

Involvement of Ion Channels in Apoptosis

Alon Meir, Ph.D.

Involvement of ion channels in apoptosis is linked to critical aspects of this complex cellular process such as coordination of the Ca^{2+} signal, cell shrinkage and mitochondrial integrity. The body of work described below demonstrates the large variety and critical involvement of ion channels in this cellular process. It highlights the emerging roles discovered for this diverse group of membrane proteins in non excitable tissue physiology and pathophysiology.

All cells of multi-cell organisms contain a genetic program ready to be activated for their death, with the actual execution of this program called apoptosis. Apoptotic pathways include several proteins with powerful enzymatic cell degrading potential (mainly belonging to the Bcl-2 and caspase families, and cytochrome C). These proteins are latent most of the cells' life (either by compartmentalization or by co-factor inhibition). The latent proteins are activated once a "death" signal has been consolidated by the cells' existing signaling mechanisms. The program also includes elements of environmental awareness as the degraded cells remaining are packed and disposed of (for review see reference 1).

Apoptosis is a very important factor in an organism's development, especially at the embryonic stage and in normal renewing tissue homeostasis (for example in epithelial and red or white blood cells).² On the other hand the same mechanism is activated in pathophysiological conditions such as neuronal or cardiac ischemia (see for example³). In addition, apoptosis prevention might be one of the necessary steps for the transformation of normal to cancerous cells.⁴

Time course and stages of apoptosis progression includes a number of well-defined cellular and morphological changes.⁵ These include cell shrinkage or apoptotic volume decrease (AVD), nuclear condensation, DNA fragmentation and formation of sub-cellular apoptotic bodies, that undergo phagocytosis by neighboring cells. Processing of the apoptotic stimulus, involve Ca^{2+} signals that lead to disruption of mitochondrial membranes. The latter leads to the release of proapoptotic factors, activating the caspase machinery that degrades the cell.⁶ In parallel to stimulus processing, the cell shrinks, involving the participation of ion channels in the secretion of salts.⁵

Methods of experimental induction of apoptosis usually mimic certain cellular process that serve as an apoptosis induction mechanism. Nevertheless, many factors bypass certain cellular pathways that are upstream of the point where they interfere with cellular function. For example, the build-up of cytoplasmic Ca^{2+} concentration probably plays a key role in the convergence of the initial apoptotic signal.⁶ Many apoptotic insults involve different ways of chronic cytoplasmic Ca^{2+} elevation. Some include inhibition of cytoplasmic Ca^{2+} sequestering into ER, like **Thapsigargin**,⁷ while others may perforate the membrane specifically for Ca^{2+} by the use of a ionophore, like **A23187**.⁸ Apoptotic inducers may be as diverse as global kinase inhibition (**Staurosporine**)⁹ or Hydrogen Peroxide (H_2O_2), mimicking the reactive oxygen species (ROS)³ produced during apoptosis. However, in many cell types apoptosis is signaled by external ligands of death receptors (such as TNF- α or Fas ligands), which in many cases were used to induce experimental apoptosis (see table).¹⁰

Ion channels are integrated membrane proteins which are exposed on both sides of the membrane. These proteins allow specific ions to cross membranes down their electrochemical gradient, usually in response to an appropriate stimulus. Ion channels are known to control a number of cellular processes including:

1. Change in a given ion concentration.
2. Changes in membrane potential (due to their charge being translocated across the membrane).
3. Changes in the cell osmotic balance.
4. Mediators of a cell's response to a wide range of both intra- and extra-cellular chemical and/or physical stimuli.¹¹

Ion channels that have been implicated in apoptosis as regulators of local and global ion content belong to a wide range of channel

families. The channels may differ regarding the ions toward which they are permeable, the stimulus that opens the channel, localization of the channel (i.e. plasma membrane, mitochondria etc.) and their overall role as pro- or anti-apoptotic agent (see table). These channels were reported to contribute to one of the following:

1. Cytosolic Ca^{2+} increase, leading to activation of Ca^{2+} dependent apoptotic machinery.
2. Ion fluxes between mitochondria and cytosol, resulting in either initiation of apoptosis or protection of apoptosis stimulated cells.
3. Permeation to large molecules such cytochrome C, facilitating their translocation from mitochondria to cytosol.
4. K^+ and Cl^- efflux (from the cytosol to the extracellular space), leading to and accompanying water efflux and cell shrinkage, also leading to reduction in cytosolic K^+ and relief of apoptotic inhibition.

Below, we will describe the possible roles played by specific ion channels in mediating processes crucial for the apoptotic signal propagation, either as mediators of the insult or as effectors activated by upstream stimuli.

Ca^{2+} permeable channels on plasma membrane

Ca^{2+} homeostasis plays a crucial role in apoptosis. It involves the generation, amplification and coordination of a diversity of Ca^{2+} signals by means of interplay between Ca^{2+} stores.⁶ One such Ca^{2+} store is the extracellular fluid, which could be accessed by the opening of Ca^{2+} permeable channels located on the plasma membrane. This role in apoptosis is reported to be taken by either P2X (ionotropic ATP receptor activated

by binding of extracellular ATP resulting in the opening of a non selective, Ca²⁺ permeable cation channel), TRP (Transient Receptor Potential, non-selective cation channels, activated by different stimuli) or by Ca_v (voltage dependent Ca²⁺ selective) channels.

P2X channels are the direct receptor of the apoptotic insult (ATP¹⁷) as well as the transducer of the ATP signal into elevation in Ca²⁺ in the cytoplasm. Therefore, these channels demonstrate the role played by Ca²⁺ influx as one of the first events which eventually lead to cell death. P2X7 channel was reported to mediate ATP induced apoptosis in blood and bone marrow derived,¹³⁻¹⁷ as well as in skin cells.¹⁸ The specific contribution of the P2X7 channel was assessed both with specific pharmacology and by the use of a natural dominant negative mutant. In thymocytes, ATP induced apoptosis, which resulted in elevated P2X1 channels.¹⁹ In neurons, P2X4 mRNA levels were elevated together with P2X7 following a starvation insult.²⁰ This demonstrates the difference between cells that readily express this ATP receptor channel (blood cells), making them vulnerable to external ATP, compared to cells that express the channel only in response to stress (neurons).

L-type voltage dependent Ca²⁺ (Ca_v1 subfamily) channel currents in chromaffin cells, were instantly upregulated as a response to high extracellular K⁺ insult (depolarization of the cell membrane among other effects), leading to apoptotic cell death.²¹ Such an observation places these voltage activated channels as first order effectors transforming membrane depolarization into Ca²⁺ influx that results in apoptosis. However, a longer process involving elevated channel expression levels was suggested in physiological context. Ca_v1 channels have been implicated as apoptosis mediators in insulin producing cells in response to diabetic patient's serum.²² In pancreatic β cells Ca_v3 (T-type) channels were upregulated in response to cytokines (that are probably contained within diabetic patients' serum).²³

TRP channels were suggested to be involved in apoptotic Ca²⁺ signaling and as direct receptors of certain insults. Two channels belonging to the TRPM subfamily of TRP cation channels were shown to mediate apoptotic insults, by conducting Ca²⁺ into cells: TRPM2 in tumor cell lines^{24,25} and TRPM7 in neurons that were protected pharmacologically from other forms of cell death.³ The latter was motivated by failure to protect against neuronal ischemic insults by blocking the excitotoxicity mediators Ca_v and ionotropic glutamate receptors. The TRPM Ca²⁺ currents develop slowly in response to apoptosis induction. However, the slow time course might represent the build-up of ROS in response to the oxygen-glucose deprivation insult, since TRPM channels are gated directly by H₂O₂.³

In experiments where apoptosis was stimulated by particulate matter (PM), mimicking air pollution, the vanilloid receptor (TRPV1 channel) was found to mediate apoptosis in epithelial airways cells and sensory neurons.²⁶ External expression of the *Drosophila* TRPL channel (homologue of the TRPC channel subfamily), supported apoptosis in prostate cancer cell line.²⁷

The initial build-up of cytosolic Ca²⁺ described above, may serve as a messenger for many cellular agents and process. For the purpose of this review we should consider Ca²⁺ activated channels, such as K_{Ca} and Cl_{Ca} channels on the plasma and/or mitochondria and the IP₃ receptor (IP₃R) on the ER membrane.

Intracellular channels on ER and mitochondrial membrane

Ca²⁺ signal convergence results in activation of intracellular channels that leads to cytochrome C release from mitochondria.⁶ This section will focus on a few more Ca²⁺ channels as well as on channels contributing to mitochondrial integrity.

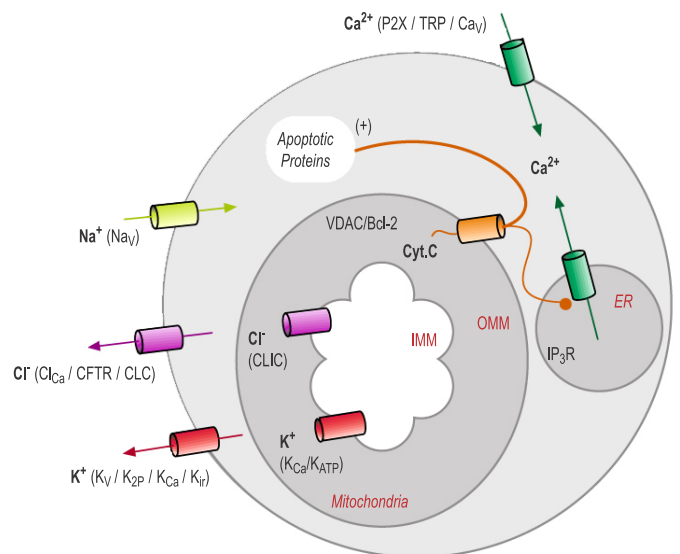
IP₃R are Ca²⁺ activated Ca²⁺ channels, on the ER membrane, which facilitate Ca²⁺ flow from ER to the cytoplasm, once the cytoplasmic Ca²⁺ level is intermediate (i.e. a bell shaped dependency in which the channel is closed both when Ca²⁺ is in the resting level and during Ca²⁺ overload) and plays a key role in Ca²⁺ homeostasis. It is also recruited to participate in an inter-organellar Ca²⁺-cytochrome C self amplifying signal, which is critical for downstream apoptotic mechanisms.^{6,28} Cytochrome C can enhance IP₃R activity as it binds the IP₃R with very high affinity and prevents

channel closure during Ca²⁺ overload.²⁸ During apoptosis, small amounts of mitochondrial cytochrome C translocate to the ER (a process that depends on IP₃R activity) and potentiate IP₃R. This leads to a bursting Ca²⁺ overload, which coordinates cytochrome C release from all the cell's mitochondria, leading to activation of the caspase cascade.²⁸

The events linking the ER released Ca²⁺ to cytochrome C release happen in the mitochondria and involve channels on both the outer and inner mitochondrial membranes (OMM and IMM respectively). The sequence of events and the exact roles played by particular channels are not fully resolved. A Ca²⁺ channel sensitive to Ruthenium Red blockade was recently suggested as the Ca²⁺ route into mitochondria. However, the exact role of the channel in apoptosis as well as its molecular identification is not yet clear.³⁰ In addition, other channels are involved in the maintenance/disruption of IMM potential and the release of cytochrome C from its "resting" location (between OMM and IMM) into the cytosol.

Bcl-2 proteins, an established apoptotic protein family, are suggested to act both as modulators of other channels and as channels themselves.^{29,31} The latter activity carried out by the Bcl-2 protein BAX, was suggested to generate the transducer of cytoplasmic Ca²⁺ elevation into cation currents disrupting the IMM potential. Other Bcl-2 proteins have been suggested to form the channel that conducts cytochrome C out of the mitochondria (for further details see³²).

However, it is more accepted that VDAC1 channels on the OMM (which are modulated by Bcl-2 proteins) actually mediate cytochrome C translocation.³³ VDAC channels are big pores, with complex behavior that are usually considered as voltage dependent anion channels. VDAC



A schematic sketch illustrating the ion channel types participating in apoptosis. See text and table for details.

Summary of Ion Channel Involvement in Apoptosis

Channel ^{reference}	Channel identification *	Method to induce apoptosis	Apoptotic interactions	Examined in cell type	Channel and/or apoptosis pharmacology **
------------------------------	--------------------------	----------------------------	------------------------	-----------------------	--

Ca²⁺ permeable channels on plasma membrane

P2X1 ¹⁹	PCR, Ab	DEX glucocorticoid, SEB super antigen.		Thymocytes (but not T-lymphocytes).	
P2X7 ^{13,18,20}	-/- mice, DN, SPh	ATP, Glucose deprivation.	Activates SPAK. Co-localized with caspase-3.	Infected macrophages, mouse macrophages (BAC1), Dendritic cells, D2SC/1, Human T-lymphocytes, Human Keratinocytes, Cerebellar granule cells (also P2X4).	BzATP (blocker), Oxidized ATP (opener/ inducer).
Ca _v 1 (L-type) ^{21,22}	UM, NSPh	Serum from diabetic patients, extracellular K ⁺ elevation.		GH3, insulin secreting and Chromaffin cells.	DHP (blocker) (±)BayK 8644 (opener/ inducer).
Ca _v 3 (T-type) ²³	UM, NSPh	Cytokines		Pancreatic cells	Ni ²⁺ (blocker).
TRPM2 ^{24,25}	AsOn, DN	H ₂ O ₂ , TNFα.	H ₂ O ₂ activates the channel	Insulinoma: RIN-5F, U937 and TF1 cells.	
TRPM7 ³	Specific RNA silencing	O ₂ ; glucose deprivation.	ROS activated channel.	Cortical neurons.	Gd ³⁺ (blocker).
TRPV1 ²⁶	-/- mice, SPh	Particulate matter.	cAMP and PKA, downstream of channel activation.	Epithelial airways cells, trigeminal ganglion neurons.	Capsazepine (blocker), Capsaicin (agonist, inducer).

Ca²⁺ channel on ER membrane

IP ₃ R-1 ²⁸	Ab	Staurosporine.	cytochrome C potentiate the channel.	HeLa and PC12 cells.	
-----------------------------------	----	----------------	--------------------------------------	----------------------	--

Channels on mitochondrial membranes

VDAC1 ³³	Ab		Release of cytochrome C, regulated by Bcl-2.	Yeast mitochondria.	
VDAC2 ³⁴	-/- mice	Staurosporine.	Protective, Inhibit BAK.	Mouse liver mitochondria.	
CLIC4 ^{35,36}	HE, AsOn	Etoposide.	Upregulated by p.53	Keratinocytes.	
K _{ATP} ^{37,38}	UM, SPh	Perfused hearts .	Protective	Cerebellar granule neurons, cardiomyocytes.	Diazoxide (opener/protector).
K _{Ca} 1.1 (BK) ³⁹	Ab, SPh	Perfused hearts.	Protective	Cardiomyocytes.	Charybdotoxin, Iberitoxin, Paxilline (blockers), NS-1619 (opener/protector).

Cl⁻, Na⁺ and gap junction channels on plasma membrane

CLCA2 ^{48,49} (also CLCA1) ⁴⁹	HE, PCR	Serum deprivation, Cyclosporin A		Mammary gland cells, HC11, Human hepatoma cell line.	
CFTR ⁵⁰	HE of w.t and DN	Cycloheximide, etoposide	Mediates acidification	Mouse mammary C127 cells.	
Swell activated CLC ^{52,53}	UM	Perfused heart		Heart.	
CLC ⁵¹	UM	CD95	Regulated by CD95.	Jurkat T cells.	Glibenclamide, IAA, DIDS, DPC (blockers)
Na _v ⁵⁴	UM, NSPh	Fas ligand	Control cell shrinkage	Jurkat T cells.	STX (blocker).
Cnx-32, Cnx-43 ⁵⁵	HE	Many apoptotic stimuli		HeLa cells.	

K⁺ channels on plasma membrane

K _v 1.3 ^{10,41}	SPh	Fas ligand, FSH.	Caspases increase channel activity.	Jurkat T cells, Porcine granulose cells.	Margatoxin, Charybdotoxin (blockers) ShK (blocked channel but not apoptosis).
K _v 2.1 ⁹	DN, HE	DTDP, Staurosporine.		Cortical neurons.	
K _v 11.1 (HERG) ⁵⁶	Ab	H ₂ O ₂ , TNFα	Conductance promotes apoptosis. Expression recruits TNFR1	HERG expressing and non expressing tumor cells.	Dofetilide (blocker).
K _{Ca} 1.1 (BK) ⁵	SPh	Staurosporine, FCCP, SNAP.		Human and rat smooth muscle cells.	Iberitoxin (blocker).
K _{Ca} 3.1 (SK4) ⁴²	SPh	A23187 (Calcimycin)		T- lymphocytes , thymocytes, erythrocyte.	Charybdotoxin (blocker) Iberitoxin, Apamin, Agitoxin no effect.
K _{2p} ⁴⁵	UM, NSPh	H ₂ O ₂ .		One-cell mouse embryo (zygote).	Quinin (blocker) Iberitoxin, Dendrotoxin, Charybdotoxin no effect.
K _{2p} 4.1 (TRAAK) ⁴⁴	Ab	Arachidonic acid.	Arachidonic acid activates the channel.	Retinal (RGC-5), PC12 cells.	
K _{2p} 3.1 (TASK-1), K _{2p} 9.1 (TASK-3) ⁴³	DN, HE	Time in culture, Staurosporine.		Cerebellar granule neurons.	Ruthenium red (blocker).
K _v 1.1 (ROMK1) ⁷	HE	Time in culture		Rat hippocampal neurons.	

*DN= dominant negative mutant, UM = unidentified molecularly, HE = heterologous expression, Ab = specific antibody, SPh = specific pharmacology, NSPh = non specific pharmacology, AsOn = antisense oligonucleotide, PCR = Polymerase Chain Reaction, w.t. = wild type, **blocker = of both channel and apoptosis, opener/inducer = of channel/apoptosis.

channels, however, may play a protective role, as was shown for the VDAC2 isoform that is an inhibitor of Bak oligomerization and therefore it helps keep this proapoptotic protein in chains.³⁴ IMM depolarization is an important factor leading to VDAC1 priming to serve as cytochrome C translocator. IMM depolarization is caused by dissipation of H⁺ gradient, probably with the participation of the Cl⁻ intracellular channel, CLIC4.^{35, 36}

The dissipation of H⁺ gradient could be compensated for by activation of K⁺ selective channels on the IMM. These include an ATP sensitive (K_{ATP}, which is a channel formed by a yet unidentified protein)^{37, 38} and a Ca²⁺ sensitive (K_{Ca}1.1)³⁹ K⁺ channels. Mitochondrial matrix ATP levels drop and Ca²⁺ load activates the two channels, respectively, with these two events accompanying H⁺ gradient dissipation. This protective pathway (involving a not fully resolved mechanism) is suggested to contribute to protective physiological phenomenon and is exploited by specific pharmacological augmentation of K_{ATP} to protect hearts during surgery.³⁸

Thus, ion channels localized on intracellular organelle membranes play a key role in consolidation of the initial apoptotic signal. However, other channels may serve to dissolve such signals and protect cells challenged by an insult.

Plasma membrane K⁺ efflux channels

K⁺ homeostasis, (high cytoplasmic compared to the low external concentration) is disrupted during apoptosis. That is, K⁺ ions must leave the cell as an obligatory step in the apoptotic pathway. However, the exact mechanism by which K⁺ depletion promotes apoptosis is not fully resolved. The activity of some apoptotic enzymes was suggested to depend on low K⁺, perhaps explaining the necessity of K⁺ depletion in apoptosis. K⁺ depletion is strongly linked to cell shrinkage, a hallmark of, but also a possible active contributor to apoptosis.⁵ Therefore, up-regulation of the activity of K⁺ channels probably lie in the basis of increased K⁺ outflow, critically contributing to apoptosis.^{5, 40}

Several reported observations suggest that any K⁺ channel is good for the job of emptying the cell of its K⁺ ions. This implies that following apoptotic stimulation the cell recruits any available K⁺ channels, in the context of the apoptotic stimulus and possible changes in expression profiles. Three arguments support this idea:

1. K⁺ channels belonging to four structurally distinct channel families were reported to be involved in apoptosis (see table).
2. There is correlation between specific channel blockers and prevention of apoptosis

in different cell types.^{41, 42}

3. Heterologous expression of several types of K⁺ channels primed the same cell type (for example, cultured hippocampal neurons^{7, 43}) to apoptosis.

It is hard to correlate the specific K⁺ channel that is activated with a specific apoptotic insult. For example, Staurosporine induced apoptosis that was mediated by K_V2.1 channels in neurons,⁹ but in smooth muscle cells it was mediated by K_{Ca}1.1 channels. The latter mediated also other apoptotic stimuli in these cells.⁵

Phenotypic differences between cell types might be correlated to the specific K⁺ channel they are using in apoptosis. For example, while expression of the two pore domain channel K_{2p}9.1 (TASK-3), is essential for apoptosis in cerebellar granule neurons,⁴³ the voltage dependent K_V2.1 carries out the same task in cortical neurons.⁹ Most granule neurons are eliminated with cerebellum maturation shortly after birth and die in culture, while cortical neurons can be maintained for long periods in primary cultures. This difference may be attributed to the particular K⁺ channel that the cell uses to mediate apoptosis. It is interesting to note that overexpression of K_{2p}9.1 in hippocampal neurons induced apoptosis and arachidonic acid activated K_{2p}4.1 (TRAAK) to induce apoptosis in PC12 cells.^{43, 44}

Since K⁺ channels are crucial to apoptosis, specific channel blockers are very indicative tools, especially as these also blocked the death process (see table).^{41, 42} For example, **Iberiotoxin**, a specific blocker of K_{Ca}1.1 channels blocks apoptosis in smooth muscle cells,⁵ but failed to influence such a process in T-lymphocytes⁴² and in a mouse embryo cell.⁴⁵ In these two cell types apoptosis was carried out by another Ca²⁺ dependent channel, K_{Ca}3.1 or by a K_{2p} channel, respectively. **Charybdotoxin**, a less specific K⁺ channel blocker, was effective in blocking apoptosis in granulose cells as did **Margatoxin**, pointing to the involvement of K_V1.3 channels.⁴¹ In T-lymphocytes Charybdotoxin also blocked apoptosis, but Iberiotoxin, **Apamin** and **Agitoxin** failed to do so, ruling-out K_{Ca}1.1, K_{Ca}2 and K_V1.3, strongly hinting at the involvement of K_{Ca}3.1.⁴²

Recently, a mechanism that links K⁺ efflux to the upstream apoptotic stimulus and mitochondrial disruption, was described in neurons.⁴⁶ It involves nitric oxide (NO) increase arising from the stimulus, leading to Zn²⁺ increase and activation of p38 MAPK. The latter activates K⁺ efflux directly, that again was found to be an obligatory step leading to cell death.⁴⁷

It also should be mentioned that compensatory mechanisms might be activated. For example, K_V1.3 was reported to mediate apoptosis but K_V1.3 null mice thymocytes exhibited normal apoptosis that was dependent on Cl⁻ channels.⁴⁷

Plasma membrane Cl⁻ and Na⁺ channels

Cl⁻ channels must be active during cell volume changes to allow the net salt transport (NaCl or KCl) that is accompanied by water movement. In epithelial mammary cells, the expression of two Ca²⁺ activated Cl⁻ channels is changed upon apoptosis induction. CLCA1, which is the dominant isoform under "normal" conditions, is down-regulated while CLCA2 expression is upregulated.⁴⁸ The latter is in agreement with the disrupted expression of this channel in apoptosis resistant tumor cells. CLCA channels were also shown to be a necessary component in apoptosis of heptoma cell line, challenged with Cyclosporin A.⁴⁹ These observations point to the importance of CLCA channels (or their loss) in apoptosis and cancer.

Epithelial cells normally secrete Cl⁻ alongside cations and a mutated Cl⁻ Channel (CFTR) is the basis of a severe epithelial disease, cystic fibrosis (CF). CFTR channels were shown to support apoptosis in mouse mammary cells, where the natural mutant channel (causing the disease), failed to support such a process.⁵⁰

One role of the CFTR channel is to create an acidic environment, which allows the DNA of dying cells to be fragmented into small pieces. When the CFTR protein cannot fulfill this task, there is an accumulation of mucus with large DNA fragments. This may be the result of a process in which CF cells are not dying "properly" (epithelial tissue must renew constantly), due to a mutation in a channel that is needed for apoptosis. A similar mechanism was suggested in Jurkat T cells, where unidentified Cl⁻ channels also mediate apoptosis and their block prevents cell acidification.⁵¹

Another, as yet unidentified, Cl⁻ channel that is activated upon cell swelling plays a role in cardiac cell apoptosis during heart surgery and/or transplantation. However, there are contradicting reports regarding its role(s) as proapoptotic or protective channel. While administration of the Cl⁻ channel blockers NPPB and IAA-94 seemed to enhance apoptosis in rabbit hearts,⁵² NPPB and DIDS protected from apoptosis in rat.⁵³

Na⁺,⁵⁴ and connexin (gap junction)⁵⁵ channels were shown to control cell shrinkage and/or apoptosis in tumor cell lines, but the exact role played in apoptosis by these channels has not been extensively studied. However, Na⁺ inflow is necessary for Jurkat T cells induced shrinkage and conducting Na_V channels are required for apoptosis as Saxitoxin (a Na_V non specific blocker), also blocked apoptosis.⁵⁴

The obligatory involvement of channels permeable to different ions in apoptosis suggests

that the disruption of ion homeostasis is a necessary component of apoptosis propagation.

The Apoptosis-Cancer paradox of ion channels

One of the main motivations for trying to understand the role of ion channels in apoptosis is derived from a possible link to the role of the channels in cancer, where apoptosis is notoriously harder to achieve. As imbalance between apoptosis and proliferation may account for cancerous phenotypes, one may expect that K⁺ channels, which must be upregulated in order for a cell to die, will be downregulated if the cell is to live forever.

In fact, this is not the case, and a few examples exist of certain K⁺ or Ca²⁺ channels that are upregulated in both cellular conditions. These include K_{2p}9.1, K_v1.3, K_{ca}1.1 and P2X7.^{43,10,5} However, K_v11.1 (HERG) channels were shown to play a dual role: in apoptosis as an active K⁺ channel and in proliferation as a membrane anchoring protein recruiting growth receptors to the membrane.⁵⁶ The duality of the K_v11.1 effect was investigated with the use of a natural mutant. It lacks the ability to conduct ions and does not support apoptosis, but retains wild-type abilities to express in the membrane and bind the growth receptor. Specific antibodies used to co-immunoprecipitate K_v11.1 and TNF1R also showed this ability. This study is particularly interesting as it was conducted in different tumor cell lines, some which express the channel and tend to die upon apoptotic insult, while others do not express the channel and are resistant to the apoptotic insult.⁵⁶

Taken together, the observations summarized here highlight the importance of ion channels in cellular mechanisms that control cellular integrity and fate. These roles played by ion channels in killing a cell are relevant in many clinical situations ranging from protection of the perfused heart during surgery, to exchanging proliferation for apoptosis as a possible tool in the fight against cancer.

References:

1. Bratton, S.B and Cohen, G.M. (2001) *Trends Pharmacol. Sci.* **22**, 306.
2. Domeni, J. (2001) *J. Apoptosis* **6**, 239.
3. Aartes, M. et al. (2003) *Cell* **115**, 863.
4. Sears, R.C. and Nevins, J.R. (2002) *J. Biol. Chem.* **277**, 11617.
5. Remillard, C.V. and Yuan, J.X.J. (2003) *Am. J. Physiol.* **286**, L49.
6. Matteson, M.P. and Chan, S.L. (2003) *Nat. Cell Biol.* **12**, 1041.
7. Nadeau, H. et al. (2000) *J. Neurophysiol.* **84**, 1062.
8. Li, H. et al. (2000) *Science* **289**, 1159.
9. Pal, S. et al. (2003) *J. Neurosci.* **23**, 4798.
10. Storey, N.M. et al. (2003) *J. Biol. Chem.* **278**, 33319.
11. Hille, B. (2001) *Ion Channels in Excitable Membranes*. 3rd edition.
12. Zheng, L.M. et al. (1991) *J. Cell. Biol.* **112**, 279.
13. Fairbairn, I.P. et al. (2001) *J. Immunol.* **167**, 3300.
14. Humphreys, B.D. et al. (2000) *J. Biol. Chem.* **275**, 26792.
15. Gu, B. J. et al. (2001) *J. Biol. Chem.* **276**, 11135.
16. Coutinho-Silva, R. et al. (1999) *Am. J. Physiol.* **276**, C1139.
17. Nihei, O.K. et al. (2000) *Blood* **96**, 996.
18. Greig, A.V.H. et al. (2003) *Journal of Investigative Dermatology* **121**, 1145.
19. Chvatchko, Y. et al. (1996) *Immunity* **5**, 275.
20. Cavaliere, F. et al. (2002) *J. Neurochem.* **83**, 1129.
21. Cano-Abad, M.F. et al. (2001) *J. Biol. Chem.* **276**, 39695.
22. Juntti-Berggren, L. et al. (1993) *Science* **261**, 86.
23. Wang, L. et al. (1999) *Endocrinology* **140**, 1200.
24. Hara, Y. et al. (2002) *Mol. Cell* **9**, 163.
25. Zhang, W. et al. (2003) *J. Biol. Chem.* **278**, 16222.
26. Agopyan, N. et al. (2003) *Am. J. Physiol.* **286**, L563.
27. Zhang, L. et al. (2003) *Cancer Gene Therapy*. **10**, 611.
28. Boehning, D. et al. (2003) *Nat. Cell Biol.* **5**, 1051.
29. Jonas, E.A. et al. (2003) *J. Neurosci.*, **23**, 8423.
30. Kirichok, Y. et al. (2004) *Nature* **427**, 360.
31. Crompton, M. (2000) *J. Physiol.* **529**, 11.
32. Degterev, A. et al. (2001) *J. Cell. Biol.* **155**, 695.
33. Shimizu, S. et al. (1999) *Nature* **399**, 483.
34. Cheng, E.H. -Y. et al. (2003) *Science* **301**, 513.
35. Fernandez-Salas, E. et al. (2002) *Mol. Cell Biol.* **22**, 3610.
36. Suh, K.S. et al. (2003) *J. Biol. Chem.* **279**, 4632.
37. Teshima, Y. et al. (2003) *Stroke* **34**, 1796.
38. McCully, J.D. and Levitsky, S. (2003) *Ann. Thorac. Surg.* **75**, S667.
39. Xu, W. et al. (2002) *Science* **298**, 1029.
40. Yu, S.P. et al. (1997) *Science* **278**, 114.
41. Manikkam, M. et al. (2002) *Biol. Reproduc.* **67**, 88.
42. Elliot, J.I. and Higgins, C. F. (2003) *EMBO Reports* **4**, 189.
43. Lauritzen, I. et al. (2003) *J. Biol. Chem.* **278**, 32068.
44. Coroneo, M.T. et al. (2002) *Invest. Ophthalmol. Vis. Sci.* **43**, E-Abstract 752.
45. Trimarchi, J.R. et al. (2002) *Am. J. Physiol.* **282**, C588.
46. Bossy-Wetzel, E. et al. (2004) *Neuron* **41**, 351.
47. Koni, P. A. et al. (2003) *J. Biol. Chem.* **278**, 39443.
48. Elble, R.C. and Auli, B.U. (2001) *J. Biol. Chem.* **276**, 40510.
49. Kim, J. A. et al. (2003) *Biochem. Biophys. Res. Commun.* **309**, 291.
50. Gottlieb, R.A. and Dosanjh, A. (1996) *Proc. Natl. Acad. Sci. USA.* **93**, 3587.
51. Szabo, I. et al. (1998) *Proc. Natl. Acad. Sci. USA.* **95**, 6169.
52. Souktani, R. et al. (2003) *Fundam. Clin. Pharmacol.* **17**, 555.
53. Mizoguchi, K. et al. (2002) *Transplantation.* **73**, 1185.
54. Bortner, C.D. and Cidlowski, J.A. (2003) *J. Biol. Chem.* **278**, 39176.
55. Kalvelyte, K. et al. (2003) *Biochem. Pharmacol.* **66**, 1661.
56. Wang, H. et al. (2002) *Cancer Res.* **62**, 4843.

Related Products:

Compound	Product #
Antibodies to Ion Channels	
Anti-P2X1	APR-001
Anti-P2X7	APR-004
Anti-P2X7 extracellular	APR-008
Anti-P2X7 extracellular-FITC	APR-008-F
Anti-Ca _v 1.2	ACC-003
Anti-Ca _v 1.3	ACC-005
Anti-Ca _v 3.1	ACC-021
Anti-TRPV1	ACC-030
Anti-IP ₃ R1	ACC-019
Anti-CLC1	ACL-005
Anti-CLC2	ACL-002
Anti-CLC3	ACL-001
Anti-CLC5	ACL-003
Anti-CLCK	ACL-004
Anti-K _v 1.3	APC-002
Anti-Kv1.3 extracellular	APC-101
Anti-Kv1.3 extracellular-FITC	APC-101-F
Anti-K _v 2.1	APC-012
Anti-K _v 11.1 (HERG)	APC-062
Anti-K _{ca} 1.1 (1098-1196)	APC-021
Anti-K _{ca} 1.1 (1118-1134)	APC-107
Anti-K _{ca} 3.1	APC-064
Anti-K _{sp} 3.1	APC-024
Anti-K _{sp} 9.1	APC-044
Anti-K _{ir} 1.1	APC-001
Anti-Na _v 1.1	ASC-001
Anti-Na _v 1.2	ASC-002
Anti-Na _v 1.3	ASC-003
Anti-Na _v 1.5	ASC-005
Anti-Na _v 1.6	ASC-006
Anti-Na _v 1.7	ASC-008
Anti-Pan-Na _v α	ASC-003
Anti-Na _v β2	ASC-007

Ion Channel Blockers

rAgitoxin-1	RTA-150
rAgitoxin-2	RTA-420
rAgitoxin-3	RTA-390
Apamin	A-200
rBeKm-1	RTB-470
Calcicludeine	C-650
Calciseptine	C-500
rCharybdotoxin	RTC-325
E-4031	E-500
rErgotoxin-1	RTE-450
FS-2	F-700
rHongotoxin-1	RTH-400
rlberiotoxin	RTI-400
rlq-2	RTL-550
rMargatoxin	RTM-325
rNoxiustoxin	RTN-340
Paxilline	P-450
Penitrem A	P-650
rSlotoxin	RTS-410
TaiCatoxin	T-800
TTX (with citrate)	T-550
TTX (without citrate)	T-500
Verrucologen	V-500

Ion Channel Openers

(±)Bay K8644	B-350
Pimaric Acid	P-270
Isopimaric Acid	I-370

Apoptosis Inducers:

The activity of the following widely used apoptosis inducers were tested for their ability to induce apoptosis in Jurkat cells as demonstrated below.

Compound	Product #
A23187	A-600
Okadaic acid (ammonium salt)	O-800
Okadaic acid (sodium salt)	O-900
Puromycin	P-540
Staurosporine	S-350
Thapsigargin	T-650

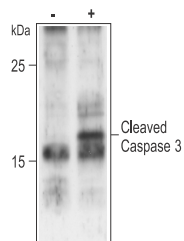
Okadaic Acid

(Ammonium Salt)

Product #: O-800

Okadaic Acid induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 1 μM Okadaic Acid or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

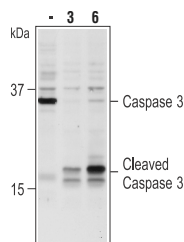


Staurosporine

Product #: S-350

Staurosporine induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM Staurosporine 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.



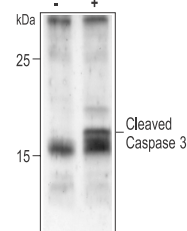
A23187 (Ca²⁺ Ionophore)

(Antibiotic A23187. Calcimycin, Calcium Ionophore A23187)

Product #: A-600

A23187 induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM A23187 or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

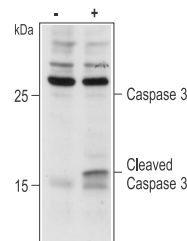


Puromycin

Product #: P-540

Puromycin induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 2 μM Puromycin or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for Caspase 3 and cleaved Caspase 3 with specific antibodies.



Thapsigargin

Product #: T-650

Thapsigargin induced apoptosis in Jurkat cells:

Jurkat cells were grown to 70% confluency. Then 1 μM Thapsigargin or vehicle were added for 6 hours. At the end of the incubation period, the cell extracts were probed for cleaved Caspase 3 with specific antibodies.

