discriminate between Ca^{2+} signals that differ in

Fig. 1: Staurosporine Induces Apoptosis in Jurkat Cells



Jurkat cells were grown to 70% confluency. $2 \mu\text{M}$ Staurosporine (#S-350) or vehicle were added for 3 or 6 hours. At the end of the incubation period, the cell extracts were probed for Caspase 3 and cleaved Caspase 3 with specific antibodies.

spike frequency, amplitude and duration.²³⁻²⁵ The CaMKI, II and IV subfamilies have been detected within the cell nucleus and suggested as mediators of nuclear Ca²⁺ signals. These kinases were implicated in control of gene transcription since they phosphorylate several transcription factors.²⁶ Several reports have indicated that both kinases negatively modulate apoptosis.^{27,28} For instance, CaMKII up-regulation increased the resistance of glioma cells to apoptosis and KN-93, a CaMKII inhibitor, annulled this effect. In cerebellar granule cells, Ca²⁺ entry through L-type voltage gated Ca²⁺ channels supported neuronal survival in a CaMKIV-dependent manner.^{29,30} During apoptosis, CaMKIV was reported to be negatively controlled by specific cleavage caused by caspase-3-like activity.³⁰ Moreover the serine-threonine protein kinase B, which is positively activated by the CaMKs-dependent

protein kinase pathway, plays an important role in neuronal survival.⁹ Similarly, in neuronal NG108 cells, expression of a peptide located in the noncatalytic N-terminal domain of CaMK-like kinase facilitated apoptosis (Fig. 2).^{31,32}

Nuclear Transcription Factor-κB in Apoptosis

Intracellular Ca²⁺ is a coordinating factor that positively regulates the activity of nuclear transcription factor-κB (NF-κB). This transcription factor is considered an anti apoptotic agent and plays a key role in cell survival by up-regulating expression of several apoptosis inhibitor genes and negative regulating the activity of caspase-3.³³⁻³⁵ Several reports illuminate the function of this intracellular pathway in prevention of apoptosis, for instance: In hippocampal pyramidal neurons, activation of glutamate receptors leads

to membrane depolarization and Ca²⁺ influx followed by activation of NF-κB.³⁶ In pancreatic β-cells, depolarization and L-type Ca²⁺ influx induce MEK/ERK dependent NF-κB related survival.³⁷

Protein Phosphatase - Calcineurin in Apoptosis

Cytosolic Ca²⁺ increase has a pivotal role in activation of the serine threonine Ca²⁺ - calmodulin-regulated phosphatase - calcineurin (also called protein phosphatase 2B). This phosphatase is a critical transducer of Ca²⁺ signals in most cell types particularly in the immune system and in the heart, due to its specific responsiveness to sustained low frequency Ca²⁺ signals. Calcineurin was suggested to be both a promoter and a supportive agent during apoptosis.

Ca²⁺ Overload Regulates Apoptosis

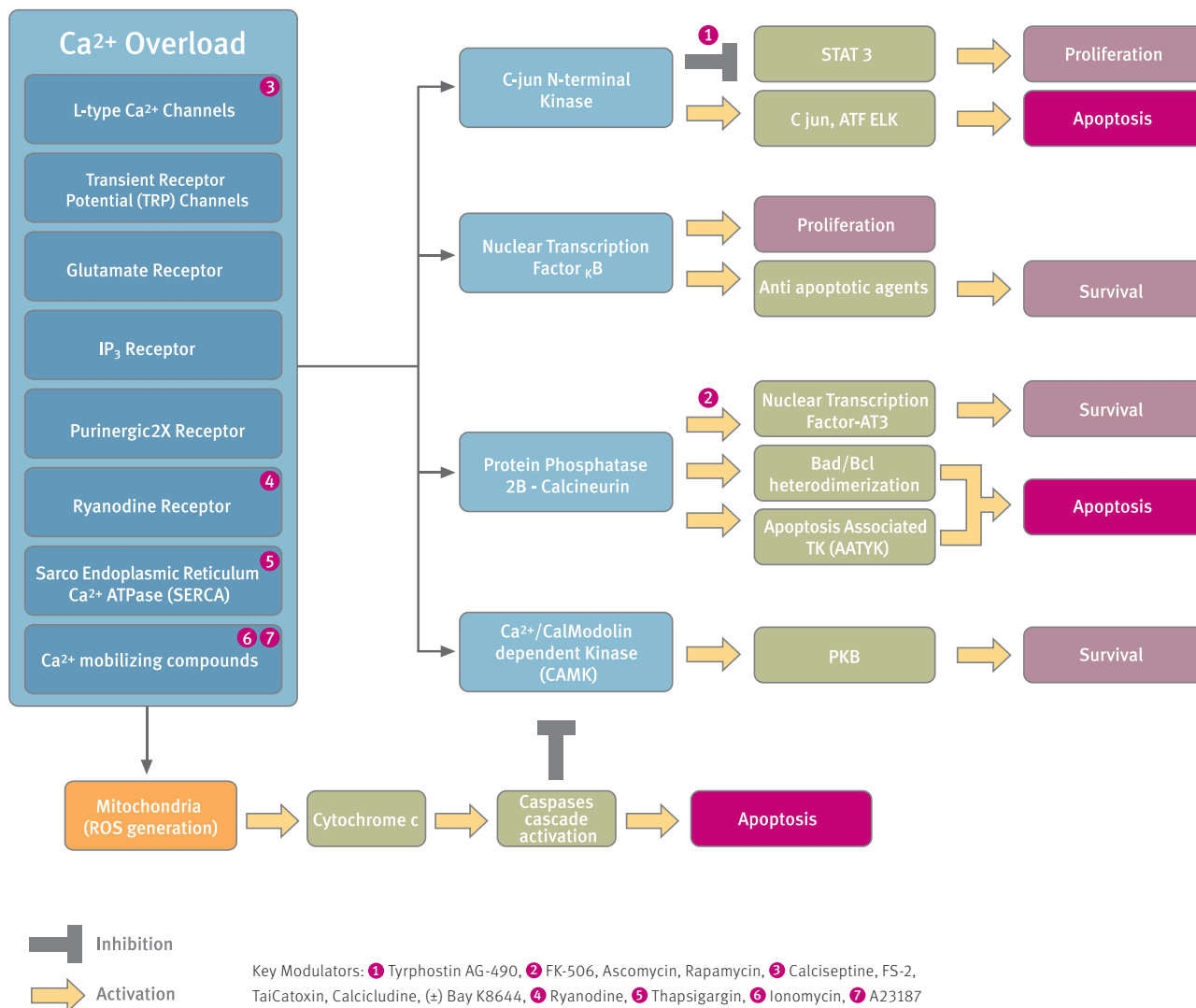
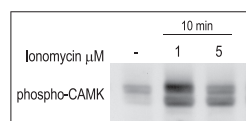


Fig. 2: Ionomycin Induces Ca²⁺ Calmodolin Dependent Kinase II (CAMK II) Phosphorylation in 3T3-L1 Cells



3T3-L1 cells were starved for 2h and then stimulated with 1 or 5 μM Ionomycin (#I-700) for 10 min. The cell extracts were blotted and probed with an antibody to phospho-(Thr₂₈₆)-CAMKII.

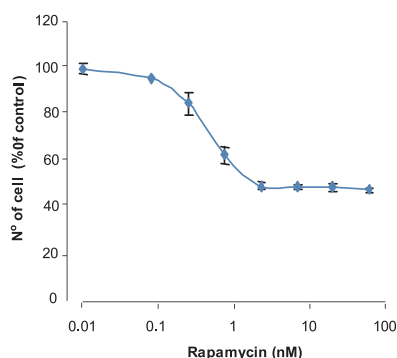
Sustained increase in cytosolic Ca²⁺ has been reported to induce apoptosis in some susceptible cell types through activation of calcineurin.³⁸⁻⁴⁰ High level forced activity of this phosphatase induced cytochrome *c*/caspase-3-dependent apoptosis in neurons⁴ and treatment with calcineurin inhibitors **Cyclosporin A** and **FK506** reduced susceptibility to apoptosis.⁴¹ Additionally, the apoptosis-associated tyrosine kinase (AATYK) is highly phosphorylated during the normal cell cycle. Activation of calcineurin in response to high extracellular K⁺ mediated L-type Ca²⁺ influx, dephosphorylates AATYK and leads to apoptotic cell death.⁴²

In hippocampal neurons, L-glutamate induced Ca²⁺ influx activates calcineurin which triggered transcription-independent apoptosis by mitochondrial targeting of BAD- a pro-apoptotic protein member of the Bcl-2 family. The Ca²⁺-induced dephosphorylation of BAD was correlated with its dissociation from the cytosol, translocation to mitochondria and enhancement of BAD Bcl-x_l heterodimerization promoting apoptosis.³⁹

Some reports suggest that the Ca²⁺-calcineurin pathway is critical in the progression of heart failure by regulating cardiomyocyte apoptosis. Moreover, induced ischemia caused more apoptosis in the hearts of transgenic mice expressing high levels of calcineurin than in the hearts of wild-type mice. Pharmacologic inhibitors of calcineurin activity block hypertrophy *in vivo* and *in vitro* and some of them (**Cyclosporine A**, **FK-506** and **Rapamycin**) (Fig. 3) were even suggested as candidates for treatment of heart disease.⁴³⁻⁴⁴

On the other hand, Ca²⁺-calcineurin activation by 2-deoxyglucose and **Staurosporine** prevents apoptosis of cardiac myocytes.⁴⁵ Calcineurin, which dephosphorylates the transcription factor NF-AT3, enables it to translocate into the nucleus, leading to prevention of apoptosis both *in vitro* and *in vivo*. Furthermore, calcineurin and protein kinase C suppressed apoptosis induced by tumor necrosis factor, through activation of NF-

Fig. 3: Rapamycin, a Calcineurin Inhibitor, Inhibits the Proliferation of WEHI B Lymphocyte Cells



WEHI B lymphocyte cells were treated with different concentrations of Rapamycin (#R-900) for 4 days; the number of live cells was measured by XTT cell proliferation assay kit, normalized to the control (100 %) and plotted against drug concentration.

κB in fibroblasts and lymphoma cells.⁴⁶ Finally, Ca²⁺-mobilizing compounds such as the Ca²⁺ ionophore **A23187** or the ER Ca²⁺ ATPase inhibitor **Thapsigargin**, which both trigger calcineurin activity, can either suppress or induce apoptosis in the same cells.⁴⁷ The reason for this dichotomy is unknown at present, but from the data summarized here we conclude that it depended on pathways downstream from calcineurin. This idea is supported by the fact that suppression or induction of apoptosis by opposing pathways such p38 and p44/42 mitogen-activated protein kinases are located downstream to Ca²⁺ activated calcineurin.⁴⁸

C-Jun N-terminal Kinase in Apoptosis

A distinctive feature of apoptosis is the requirement for *de novo* RNA synthesis. A key transcription factor for apoptosis is c-Jun, an immediate-early gene.⁴⁹ Ca²⁺ influx has been reported to be involved in c-jun N-terminal kinase (JNK) signaling pathway mediated IL-1β-induced apoptosis. Pharmacological blockers of L- and T-type Ca²⁺ channels, such as dihydropyridine, suppressed IL-1β-induced c-jun phosphorylation. Treatment with Ca²⁺ mobilizing compounds such as **A23187** and **Ionomycin** caused an amplification of IL-1β-induced JNK activation and lead to apoptosis.⁵⁰

Regulation of Intracellular Ca²⁺ Levels and Apoptosis

Control of cytosolic Ca²⁺ levels involves coordination of diverse intracellular and extracellular Ca²⁺ currents and it plays a vital role in the initiation of the apoptotic process.

Manipulation of cytosolic Ca²⁺ by different experimental methods may interfere with cellular process to induce apoptosis. For example, sustained increase of Ca²⁺ by Ca²⁺-mobilizing compounds such as the Ca²⁺ ionophore **A23187** (Fig. 4) and **Ionomycin** were reported to act as apoptosis inducers in some cell lines.^{47,51,8}

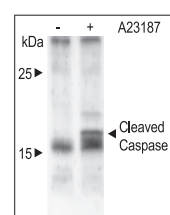
Ligand Gated Ca²⁺ Permeability

Some receptors that function as ligand gated Ca²⁺ permeable channels play a key role in Ca²⁺ homeostasis and have been formally implicated in mediating apoptosis. This role is reported to be taken by the purinergic receptor P2X (P2X R)⁵², inositol 1,4,5-triphosphate receptor (IP₃R),⁵³ and ryanodine receptor (RyR).⁵⁴ Whereas the P2XR_s are cell surface membrane Ca²⁺ channel, the IP₃R and RyR function as Ca²⁺ release channels in the ER membrane.

The P2XR_s are non selective cation channels with significant permeability to Ca²⁺. They induce signaling by allowing Ca²⁺ entry relative to the extracellular concentration of ATP. The first clue to the involvement of P2XR_s was the finding that high concentrations of ATP induced apoptosis by increasing the intracellular Ca²⁺ level.^{55,56} P2X1 channel proteins were up-regulated in thymocytes during dexamethasone-induced apoptotic cell death, while pharmacological inhibition of the P2XR1 protect thymocytes from apoptosis.^{52,55} P2X channels were reported to mediate the apoptotic ATP signal by Ca²⁺ overload in blood, brain and skin cells.⁵⁷

Intracellular organelles such as the ER and mitochondria have higher Ca²⁺ concentrations than the cytosol and they are considered cellular Ca²⁺ stores. Regulation of ER Ca²⁺ release controls many cellular functions. Enzymatic cascades which are dependent on Ca²⁺ concentration in the ER lumen integrate rapid Ca²⁺ signaling with long-lasting adaptive responses through modifications of protein synthesis and processing. Several

Fig. 4: A23187 Induced Apoptosis in Jurkat Cells



Jurkat cells were grown to 70% confluency. A23187 (#A-600) (2 μM) or vehicle were added to cell culture for 6 hours. At the end of the incubation, the cells were extracted and blotted. Apoptosis was probed by detection of cleaved Caspase 3 using Caspase 3 specific antibodies.

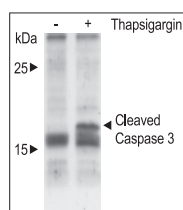
studies have suggested a central role for participation of ER and intracellular Ca^{2+} levels in the regulation of mitochondrial cytochrome *c* release that leads to induction of apoptosis.⁵⁸

IP_3 Rs are intracellular ion channels that mediate the release of Ca^{2+} from the ER and play a central role in control of intracellular Ca^{2+} homeostasis. Cytosolic IP_3 is produced in response to GPCR stimulated phospholipase C. Binding of IP_3 to the receptor induces transient opening of the receptor channel, facilitating Ca^{2+} flow from the ER lumen into the cytoplasm, thereby producing a transient elevation of cytosolic Ca^{2+} that in turn activates signal transduction kinases. Although IP_3 Rs are mostly located on the ER membrane, evidence suggests that they are also located on the plasma membrane, enabling the IP_3 R to mediate entry of extracellular Ca^{2+} into the cytoplasm. The IP_3 R has been suggested to be involved in adrenal corticosteroid related apoptosis^{58,59} through its downstream target, calcineurin.⁶⁰ Cytosolic Ca^{2+} levels are tightly controlled by IP_3 R channel activity. Intermediate Ca^{2+} levels facilitate the channel closure, and prevent Ca^{2+} overload.^{54,62}

As a response to a pathological stimulus, Ca^{2+} together with cytochrome *c* participates in self amplifying feedback to promote apoptosis.⁶¹ Low levels of cytochrome *c* prevent the closing of the IP_3 R channel during Ca^{2+} overload, leading to a burst of Ca^{2+} flow from the ER lumen into the cytosol. This in turn potentiates release of all the mitochondrial cytochrome *c*, which is sufficient to operate the caspase cascade to promote apoptosis.⁶¹

RyRs operate as Ca^{2+} release channels particularly in skeletal and cardiac ER, they are also located in the cell membrane of a variety of cells and are highly expressed in T- and B-lymphocytes. These channels provide a pathway for fast Ca^{2+} release from intracellular stores into the cell cytosol. In some cases, RyR channels transmit Ca^{2+} signals directly to closely associated mitochondria.⁵⁴ In

Fig. 5: Thapsigargin Induces Apoptosis in Jurkat Cells



Jurkat cells were grown to 70% confluency. Then 1 μ M Thapsigargin (#T-650) or vehicle were added for 6 hours. At the end of the incubation period, the cells extracts were detected for cleaved Caspase 3 with Caspase 3 specific antibodies.

pancreatic β -cells RyR2 was found to be involved in buffering of Ca^{2+} influx during stimulation with glucose or high K^+ . Blocking of Ca^{2+} flux through the type 2 RyR in these cells markedly increased apoptosis in calpain-dependent death pathways.⁶² On the other hand, depletion of intracellular Ca^{2+} stores and cytosolic Ca^{2+} overload via caffeine or **Ryanodine** activated RyR channels in CHO cells, observed to enhance translocation of the proapoptotic factor Bax into the mitochondrial membrane and induction of apoptosis.^{3,63,64}

The maintenance of ER Ca^{2+} stores is result of equilibrium between Ca^{2+} release and Ca^{2+} refill which involves opening of cation channels as well as activity of Ca^{2+} pumps. The Sarco Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) is a critical molecule which pumps Ca^{2+} from the cytosol into the ER lumen in order to maintain low intracellular Ca^{2+} .⁶⁵ Dysfunction of this pump by inhibition with **Thapsigargin** caused ER Ca^{2+} depletion and cytosolic Ca^{2+} overload, followed by extensive DNA fragmentation and apoptosis in a variety of cells (Fig. 5).⁶⁶ Increase of the cholesterol phospholipid ratio which occurs under pathological conditions changes the fluidity of the ER membrane and inhibits SERCA. This effect can be avoided by pharmacologic manipulations that block cholesterol trafficking to the ER.⁶⁷

Ca^{2+} Channels

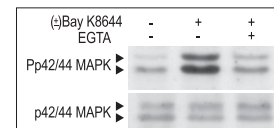
Ca^{2+} influx in nonexcitable cells regulates such diverse processes as exocytosis, enzyme activity, gene regulation and cell proliferation. Overload of extracellular Ca^{2+} can damage tissues, this effect may attribute to apoptotic effect caused by Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels and by Transient Receptor Potential (TRP) non selective ion channels.

L-type Ca^{2+} channels were exclusively upregulated as a response to chronic cell membrane high K^+ depolarization, leading to cell apoptosis in some cell lines.⁶⁸ Such an observation might be explained by the fact that a steep rise in intracellular Ca^{2+} is buffered to some degree by mitochondrial Ca^{2+} uptake. However, a continuous increase in cytosolic Ca^{2+} , such as in the case of channel upregulation, revokes the buffering capacity and causes dysfunction of these organelles.⁶⁹⁻⁷¹ On the other hand, high K^+ induced transmembrane Ca^{2+} -influx was observed to be essential in the maintaining of nerve and renal cells since blockage of Ca^{2+} influx might prevent cell survival. Moreover, the L-type Ca^{2+} channel agonist - (\pm)**Bay K 8644** is a potent agent that prevents nerve cell loss during low K^+ enhanced apoptosis (Figs.6-7).⁷²⁻⁷⁴

The TRPM2 channels which belong to the TRP family were shown to contribute to apoptotic cell death. This Ca^{2+} permeable channel was reported to be gated by adenosine diphosphate-ribose

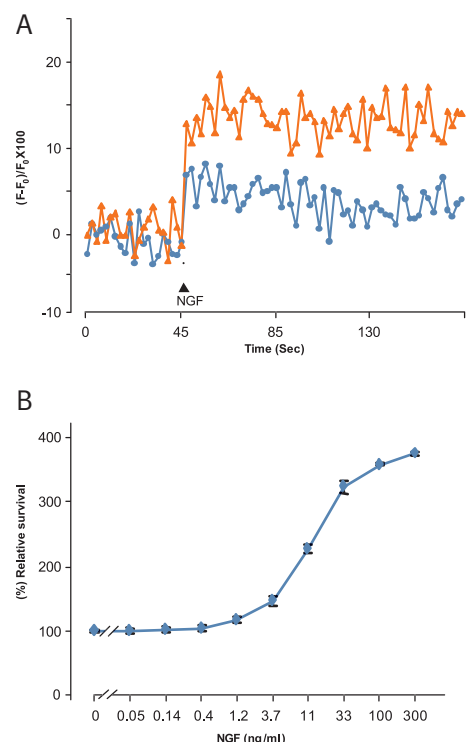
(ADP-ribose) and H_2O_2 which are both evoked during oxidative stress, resulting in a disruption of intracellular free Ca^{2+} homeostasis.^{75,76}

Fig. 6: (\pm)-Bay K 8644 Promotes Phosphorylation and Activation of Mitogen Activated Protein Kinase (MAPK- ERK 1/2) in Jurkat Cells



Cells were preincubated with or without 2 mM EGTA to chelate Ca^{2+} for 20 min then stimulated with 50 μ M (\pm)-Bay K 8644 (#B-350) for 10 min. The cell proteins were resolved by SDS PAGE and probed with Anti-Phospho-p42/44 MAPK (upper panel) or with Anti-p42/44 MAPK (lower panel).

Fig. 7: Nerve Growth Factor (NGF) Promotes L-Type Ca^{2+} Influx and Prevents Apoptosis in PC12 Cells



A. Ca^{2+} traces from Fura-2 AM loaded PC12 cells treated with 300 ng/ml mNGF 2.5S (Grade I) (#N-240) in presence (blue line) or absence (orange line) of 300 nM L-Type Ca^{2+} channel blocker- Calcicludine (#C-650). B. PC12 cells were grown in absence of serum (to induce apoptosis) and in presence of different concentrations of mNGF 2.5S. The cell survival as function of NGF concentration was measured after 4 days using the XTT-method calculated as relative percentage of the control without NGF and plotted against NGF concentrations.

Extracellular Ca²⁺ Sensing Prevents Apoptosis

Ca²⁺ sensing plays a key role in regulation of the calcitropic hormone PTH in tissues that maintain systemic Ca²⁺ homeostasis. The G protein-coupled extracellular Ca²⁺-sensing receptor (CaR) has been elucidated as responsible for recognizing and responding to small changes in extracellular Ca²⁺ level. It is becoming clear that the CaR also participates in a variety of non-Ca²⁺ homeostatic functions, such as control of ion channels, hormone secretion, and regulation of cell-cycle events.^{77,78} The role of CaR in apoptosis has been demonstrated when it was shown that a rise in extracellular Ca²⁺ protected AT-3 prostate carcinoma cells and CaR-transfected HEK293 cells from apoptotic cell death. Furthermore, anti apoptotic effect of extracellular Ca²⁺ was also observed in *c-myc* overexpressing/serum deprived cells (all the cells were shown to express CaR).⁷⁹ PI3K and p38 MAPK were suggested to be the downstream pathways that mediate the mitogenic response to extracellular Ca²⁺ activated CaR.⁸⁰ However, the downstream mechanism by which CaR prevents apoptosis has not been proved yet.

Ca²⁺ and Mitochondrial Apoptosis

Ca²⁺ plays a central role in mitochondrial function and mitochondrial regulation and it acts on several levels within the organelle to stimulate ATP synthesis. Although the luminal level of Ca²⁺ in mitochondria is high, most of the mitochondrial effects of Ca²⁺ require its entry across the double membrane into the matrix. The mitochondrial outer membrane was thought to be permeable to Ca²⁺, even though recent evidence suggests that the outer membrane Voltage-Dependent Anion Channel (VDAC) is a ruthenium red -sensitive Ca²⁺ channel. This Mitochondrial Permeability Transition (MPT) pore was also proposed to conduct non specific low molecular insults movement.^{81,82} However, dysregulation of mitochondrial Ca²⁺ homeostasis is now recognized to play an active role in apoptosis by switching the mitochondrial Ca²⁺ signaling to facilitate Ca²⁺-induced opening of the MPT pore.⁸¹⁻⁸³ For example, mitochondrial matrix Ca²⁺ overload enhances generation of Reactive Oxygen Species (ROS) which trigger the opening of the MPT pore and release of apoptogenic factors such as cytochrome c.⁵⁹ Because mitochondrial cytochrome c release during apoptosis is an “all-or-nothing” event occurring within a rapid time frame³, it has been suggested that the Ca²⁺ spike coordinates cytochrome c release by regulation of the VDAC opening.⁸⁴ This paradigm is supported by the observation that increased expression of this channel augments the mitochondrial sensitivity to ER-stress caused by **thapsigargin**-induced apoptosis.^{85-87,22}

The primary ROS manufactured by the mitochondria is superoxide (O₂⁻), which is converted to H₂O₂ either by spontaneous dismutation or by the enzyme superoxide dismutase.²² Apoptosis is reported to be accompanied by a burst of ROS which damages cellular components such as proteins, lipids, and DNA.⁸⁸ At the heart of understanding how Ca²⁺ can be both a physiological and a pathological effector of mitochondrial function is the fact that Ca²⁺ modulates mitochondrial ROS generation. The normal production of ROS is limited only to the amount required for microdomain cell signaling⁸⁹ whereas overload of mitochondrial Ca²⁺ during pathological conditions upregulates the production of QH - a ROS precursor, thereby causing an increase in ROS production. Alternatively, Ca²⁺ can stimulate nitric oxide generation,⁵ another generator of ROS, to generate apoptosis.⁹⁰

Conclusion

Apoptotic processes are an integral part of the life cycle: development, differentiation, proliferation, homeostasis, regulation and function of the body systems and in the removal of defective and therefore harmful cells. Thus, dysfunction or dysregulation of the apoptotic program is implicated in a variety of pathological conditions. Due to its importance in such varied biological processes, programmed cell death is a widespread phenomenon investigated in many areas of biology.

Pathological elevation in cytosolic Ca²⁺ concentrations (above 10 μM) is deleterious and can modulate apoptotic cell death through multiple mechanisms in a specific cells. Understanding the dual role played by Ca²⁺ signaling in initiation of apoptosis is relevant in many clinical situations such inherited muscle diseases and autoimmune diseases.^{91,5}

In view of data summarized here we conclude that different Ca²⁺ stimuli have distinct effects on stimulation of apoptotic process, dependent on cell type, cell environment, Ca²⁺ source and Ca²⁺ levels. Furthermore, cell apoptosis is strongly dependent on the biochemical situation or pathways that are already activated at the time of the apoptotic stimulus. Moreover, we speculate that Ca²⁺ can serve as either positive or negative trigger, but not as an exclusive factor, for driving apoptosis. The final result will be dictated by the cell situation, which is an integration of the levels, availability and location of certain cell components as well as equilibrium among different signaling pathways that take place during apoptosis modulation.

On the other hand, during progression of the apoptosis process, Ca²⁺ is an active factor that promotes the conversion of proapoptotic components into apoptotic conformation.

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Voltage-Gated Ca²⁺ Channel Blockers

ω-Agatoxin IVA	A-500
ω-Agatoxin TK	A-530
Calcicludine	C-650
Calciseptine	C-500
ω-Conotoxin GVIA	C-300
ω-Conotoxin MVIIA	C-670
ω-Conotoxin MVIIC	C-150
ω-Conotoxin SVIB	C-570
FS-2	F-700
ω-Grammotoxin SIA	G-450
PLTX-II	P-510
SNX-482	S-500
TaiCatoxin	T-800

Ca²⁺ Channel Blockers EconoKit

A Collection of Ca ²⁺ Channel Blockers	EK-400
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Voltage-Gated Ca²⁺ Channel Opener

(±)Bay K 8644	B-350
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Intracellular Ca²⁺ Mobilizers

A23187	A-600
Anhydriyandine	A-510
Cyclopiazonic Acid	C-750
Imperatoxin A	I-300
Ionomycin	I-700
Ochratoxin A	O-400
Ryanodine	R-500
tBuBHQ	T-220
Thapsigargin	T-650
Thapsigargin Epoxide	T-670

Intracellular Ca²⁺ Modulators EconoKit

A Collection of Ca ²⁺ Modulators	EK-100
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Cell Signaling

Anisomycin	A-520
Ascomycin	A-800
Cyclosporin A	C-900

FK-506 (Tacrolimus)	F-900
Oligomycin	O-300
PD 98059	P-260
Rapamycin	R-900
SB 203580	S-370
Staurosporine	S-350
Tryphestin AG-490	T-700
U0126	U-400

Ion Channel Antibodies

Anti-IP3R1	ACC-019
Anti-Ca _v 1.2 (a1C)	ACC-003
Anti-human Ca _v 1.2 (a1C)	ACC-022
Anti-Ca _v 1.2a (a1C Cardiac)	ACC-013
Anti-Ca _v 1.3 (a1D)	ACC-005
Anti-P2X1	APR-001
Anti-P2X2	APR-003
Anti-P2X3	APR-016
Anti-P2X4	APR-002
Anti-P2X5	APR-005
Anti-P2X6	APR-013
Anti-P2X7	APR-008
Anti-P2X7	APR-008-F
Anti-P2X7	APR-004
Anti-TRPC1	ACC-010
Anti-TRPC3	ACC-016
Anti-TRPC4	ACC-018
Anti-TRPC5	ACC-020
Anti-TRPC6	ACC-017
Anti-TRPV1	ACC-030
Anti-TRPV4	ACC-034
Anti-TRPV5	ACC-035
Anti-TRPV6	ACC-036

Related Products

Compound	Product #
Nerve Growth Factors	
mNGF 2.5S (Grade I)	N-240
mNGF 2.5S (Grade II)	N-100
mNGF 7S	N-130
hβ-NGF	N-235

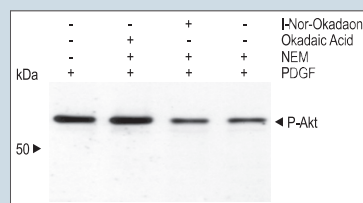
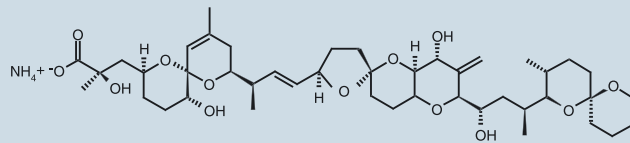
Okadaic Acid Protein Phosphatase Inhibitors

Okadaic acid is a polyether compound, isolated from a black sponge, *Halichondria okadaei*. It is previously demonstrated as a non-TPA-type tumor promoter and then as a cell-permeable, potent and specific inhibitor of serine/threonine protein phosphatases 1 and 2A. In higher concentrations Okadaic acid induced apoptosis (IC₅₀ = 0.5 μM).¹⁻⁴

Okadaic acid down-regulates the TNF receptor, induces the release of Ca²⁺ from intracellular stores in ECV304 endothelial cells and inhibits GH₄ cell proliferation in a concentration-dependent manner (IC₅₀ = 5 nM).^{5,6}

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Okadaic Acid (#O-800 or #O-900) prevents dephosphorylation of Akt by Protein Phosphatase 2A (PP2A) in 3T3-L1 cells. 3T3-L1 cells were grown to confluency then serum starved for 18 h. For *in vivo* phosphates activity measurement, the growth medium was replaced with HENK'S buffer and the cells were preincubated for 30 min in the presence or absence of 100 nM Okadaic Acid or 100 nM I-Nor-Okadaone (#N-750) (negative control). For PP2A activation the cells were preincubated with 20 mM NEM as indicated in the picture. Then the cells were stimulated for Akt phosphorylation with 5 ng/ml PDGF AA for 30 min. The Akt phosphorylation level was detected using Ser₄₇₃ phospho-specific antibody.