Activation of K\textsuperscript{+} channels: an essential pathway in programmed cell death

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Invited Review

Am J Physiol Lung Cell Mol Physiol 286: L49–L67, 2004; 10.1152/ajplung.00041.2003.—Cell apoptosis and proliferation are two counterparts in sharing the responsibility for maintaining normal tissue homeostasis. In recent years, the process of the programmed cell death has gained much interest because of its influence on malignant cell growth and other pathological states. Apoptosis is characterized by a distinct series of morphological and biochemical changes that result in cell shrinkage, DNA breakdown, and, ultimately, phagocytic death. Diverse external and internal stimuli trigger apoptosis, and enhanced K\textsuperscript{+} efflux has been shown to be an essential mediator of not only early apoptotic cell shrinkage, but also of downstream caspase activation and DNA fragmentation. The goal of this review is to discuss the role(s) played by K\textsuperscript{+} transport or flux across the plasma membrane in the regulation of the apoptotic volume decrease and apoptosis. Attention has also been paid to the role of inner mitochondrial membrane ion transport in the regulation of mitochondrial permeability and apoptosis. We provide specific examples of how deregulation of the apoptotic process contributes to pulmonary arterial medial hypertrophy, a major pathological feature in patients with pulmonary arterial hypertension. Finally, we discuss the targeting of K\textsuperscript{+} channels as a potential therapeutic tool in modulating apoptosis to maintain the balance between cell proliferation and cell death that is essential to the normal development and function of an organism.

Apoptosis; ion channels; cell volume regulation; pulmonary artery smooth muscle cells; pulmonary hypertension

CELL DEATH IS CRITICAL for the normal development and function of multicellular organisms. For a tissue to function properly, removal of excess cells or of cells with genetic damage or improper developmental mutations is crucial. Cancer (59), hypertension (151), cardiac disease (36), viral infections (11, 125), and autoimmune (48) and neurodegenerative disorders (189) are all characterized by abnormal cell death regulation. The cellular turnover that results from the balance between cell death and proliferation is important in maintaining tissue homeostasis. Ion channels in both the sarcolemmal and mitochondrial membranes have been implicated in the signal transduction cascades that regulate apoptosis (64). This review focuses on the role played by K\textsuperscript{+} and K\textsuperscript{+} transport in the onset and development of cellular changes typical of the apoptotic process, especially in pulmonary vascular smooth muscle cells, and how modulation of K\textsuperscript{+} efflux and K\textsuperscript{+} channel function by both pro- and antiapoptotic proteins is a potential therapeutic target for cardiopulmonary diseases.

APOPTOSIS: AN OVERVIEW

Apoptosis, or programmed cell death, allows individual cells to die according to a highly controlled series of morphological and biochemical changes (Fig. 1). In the earliest stage of apoptosis, cells undergo shrinkage, the apoptotic cell shrinkage, with little or no change in the structure of intracellular organelles. As will be discussed later, the enhanced activation of ion-selective channels and water-permeable channels (aquaporins) modulates the apoptotic volume decrease (AVD). Nuclear condensation and DNA fragmentation within the nucleus typically occur after apoptotic stimulation and the onset of AVD, as early as 1–4 h after the apoptotic stimulation in the case of human pulmonary artery vascular smooth muscle cells (129), human leukemia HL-60 cells (31), neurons (20), human lymphoid cells (103), and thymocytes (178). The formation of apoptotic bodies (membrane-bound vesicles that pinch off from the dying cell) containing organelles and nuclear fragments constitutes the final step before phagocytosis by resident macrophages and neighboring cells. Apoptotic bodies are then degraded after phagocytosis. Unlike cellular necrosis, apoptosis does not result in an inflammatory response since the intracellular contents are not exposed to the environment prior to phagocytosis, thereby minimizing damage to adjoining healthy cells.

Increased proteolytic activity following AVD is a key element in the steps leading to nucleotide fragmentation. The biochemical changes inherent to apoptosis are due to the activation of cytoplasmic proteolytic enzymes, the caspases, by proapoptotic stimuli (41, 68, 161) via one of two pathways (Fig. 2). The extrinsic pathway, or the death receptor pathway, is initiated by the activation of transmembrane death receptors by the binding of proteins such as CD95, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), and Fas ligand. Activation of the death

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receptors activates the membrane proximal initiator caspase-8 (and/or caspase-10), which then cleaves procaspase-3 to generate the active effector caspase-3. The intrinsic pathway, or the mitochondrial death pathway, requires disruption of the mitochondrial membrane [e.g., by staurosporine (ST), actinomycin D, peroxide, ultraviolet (UV) radiation] and/or the release or translocation of cytochrome c (cyt-c) (81, 183) and other apoptosis-inducing factors from the mitochondrial intermembrane space to the cytoplasm. The precise triggering mechanism for cyt-c release is under investigation. There are suggestions that its release results from 1) physical disruption of the mitochondrial membrane, 2) mitochondrial membrane depolarization, and 3) increased mitochondrial permeability transition (10, 60, 67, 81). Whatever its extrusion mechanism, the released cyt-c binds to apoptotic protease-activating factor 1 (APAF-1) and forms a heptameric APAF-1-cyt-c complex with deoxyadenosine triphosphate/adenosine triphosphate (dATP/ATP), the apoptosome. The apoptosome activates procaspase-9, which in turn activates the downstream effector caspases (caspase-3, -6, -7) in the cytoplasm. Activation of the effector caspases-3/-6/-7 by either death receptor stimulation or mitochondrial disruption leads to chromatin degradation and ultimately to apoptosis.

Two other mitochondrial proteins, Smac/Diablo (39, 165) and apoptosis-inducing factor (AIF) (77), can be released into the cytoplasm and cause apoptosis via caspase-independent pathways. The initiator caspase-8, initially activated by the extrinsic pathway, can also truncate the cytosolic Bid protein, leading to cyt-c release and then to the activation of the effector caspases-3/-6/-7 via the intrinsic pathway. Mitochondrial proteins released into the cytoplasm, such as Smac/Diablo and Omi/HtrA2 (39, 66, 156, 165, 166), can antagonize the actions of the inhibitors of apoptosis, which directly inhibit caspase activity (35, 175). The death receptor and mitochondrial pathways cross talk with each other in multiple steps to achieve the final goal, activation of the effector caspases.

In summary, apoptosis is a process that plays a critical role in embryonic development and tissue homeostasis. The programmed cell death cascade due to activated death receptors can be divided into at least three functionally distinct stages (60, 104): 1) the initiation or signaling phase in which death-promoting molecules (e.g., TNF-α and Fas ligand) bind to death receptors on the cell surface with subsequent recruitment of death domain proteins for activation of caspase-8; 2) the effector phase during which depolarization of mitochondrial membrane potential (ΔΨm), release of cyt-c from the mitochondrial intermembrane space to the cytoplasm, and/or activation of cytoplasmic caspases take place; and 3) the structural alteration and DNA degradation phase in which activated effector caspases lead to the cleavage of the lamin proteins that make up the nuclear lamina (the rigid structure that underlies the nuclear membrane and is involved in chromatin organization) and ICAD [an inhibitor of the caspase-activated deoxyribonuclease (CAD or DFF) responsible for DNA fragmentation], and to the fragmentation and degradation of genomic DNA (161). Indeed, it has been well documented that cells undergoing apoptosis show cell shrinkage, chromatin (nuclear) condensation with subsequent internucleosomal fragmentation of DNA, and membrane redistribution of phospholipids.

**REGULATION OF CELL VOLUME**

Mammalian cellular membranes are highly permeable to water. The movement of water across the membrane occurs via water channels, or aquaporins, which are highly expressed in virtually all cell types (80, 137). Of the eleven known aquaporins, for example, eight have been identified in human pulmonary artery smooth muscle cells (PASMC) to varying degrees (I. Fantozzi and J. X.-J. Yuan, unpublished observations). Animal cell membranes cannot tolerate the hydrostatic pressure gradients produced by the passive transport of water according to its concentration gradient. Therefore, water movement is largely regulated by osmotic gradients across the cell membrane and is rarely a limiting factor in cellular volume changes. In fact, alterations of intra- or extracellular osmolarity typically precede the movement of water and cellular volume changes (93, 94).

Ion transport contributes greatly to the regulation of the transmembrane osmotic gradient (Fig. 3). Most cells achieve and maintain a physiological osmotic balance through the continuous activity of an electrogenic Na⁺-K⁺-ATPase pump (3 Na⁺ out: 2 K⁺ in), which creates an intracellular environ-
ment high in K\(^+\) (~140 mM) and low in Na\(^+\) (~10 mM) (79, 181), as well as various anion and cation cotransporters (108, 121). In most excitable and nonexcitable cells, K\(^+\) is the dominant cytoplasmic cation (being ~30-fold more concentrated within the cytoplasm than the intercellular space), whereas Na\(^+\) and free Ca\(^{2+}\) are more concentrated in the extracellular space (Table 1). Cl\(^-\) is the major anion in these cells; the cytoplasmic Cl\(^-\) concentration ([Cl\(^-\)]
_cyt\_)
 usually ranges from 5 to 15 mM in many excitable cells, although larger variations can be found in smooth muscle cells, neurons, and cardiac muscle depending on species and tissue type (76, 131, 167). [Cl\(^-\)]
_cyt\_ in smooth muscle cells, especially vascular smooth muscle cells, however, can be as high as 50 mM (76), suggesting that, in addition to organic anions (such as HCO\(_3\)\(^-\) and nitrates), Cl\(^-\) is a dominant cytoplasmic anion in smooth muscle cells.

Because the cell membrane is permeable to K\(^+\) under resting conditions, i.e., the permeability to K\(^+\) is much greater than the permeability to other ions, the activity of membrane K\(^+\) channels plays a critical role in the regulation of cellular volume. Whole cell K\(^+\) current (\(I_K\)) at any given time is determined by the following equation

\[ I_K = N \times i \times P_{open} \]

where \(N\) denotes the total number of functional K\(^+\) channels expressed in the plasma membrane; \(i\) is the current through a single K\(^+\) channel; and \(P_{open}\) is the steady-state open probability of a K\(^+\) channel. Therefore, when K\(^+\) channels open (i.e., \(i\) or \(P_{open}\) rises) and/or the number of functional K\(^+\) channels in the plasma membrane increases (i.e., \(N\) increases due to upregulation of K\(^+\) channel gene expression), the whole cell \(I_K\) or transmembrane K\(^+\) efflux is increased, which would induce or enhance cell volume decrease. In contrast, when K\(^+\) channels close (i.e., \(i\), \(N\), or \(P_{open}\) decline), the whole cell \(I_K\) or transmembrane K\(^+\) efflux is decreased, which would inhibit cell volume decrease.

The efflux of K\(^+\) thereby creates a positive potential outside the cell, which would drag Cl\(^-\) out of the cell according to its electrochemical gradient, and the membrane hyperpolarization induced by K\(^+\) efflux would also activate membrane Cl\(^-\)

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**Fig. 2.** The two major apoptotic pathways involve either membrane death receptor stimulation (extrinsic pathway) and/or mitochondrial disruption (intrinsic pathway). The major proteins involved are shown, as well as the modulatory sites for selected regulatory proteins. AIF, apoptosis-inducing factor; AKT, protein kinase B; APAF-1, apoptotic protease-activating factor 1; ARC, apoptosis repressor with caspase recruitment domain; tBid, truncated Bid; cyt-c, cytochrome c; DIABLO, direct IAP-binding protein with low pI; FADD, Fas-associated death domain protein; c-FLIP, FADD-like ICE (caspase-8) inhibitory protein; IAPs, inhibitors of apoptosis; ROS, reactive oxygen species; Smac, second-mitochondrial-derived activator of caspase; \(\Delta \Psi_m\), mitochondrial membrane potential.
channels and further enhance Cl⁻ efflux (192). The resultant accumulation of KCl outside the cell thus shifts the osmotic balance such that water is also extruded from the cell in an attempt to reestablish a normal osmotic gradient. The subsequent cell shrinkage may be functionally important since a doubling of extracellular osmolarity has been shown to trigger apoptosis in lymphocytes (15). According to the modulation of K⁺ and Cl⁻ movement as well as K⁺ and Cl⁻ channel activity is thus crucial in initiating and regulating the apoptotic volume decrease in cells undergoing apoptosis.

Although Na⁺, K⁺, and Cl⁻ ions have been implicated in cell shrinkage, Ca²⁺ ions may also play a role in the regulation of cell volume. After cell swelling, intracellular Ca²⁺ concentration ([Ca²⁺]i) increases in some cell types, either due to enhanced sarcolemmal Ca²⁺ influx or due to Ca²⁺ release from intracellular stores (93). Although increased [Ca²⁺]i itself may not have a direct role on the regulation of cell volume, it may affect cytoskeletal elements such as the actin filaments or serve as a signal transduction element to activate other membrane ion channels (e.g., Ca²⁺-activated K⁺ and Cl⁻ channels) and transporters. Obviously, both cell swelling and shrinkage result in significant changes in the cytoskeletal architecture. Actin filament networks are known to be depolymerized in swollen cells, possibly due to Ca²⁺ binding to gelsolin (93). An intact actin filament network is required for the activation of some volume regulatory mechanisms. Disruption due to Ca²⁺-mediated depolymerization will affect many processes, including 1) Na⁺ channel activity, 2) insertion of volume regulatory channels into the membrane, 3) regulation of channels by kinases and phospholipids, 4) activation of mechanosensitive anion channels by membrane stretch, and 5) activation of the Na⁺/H⁺ exchanger and the Na⁺/K⁺/2Cl⁻ cotransporter, resulting in volume deregulation (93).

**MODULATION OF APOPTOTIC STAGES BY K⁺ FLUX ACROSS THE PLASMA MEMBRANE**

Cell shrinkage is an early hallmark of apoptosis. Apoptotic cell shrinkage occurs in two distinctive stages: the initial phase starting before formation of the cyt-c/APAF-1/caspase-9 apoptosome and cell fragmentation and the late phase that is associated with cell fragmentation (123). The early phase of the apoptotic cell shrinkage when cells undergo apoptosis is mainly regulated by the activity of membrane ion channels and transporters (123, 185, 186). The time courses of the effect of apoptosis inducers on morphological changes, cyt-c translocation, caspase activation, and DNA/cell fragmentation have demonstrated that the initial phase of AVD occurs before the release of cyt-c, activation of cytoplasmic caspases, and breakage of cell nuclei. However, a rise in cytoplasmic cyt-c and an increase in active caspases in the cytoplasm have also been demonstrated to contribute to the apoptotic cell shrinkage, mainly the late phase of the volume decrease (73, 129, 143, 169, 180). These results suggest that the early and late phases of cell shrinkage in apoptotic cells may result from different mechanisms. In both stages, membrane ion channels and transporters appear to be involved.

The apoptotic cell shrinkage has been demonstrated to correlate with increased K⁺ and Cl⁻ efflux and activation of K⁺ channels (13, 45, 87, 88, 172, 188). Because high cytoplasmic K⁺ ([K⁺]cyt) is required to maintain cytoplasmic ion homeostasis and cell volume, any changes of K⁺ efflux or influx will influence plasma membrane permeability and cell volume. The link between K⁺ efflux and apoptosis has been further established by experiments using ionophores. Valinomycin, a K⁺ ionophore that allows K⁺ efflux based on the K⁺ electrochemical gradient, can induce apoptosis in many cell types, including neurons (188), thymocytes (4, 29, 30), ascites hepatoma cells (74), and PASM (87).

K⁺ uptake (from extracellular fluid to the cytoplasm) is modulated primarily by the ouabain-sensitive Na⁺/K⁺ -AT-Pase, or Na⁺ pump (181). Recent studies have shown that anti-Fas and dexamethasone treatments inactivated an ouabain-sensitive Na⁺/K⁺-ATPase pump in lymphocytes (17) and thymocytes (106), significantly decreasing K⁺ uptake, irre-

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**Table 1. Ionic distribution in excitable tissues**

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>[Na⁺]</th>
<th>[Na⁺]</th>
<th>[K⁺]</th>
<th>[K⁺]</th>
<th>[Cl⁻]</th>
<th>[Cl⁻]</th>
<th>[Ca²⁺]</th>
<th>[Ca²⁺]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian</td>
<td>VSM*</td>
<td>12–47</td>
<td>132–198</td>
<td>110–175</td>
<td>5–6</td>
<td>8–66</td>
<td>134–145</td>
<td>0.06–1.2</td>
<td>1.2–2.5</td>
<td>76</td>
</tr>
<tr>
<td>Mammalian</td>
<td>neuron†</td>
<td>18</td>
<td>140–150</td>
<td>155</td>
<td>2–6</td>
<td>−40</td>
<td>99–150</td>
<td>0.05–0.15</td>
<td>2.4</td>
<td>51, 55, 82, 131, 135, 144</td>
</tr>
<tr>
<td>Squid</td>
<td>giant axon</td>
<td>50</td>
<td>440</td>
<td>400</td>
<td>20</td>
<td>40–150</td>
<td>560</td>
<td>0.4</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>Mammalian</td>
<td>skeletal muscle‡</td>
<td>7–10</td>
<td>109–145</td>
<td>124–155</td>
<td>2.2–4</td>
<td>1.5–4</td>
<td>77–131</td>
<td>10⁻⁴</td>
<td>1.5–2.1</td>
<td>61</td>
</tr>
<tr>
<td>Mammalian</td>
<td>cardiac muscle§</td>
<td>10–100</td>
<td>75–148</td>
<td>48–155</td>
<td>4.5–5.4</td>
<td>4–82</td>
<td>123–131</td>
<td>0.1–0.36</td>
<td>2–4</td>
<td>84, 167</td>
</tr>
</tbody>
</table>

All concentrations are given in mM. *Vascular smooth muscle (VSM) from aorta (rat, rabbit), portal vein (rat, rabbit), pulmonary artery (rabbit), and carotid artery (dog, sheep); †hippocampus (rat, mouse) and pyramidal cells (human, guinea pig); ‡skeletal muscle (frog); §purkinje fibers (dog), and ventricle (dog).
versely depolarizing the cell membrane, activating voltage-dependent K+ and Cl− channels, and causing apoptosis. What follows is an overall review of the modalities of cytoplasmic K+ efflux and how they regulate apoptosis.

ACTIVATION OF K+ CHANNELS INDUCES APOPTOTIC CELL SHRINKAGE

Enhancement of K+ efflux-mediated cell shrinkage is considered to be one of the earliest signs of apoptosis in many cells. The role of K+ channels in apoptosis was proposed by the original work of Yu et al. in 1997 (188) and subsequently supported by other investigators (18, 30, 56, 87, 103, 169). Bortner et al. (18, 169) showed a correlation between the significant decrease in [K+]cyt and the number of shrunken cells when lymphocyte apoptosis was induced by Fas ligand, dexamethasone, ST, and anisomycin (a protein synthesis inhibitor), thereby establishing a link between K+ efflux and AVD. These studies were further reinforced by observations that raising extracellular K+ ([K+]o), which reduces transmembrane K+ concentration gradient and reduces K+ efflux, can inhibit AVD and apoptosis induced by valinomycin, Fas ligand, carbonyl cyanide-p-trifluoromethoxyphenyl hydrazone (FCCP), and ST in neurons (188), PASM C (86, 87), and lymphocytes (18, 56). Inhibition of K+ channel activity with quinine or Ba2+ also prevents cell shrinkage induced by ST or TNF-α/cycloheximide (103). In addition, treatment of lymphocytes and cortical neurons with tetrpentalammonium (TPA) inhibits the early stages of apoptosis before caspase activation has occurred (30, 176). These results indicate that an increased K+ efflux via sarcolemmal K+ channels is thus a central mediator of AVD and apoptosis.

Okada and Maeno (123) classified the apoptotic cell shrinkage into two stages: the early volume decrease that occurs before cyt-c release and caspase activation and the late volume decrease that occurs concurrently with DNA fragmentation and nuclear breakage. In PASMC, our recent results suggest that, when cells are treated with ST, voltage-gated K+ current (IK(V)) increases within 30 min immediately followed by a reduction of cell size/volume (Fig. 4) (129). The ST-mediated nuclear breakage and condensation, determined by 4′,6′-diamidino-2-phenylindole (DAPI) staining (and terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL) assay, occurs after the ST-mediated increase in IK(V) and cell volume decrease (Fig. 4) (129). Furthermore, cytoplasmic application of recombinant cyt-c enhances 4-amino- pyridine (4-AP)-sensitive voltage-gated K+ (KV) currents in these cells (Fig. 5) independently of caspase-9 activation (129), suggesting that cyt-c-mediated opening of K+ channels precedes caspase-9 activation. These observations indicate that activation of K+ channels is involved in both the early and late volume decrease. In cells challenged by apoptosis inducers (e.g., ST, TNF-α, UV light, dexamethasone) or death triggers, K+ channels may be activated by an unknown mechanism to initiate the early volume decrease and further activated by cyt-c to maintain the early stage of cell shrinkage. The cyt-c-mediated KV channel activation may also play an important role in initiating the late volume decrease associated with cell fragmentation.

ACTIVATION OF K+ CHANNELS INDUCES APOPTOSIS

In addition to K+-permeable channels, K+ efflux is also controlled by many different mechanisms in excitable cells, such as an electroneutral K+−Cl− symporter and a K+−H+ and Cl−−HCO3− exchanger system (121). Enhanced K+ efflux through K+ channels, however, is a major pathway for K+
loss. Five classes of K\(^+\) channels have been identified in excitable cells: K\(_V\) channels, Ca\(^{2+}\)-activated K\(^+\) (K\(_{Ca}\)) channels, ATP-sensitive K\(^+\) (K\(_{ATP}\)) channels, inwardly rectifying K\(^+\) (K\(_{IR}\)) channels, and tandem pore K\(^+\) (K\(_{T}\), two-pore six-domain) channels (97, 105, 115). The enhanced activity of four of these channels has been implicated in apoptosis induced by the stimulation of either the mitochondrial or death receptor apoptotic pathways, as is discussed below.

ST, a potent apoptosis inducer in almost all cell types, enhances the activity of a 4-AP-sensitive K\(_V\) channel in human and rat PASMC (Fig. 6A) (37, 85) and of a tetraethylammonium (TEA)-sensitive K\(^+\) channel in mouse neocortical neurons (188). Activation of the 4-AP-sensitive K\(_V\) channels by the nitric oxide (NO) donor S-nitroso-N-acetyl-penicillamine (SNAP) also causes apoptosis in PASMC (Fig. 6B). In rat and human PASMC treated with ST, the maximum enhancement of K\(_V\) currents occurs within 6 h, whereas apoptosis is maximal after ~24 h of treatment (Fig. 4C), suggesting that K\(_V\) channel activation occurs rapidly following the challenge of apoptotic inducers or death triggers and likely precedes caspase activation and DNA degradation (43, 86, 129). A similar 4-AP-sensitive K\(^+\) current is activated by UV radiation in myeloblastic leukemia cells (173). In rat fetal neurons, the sulfhydryl-oxidizing agent 2,2'-dithiodipyridine activates a TEA-sensitive K\(^+\) current with similar kinetics to that produced by the 4-AP-sensitive K\(_V\) channels (109), whereas neuronal apoptosis is associated with a significant increase in K\(_V\) currents (188). K\(^+\) currents sensitive to TPA, a TEA analog, were also detected in thymocytes and cortical neurons treated with dexamethasone and ST, respectively (30, 176). In thymocytes, TPA prevented all characteristics of dexamethasone-induced apoptosis, including \(\Delta\Psi_m\) dissipation, cytosolic K\(^+\) efflux, chromatin condensation, and caspase and endonuclease activation (30). These results using TPA as a K\(^+\) channel inhibitor should be interpreted with caution, as the compound has been shown to have multiple nonspecific effects on voltage-dependent Ca\(^{2+}\) and Na\(^+\) channels' activity, as well as on K\(^+\) channel activity, in cortical neurons (176).

In addition to K\(_V\) channels, activation of K\(_{Ca}\) channels has also been implicated in AVD and apoptosis. In vascular smooth muscle cells, for example, FCCP, which dissipates the...
proton gradient across the inner mitochondrial membrane (IM) and disrupts the $\Delta Y_{m}$, causes an increase in cytoplasmic free Ca$^{2+}$ concentration and enhances K$^+$ efflux via iberiotoxin- and TEA-sensitive K$_{Ca}$ channels (Fig. 7A) (38, 87). Activation of K$_{Ca}$ channels by the NO donor SNAP (Fig. 7B) and by dihydroepiandrosterone (DHEA) also induces apoptosis in human PASMC (86, 88). TNF-$\alpha$, a death receptor agonist, activates Ca$^{2+}$-dependent and protein kinase C (PKC)-activated K$_{Ca}$ channels, increases K$^+$ currents and efflux, and induces apoptosis in rat liver HTC cells (120). Furthermore, hydrogen peroxide (H$_2$O$_2$)-mediated apoptosis (134) is associated with apoptosis in rat liver HTC cells (120). Furthermore, hydrogen peroxide (H$_2$O$_2$)-mediated apoptosis (134) is associated with activation of TRESK K$_T$ channels (162), whereas cromakalim induces neuronal apoptosis by activating K$_{ATP}$ channels (188).

Conductance of the human ether-a-go-go channels markedly promotes H$_2$O$_2$-induced apoptosis in various tumor cell lines (170). Nevertheless, inhibition of K$^+$ channels by Ba$^{2+}$ and quinine attenuates apoptosis and increases viability of ST- or TNF-$\alpha$/cytochalasin-treated cells in human lymphoid (U-937) and epithelial (HeLa) cells, hybrid neuroblastoma/glioma (NG108-5) cells, rat pheochromoytoma (PC12) cells (103), and liver HTC cells (120).

There is evidence that the proapoptotic stimulation of K$^+$ channels may be mediated by auxiliary modulatory proteins and kinases. KChAP (K$^+$ channel-associated protein/protein inhibitor of activated STAT) is a K$^+$ channel modulatory protein belonging to the protein inhibitor of the STAT family, members of which are known to interact with transcription factors such as the proapoptotic p53 protein (90, 179). KChAP induces apoptosis in prostate cancer cell lines by increasing K$^+$ efflux and causing cell shrinkage; KChAP is also increased by ST treatment (179). On the basis of the latter study, it is believed that KChAP increases p53 levels and stimulates phosphorylation of p53 residue serine 15, leading to elevation of p21 levels and apoptosis (38, 179). Although PKC is involved in numerous cell functions, its role in modulating apoptosis is unclear. There is evidence that the enhanced K$^+$ efflux produced by Fas and TNF-$\alpha$ can be blocked by PKC stimulation (56, 120), i.e., PKC inhibition promotes cell shrinkage. Tyrosine kinase-mediated phosphorylation appears to play a more important role in modulating cell survival than PKC. In cortical neurons (187) and lymphocytes (157), tyrosine kinase inhibition (by herbimycin A, lavendustin A, or genistein) attenuates Fas- and ceramide-induced apoptosis and upregulation of N-type and delayed-rectifier K$^+$ channels. A more recent study showed that inhibition of tyrosine phosphorylation also suppresses the activity of the N$^+$-K$^+$-ATPase pump in cortical neurons, leading to apoptosis (177). Although kinases may modulate K$^+$ channel activity, kinase stimulation also may be dependent on K$^+$ channel activation. For example, in myeloblastic leukemia cells, UV-stimulated K$^+$ currents subsequently activate the JNK/SAPK signaling pathway to cause apoptosis (177). These observations indicate that phosphorylation of apoptotic proteins and of membrane channels plays an important role in regulating cell survival and, in particular, in enhancing the proapoptotic role of K$^+$ channels.

**MODULATION OF CYT-C RELEASE AND CASPASE ACTIVATION BY K$^+$ EFFLUX**

The release of cyt-c from the mitochondrial intermembrane space is pivotal to apoptosis, since formation of the cyt-c/ APAF-1/caspase-9 apoptosome triggers the activation of the effector caspases-3/-6/-7. ST (86, 103), TNF-$\alpha$/cytochalasin (103), NO (88), Fas ligand (56), etoposide (183), and UV irradiation (52, 90) all cause cyt-c release into the cytosol. Mitochondrial membrane depolarization induced by FCCP and NO also causes cyt-c release (10, 67). In many of these cases (except Fas ligand), caspase inhibitors do not prevent the release of cyt-c, indicating that cyt-c release occurs before activation of caspase-3 (19, 60, 78, 81, 129, 164, 183).

Both cyt-c release and caspase-3 activation are readily attenuated by inhibition of sarcolemmal K$^+$ channels by quinine and Ba$^{2+}$ (103), suggesting that activation of K$^+$ channels occurs before cyt-c release and caspase activation in apoptotic...
cells. In addition, a decrease in [K+]cyt enhances caspase activation and limits cyt-c release in lymphocytes (16). Physiological [K+]cyt also inhibits formation of the APAF-1/cyt-c/caspase-9 apoptosome (21), while increasing [K+]j, (which reduces the driving force for K+ efflux) inhibits death receptor-mediated apoptosis before cyt-c release and caspase-8 activation can occur (21, 160). Therefore, maintenance of physiological and high [K+]cyt not only inhibits AVD but also suppresses cyt-c release from the mitochondria and inhibits cytoplasmic caspase activation, the deciding factors in cell death.

MODULATION OF CASPASE ACTIVITY AND DNA FRAGMENTATION BY CYTOPLASMIC K+ 

The final phase of apoptosis involves degradation of the nucleus and its contents. Internucleosomal DNA fragmentation is typically visualized as DNA laddering, i.e., DNA fragments that migrate as multiples of ~200 bp during agarose gel electrophoresis correspond to strands of DNA cleaved in internucleosomal sites (112). Cytoplasmic K+ in physiological concentration (~140 mM) inhibits chromatin condensation and DNA fragmentation, likely through suppression of caspase and endonuclease activities (29). The suppression of endonucleases and caspases is mimicked by sarcolemmal K+ and endonuclease activities (29). The suppression of endonucleases and caspases is mimicked by sarcolemmal K+ channel inhibition (30) during apoptotic stimulation by dexamethasone (a glucocorticoid receptor agonist) and etoposide (a topoisomerase inhibitor and genotoxic agent). Similar effects are also observed with quinine and Ba2+ in ST- or TNF-α/cycloheximide-treated U-937, HeLa, PC12, and NG108-15 cells (103).

Although K+ efflux may also occur via K+-Cl- cotransporters and the combined K+--H+ exchange/Cl-/HCO3- exchange system, most efflux occurs via K+ channels. Decreased [K+]cyt, due to enhanced K+ efflux through open sarcolemmal K+ channels, also enhances endonuclease activity. This suggests that the [K+]cyt, transmembrane K+ gradient, and function and expression of sarcolemmal K+ channels all contribute to regulating the early (e.g., by modulating AVD) and late (e.g., by modulating caspase activity) stages of apoptosis (16, 112). Indeed, in a cell-free system (isolated nuclei), a decrease in [K+] from 140 to 80 mM caused a 1.6-fold increase in apoptosis induced by the apoptosis-inducing factor (29). In lymphocytes, an 8-h treatment with ST decreased [K+]cyt from 140 to 50 mM, whereas a decrease in [K+] in assay buffer from 150 to 80 mM caused a 2.4-fold increase in DNA degradation in isolated nuclei (72). Similar experiments performed in rat PASMC show that increasing [K+] in the assay buffer enhanced caspase-3 activity (Fig. 8A) (105). The NO-induced apoptosis (Fig. 6B) and increase in caspase-3 (Fig. 6B) were both attenuated by K+ channel inhibition by 4-AP, TEA, and high [K+]j. These results provide evidence that a high [K+]cyt is required to suppress the activation of apoptotic processes (e.g., activation of caspases and endonucleases), whereas K+ efflux relieves the inhibition on cytoplasmic caspases and nucleases, thereby enhancing apoptosis.

CL- EFFLUX ALSO AFFECTS APOPTOSIS

As was discussed earlier, Cl- efflux is tightly coupled to K+ efflux, especially in cells undergoing apoptosis. It is therefore not surprising that numerous apoptotic inducers can trigger Cl- channel activity. ST-induced AVD and apoptosis are significantly reduced by Cl- channel inhibitors such as 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS), phloretin, and 4-acetamido-4'-isothiocyanostilbene in HeLa, U-937, PC12, and NG108-15 cells (103). Apoptosis induced by TNF-α treatment of rat liver HTC cells is reversed by NPPB and N-phenylthranilic acid (DPC) (120). However, at least in HeLa and U-937 cells, application of anthracene-9-carboxylate and furosemide does not prevent ST-induced apoptosis, thereby eliminating cAMP-activated cystic fibrosis transmembrane regulator (CFTR) channels and Na+-K+-2Cl- and Na+-Cl- symporters as possible Cl- extrusion pathways (103), although the role of CFTR in apoptosis is under debate (58, 110). Fas ligand/CD95 binding-mediated apoptosis is partially inhibited by indanyloxycetic acid, DPC, and DIDS in lymphocytes (158). Many of these Cl- channel antagonists also attenuate cyt-c release, caspase-3 activation, and DNA fragmentation in the same cells (103, 136). The Cl- channels involved in apoptosis may possibly be members of the Ca2+-activated or volume-sensitive channel families identified in mammalian cells based on their pharmacological properties (95, 152).

MODULATION OF K+ CHANNEL ACTIVITY BY ANTIAPOPTOTIC PROTEINS

The important role played by [K+]cyt and K+ channel activity in AVD and apoptosis is further enhanced by the fact that the antiapoptotic proteins Bcl-2, an antiapoptotic member of the Bcl-2 family, and ARC (apoptosis repressor with caspase recruitment domain), an antiapoptotic protein in cardiac and skeletal myocytes, modulate sarcolemmal K+ channel function (43, 44).

Bcl-2 is a large family of proteins with contrasting effects on apoptosis. Although structurally similar, some members of the family (Bax, Bak, Bad, Bid, Bim, PUMA, Noxa, Blk, Bik/Nbk, Hrk/DPS, Bok/Mbl, Bcl-xL) promote apoptosis, whereas others (Bcl-2, Bcl-xL, Bcl-2, A1, Mcl-1, Boo) inhibit apoptosis (1, 154). Proapoptotic Bcl-2 proteins are cytoplasmic and activate only with apoptotic stimulation. Some (like Bak, Bax, and...
truncated Bid) can translocate and insert themselves into the mitochondrial membrane upon apoptotic stimulation, thereby enhancing cyt-c release and causing apoptosis (62, 98, 101). The antiapoptotic protein Bcl-2 is mainly located in the endoplasmic reticulum (ER) membrane, the nuclear envelope, and the outer mitochondrial membrane (OM). The designation of Bcl-2 and Bcl-xL as antiapoptotic proteins has been blurred by recent evidence that cleavage by caspase-3 converts them into proapoptotic proteins similar to Bax (22, 23). Furthermore, mitochondrial Bcl-2 can cause apoptosis, whereas ER Bcl-2 protects against apoptosis induced by Bax overexpression (174). Therefore, the physical origin or location of Bcl-2 may be an important regulator in apoptosis.

Bcl-2 genes are regulated by cytokines and other death-survival signals at different levels. For example, antiapoptotic genes are induced transcriptionally by certain cytokines, whereas antiapoptotic Bax genes are induced as part of the p53-mediated damage response (3, 14). In addition, Bcl-2 can protect against apoptosis induced by γ- and UV-irradiation, cytokine withdrawal, glucocorticoid treatment, and ST (25), but not against apoptosis induced by ligand binding to CD95 death receptors in lymphocytes (153).

Bcl-2 inhibits apoptosis primarily by blocking cyt-c release into the cytoplasm (81, 183), although it can also protect against apoptosis via (1) inhibition of some proapoptotic proteins (see Fig. 2) (62, 98, 101, 155), (2) restoration of the high ATP-to-ADP ratio in the cytosol by facilitating mitochondrial ATP/ADP exchange (163), (3) direct antioxidant effects (24, 69), (4) regulation of Ca²⁺ content in the mitochondria and sarcoplasmic reticulum (65, 91, 197), and (5) maintenance of a negative ΔΨm via enhanced proton efflux or formation of mitochondrial cation channels (5, 142, 145). In addition to these more well-characterized effects, the antiapoptotic Bcl-2 protein has been shown to prevent apoptosis by, at least in part, acting on sarcolemmal Kᵥ channels in vascular smooth muscle cells. In rat PASMC, overexpression of the human bcl-2 gene using an adenoviral vector (5) markedly increases the protein expression of Bcl-2 (Fig. 9Aa), decreases current density of the 4-AP-sensitive Kᵥ channels (Fig. 9, Ab–d), downregulates mRNA expression of Kᵥ1.1, Kᵥ1.5, and Kᵥ2.1 channels as well as their representative whole cell Kᵥ currents (Fig. 9B), and (4) inhibits ST-mediated apoptosis (Fig. 9C) (43). These results suggest that inhibition of Kᵥ channel activity may serve as an additional mechanism involved in the Bcl-2-mediated antiapoptotic effect in vascular smooth muscle cells. The precise mechanisms by which Bcl-2 downregulates mRNA expression of Kᵥ channels and inhibits Kᵥ channel activity are unknown.

The recruitment and activation of caspases is central to the regulation of apoptosis. One of the protein–protein interaction motifs involved in death receptor-mediated apoptosis involves a caspase recruitment domain (CARD). ARC is a cardiac and skeletal muscle CARD-containing protein that binds to the initiator caspases-8/9 and significantly attenuates death receptor-induced apoptosis (83, 161). Multiple mechanisms are involved in the antiapoptotic effect of ARC on cardiomyocytes: (1) inhibition of caspase activation (83); (2) blockade of hypoxia/ischemia-induced cyt-c release (42); and (3) prevention of H₂O₂-mediated loss of membrane integrity and disruption of the ΔΨm (116).

Fig. 9. Overexpression of antiapoptotic Bcl-2 inhibits Kᵥ channel expression and function. A: Western blot analysis of human Bcl-2 protein levels in rPASMC infected with an empty adenoviral vector (−bcl-2) and an adenovirus carrying the human bcl-2 gene (+bcl-2). Aa: Families of currents were elicited by test pulses ranging between −40 and +80 mV (−70 mV holding potential) in control (left) and Bcl-2-infected (right) cells (b). Summarized results showing the current (I)-voltage (V) relationship curves in control (c) and bcl-2-transfected (d) cells (c). Current density of Iᵥ(V), at +80 mV is significantly decreased in cells infected with bcl-2 (d). B: single cell RT-PCR amplified products (a) for human Bcl-2 (267 bp) and rat Kᵥ1.1 (298 bp), Kᵥ1.5 (196 bp), Kᵥ2.1 (269 bp), and β-actin (267 bp) as well as the corresponding whole cell Iᵥ (b) in a control cell (−bcl-2) and a bcl-2-infected cell (+bcl-2). **RT, RT performed in the absence of reverse transcriptase. M, 1 kb plus DNA ladder marker. C: ST (0.02 μM)-induced apoptosis is inhibited in cells infected with bcl-2. ***P < 0.001 vs. −bcl-2. [From Ekhterae et al. (43).]
In addition to the inhibitory effects on cyt-c release (42) and mitochondrial disruption (116), overexpression of ARC in cardiomyocytes 1) blocks sarcolemmal Kv channels (Fig. 10A), which possibly contributes to inhibition of the apoptotic cell shrinkage, and 2) inhibits ST-mediated activation of Kv channels (Fig. 10B) and apoptosis (Fig. 10C) (44). The precise mechanism(s) by which ARC blocks Kv channels remains unclear. The ARC-mediated inhibition of ST-induced increase in IK(V) may be partially due to its inhibiting cyt-c release (42), because cytoplasmic dialysis of cyt-c increases IK(V) in vascular smooth muscle cells (Fig. 5) (129).

The inhibitory effects of the antiapoptotic proteins Bcl-2 and ARC on plasmalemmal K+ channels in smooth muscle cells and cardiomyocytes further support the theory that activation of K+ channels is a critical step for cells to undergo apoptosis, whereas inhibition of K+ channels attenuates apoptosis, which would facilitate cell proliferation and cause tissue remodeling.

**ROLE OF MITOCHONDRIAL ION FLUX IN APOPTOSIS**

Changes in mitochondrial membrane permeability (MMP) determine the ultimate fate of cells irrespective of the nature of the proapoptotic stimuli. Therefore, the mitochondria play a central role in modulating apoptosis by integrating different signal transduction cascades to a common pathway (47) initiated by MMP alterations and 2) releasing soluble proteins (i.e., cyt-c, Smac/Diablo, AIF, procaspases) from the mitochondrial intermembrane space into the cytosol.

The two well-defined compartments (i.e., intermembrane space and matrix) within the mitochondria regulate its activity. Under physiological conditions, the folded IM (Fig. 11) is almost impermeable, allowing the respiratory chain within the matrix (the region surrounded by the IM) to generate an electrochemical gradient that regulates the highly negative (−150 to −200 mV) ΔΨm via the production and translocation of H+. Disruption of MMP may result from defective ATP/ADP exchange between the matrix and cytosol mediated by the adenine nucleotide translocase (ANT) on the IM and voltage-dependent anion channels (VDAC) on the mitochondrial OM. Persistent membrane impermeability to ATP/ADP exchange ultimately results in loss of OM integrity, rendering it permeable to soluble proteins. IM permeabilization (visualized by cytofluorometry) results from disruption of ΔΨm.

The numerous channels and exchangers that populate the mitochondrial IM and OM play significant roles in the modulation of ΔΨm and apoptosis. What follows is a brief discussion of selected mitochondrial ion channels and how ion permeability within the mitochondria also contributes to cyt-c release and apoptosis. A focused review of mitochondrial K+ channels will follow in a separate section. For more in-depth information, readers should refer to recent publications dealing with mitochondrial cation transport, ΔΨm regulation, and control of cellular function (12, 47, 89, 118, 124, 175).

The VDAC, or mitochondrial porin, is a large-diameter OM channel serving as a voltage-dependent permeability pathway for large uncharged molecules (≤−5 kDa) such as NADH and metabolites. Together with the ANT on the IM, the VDAC forms the so-called mitochondrial permeability transition pore, a nonselective channel that, when opened, allows for the equilibration of ions within the matrix and intermembrane space, thereby dissipating the H+ gradient and disrupting or depolarizing ΔΨm (60, 89, 147). VDAC activity is required for apoptotic ΔΨm loss or depolarization to occur (146). Alone, the VDAC is weakly anion selective (i.e., permeable to Cl−, DIDS sensitive) (146).

Bax channels are formed on the OM when soluble Bax proteins translocate from the cytosol upon apoptotic stimulation. Bax channels exhibit cation (K+ , Na+) selectivity and, like VDAC, do not allow cyt-c release from the mitochondrial intermembrane space to the cytosol (146). However, both Bax and Bak proteins can interact with OM VDAC to form a large pore. In consequence, disruption of VDAC integrity allows cyt-c to pass into the cytosol at a rate of ~10 molecules·s−1·channel−1 (68, 146).

A mitochondrial intracellular Cl− channel (mtCLIC) has been identified on the mitochondrial IM (46); no other mitochondrial IM Cl− channels have been identified to date. Expression of mtCLIC is regulated by p53 and TNF-α, two potent proapoptotic agents, suggesting that it may be a common

![Fig. 10. Overexpression of ARC decreases Kv channel activity in embryonic rat heart H9c2 cells. A: Western blot analysis (top) showing the ARC protein levels in Neo cells (empty vector) and cells stably transfected with the human ARC-5 gene. Representative currents (bottom), elicited by test potentials between −60 and +80 mV (holding potential −70 mV), in a Neo cell and an ARC-5-transfected cell. B: averaged currents at +80 mV (holding potential, −70 mV) in Neo cells and ARC-5-transfected cells before (Cont) and after (ST) treatment with ST (0.02 μM). C: summarized data showing the percentage of cells undergoing apoptosis in Neo and ARC-5-transfected cells before (Cont) and after (ST) exposure to ST. **P < 0.001 vs. Neo. [From Ekhtera et al. (44)].](http://ajplung.physiology.org/)

![Fig. 11. Cross-sectional view of the mitochondria and the inner and outer membrane channels contribute to the regulation of apoptosis. IM, inner mitochondrial membrane; OM, outer mitochondrial membrane; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; mtKCa, mitochondrial KvCa channel, mtKATP, mitochondrial KATP channel, mtCLIC, mitochondrial intracellular Cl− channel.](http://ajplung.physiology.org/)
downstream effector for these two apoptotic stimulants. It has been suggested that changes in mitochondrial IM permeability to Cl\(^-\) via mtCLIC and changes in \(\Delta \Psi_m\) lead to activation of the mitochondrial permeability transition pore and apoptosis.

Bcl-2 channels on the mitochondrial OM are mostly closed at neutral pH. At more acidic pH (pH 5.4), Bcl-2 forms cation channels (142). The antiapoptotic Bcl-2 and Bcl-X\(_L\) channels increase cell survival by causing \(\Delta \Psi_m\) hyperpolarization, leading to 1) decreased cyt-c release, 2) increased mitochondrial uptake of cationic fluorescent dyes (e.g., rhodamine-123), 3) increased Ca\(^{2+}\) uptake, 4) increased resistance to disruption of \(\Delta \Psi_m\), 5) enhanced H\(^+\) efflux in the presence of \(\Delta \Psi_m\)-depolarizing stimuli (without influencing K\(^+\) efflux from the mitochondria), 6) maintained mitochondrial osmotic homeostasis, 7) VDAC closure, and 8) prevention of Bak/Bax dimerization and translocation to the OM (2, 62, 142, 145, 147, 164, 174).

The idea that \(\Delta \Psi_m\) depolarization is required for translocation of cyt-c from the mitochondria is not, however, universally accepted (19). The aforementioned effects are specific to local mitochondrial regulation. Bcl-2 can also modulate MMP and \(\Delta \Psi_m\) indirectly via control of sarcolemmal K\(^+\) permeability. When plasma membrane potential (\(E_m\)) is more depolarized, \(\Delta \Psi_m\) should be more hyperpolarized to maintain mitochondrial membrane integrity and to contain cyt-c within the mitochondria. Because antiapoptotic Bcl-2 can decrease \(K_v\) currents and cause \(E_m\) depolarization, it is not surprising that release of cyt-c is also inhibited by Bcl-2 (43, 146). One must not, however, assume that modulation of sarcolemmal K\(^+\) channels by apoptotic agents is itself linked to changes in \(\Delta \Psi_m\). For example, in PASMC, ST-induced \(\Delta \Psi_m\) depolarization (visualized as increased rhodamine-123 fluorescence) is not blocked by decreased K\(^+\) efflux due to increased [K\(^+\)]\(_i\) (from 5 to 40 mM) (86). Rapid FCCP-induced depolarization of \(\Delta \Psi_m\) is not mimicked by enhanced [K\(^+\)]\(_i\) (from 5 to 25 mM), or by inhibition of \(K_a\) channels by TEA or iberiotoxin (Fig. 12) (87). In the latter example, FCCP depolarization of \(\Delta \Psi_m\) likely causes Ca\(^{2+}\) release from the mitochondria to the cytosol, thereby activating \(K_a\) channels and contributing to K\(^+\) efflux, AVD, and apoptosis. Finally, SNAP induces apoptosis and gradual \(\Delta \Psi_m\) depolarization, the latter being unaffected by 40 mM [K\(^+\)]\(_i\) or iberiotoxin (88).

**K\(^+\)** CHANNELS IN THE MITOCHONDRIA

As previously mentioned, sarcolemmal K\(^+\) channel activity does not appear to contribute directly to \(\Delta \Psi_m\) regulation. Therefore, it is possible that K\(^+\) flux within intracellular organelles may modulate \(\Delta \Psi_m\). Mitochondrial mtK\(_{ATP}\) (mtK\(_{ATP}\)) channels have been identified in mitochondrial IM from neurons (33, 99, 118), cardiac cells (71, 100), and liver (75, 126). The primary functions of mtK\(_{ATP}\) channels are to control mitochondrial volume by maintaining K\(^+\) homeostasis and to enable the formation of the pH gradient and the transmembrane electric potential on the IM (33, 53). Based on pharmacological similarities, it has been hypothesized that the molecular structures (sulfonylurea receptor + Kir 6.x channel) of sarcolemmal and mtK\(_{ATP}\) channels are similar (53, 169). Nonetheless, mtK\(_{ATP}\) channels differ in that they are selectively activated by diazoxide and inhibited by 5-hydroxydecanoate (33, 100), a distinction that has proven essential in determining the physiological role of mtK\(_{ATP}\) channels.

In one scenario, activation of neuronal mtK\(_{ATP}\) channels by diazoxide causes K\(^+\) influx into the matrix, thereby causing \(\Delta \Psi_m\) depolarization (12), mitochondrial matrix swelling, cyt-c release, caspase activation, and apoptosis (33, 118, 149). Presumably, \(\Delta \Psi_m\) depolarization leads to more than just the release of cyt-c, since, in the same cells, diazoxide-induced mtK\(_{ATP}\) activation can also protect against apoptosis during ischemia, hypoxia, or ST treatments (63, 100). The protective mechanism mediated by mtK\(_{ATP}\) channel opening (which would induce \(\Delta \Psi_m\) depolarization) may involve 1) relief of the mitochondrial Ca\(^{2+}\) overload typical in cardiac ischemia-reperfusion injury (71), 2) gradual oxidation of cardiac cells and neurons (57, 100, 141, 195), 3) elevated Bcl-2 suppressing both Bax translocation to the mitochondrial membrane and cyt-c release in neurons (99), 4) enhanced ATP production (85), or 5) altered reactive oxygen species production (50, 184).

Recently identified neural (148) and cardiac (182) IM mitochondrial large-conductance \(K_a\) (mtK\(_{Ca}\)) channels may also have dual effects, mediating both apoptosis and cardioprotection. The biophysical (unitary conductance and Ca\(^{2+}\) and voltage dependence) and pharmacological (charybdotoxin sensitivity) properties of mtK\(_{Ca}\) channels are similar to those of sarcolemmal maxi-K\(_{Ca}\) channels identified in many smooth muscles (115). Cardiac mtK\(_{Ca}\) channel activity is detectable at physiological (~200 nM) and high (40 \(\mu\)M) cytosolic free Ca\(^{2+}\) concentrations (182), suggesting that it may play an important role in modulating mitochondrial function under physiological situations or during conditions of Ca\(^{2+}\) overload, such as during ischemia. During neuronal apoptosis, activation
of mtKCa might be expected to cause complete and irreversible uncoupling of the mitochondria, thereby promoting the effect of mitochondrial apoptosis. However, evidence using isolated perfused hearts suggests that, as for activated mtKATP channels, preischemic exposure to NS-1619, an mtKCa channel opener, results in ~50% protection against myocardial infarction (182).

It appears, therefore, that mitochondrial K⁺ channels may play a more protective role against ischemia-induced apoptosis, at least in cardiac myocytes. Despite this evidence, it is still unclear as to which trigger, if any, determines the ultimate outcome of mitochondrial membrane depolarization. Further study of the mtKATP and mtKCa channels is required to address the following issues: 1) Do different cell populations within a tissue contribute to the antiapoptotic response? and 2) Does the activation of mtKATP and/or mtKCa channels by environmental (e.g., ischemia/hypoxia) or chemical (e.g., ST) stresses result in protective preconditioning or apoptosis? Although mitochondrial K⁺ channels have not yet been discovered in vascular smooth muscle cells, it is possible that the relative contributions of sarcolemmal and mitochondrial K⁺ channel activity to [K⁺]cyt regulation may determine the fate of cells under pathological situations. Figure 13 provides a schematic overview of the roles of sarcolemmal and mitochondrial channels in cell survival and death. Despite the stark contrast between the proposed roles of mitochondrial and sarcolemmal K⁺ channels in the regulation of cell death, mitochondrial K⁺ channels should be regarded as potential therapeutic targets for stroke and neurodegenerative conditions (159).

**INHIBITION OF APOPTOSIS CONTRIBUTES TO THE DEVELOPMENT OF PULMONARY VASCULAR REMODELING IN PATIENTS WITH PRIMARY PULMONARY HYPERTENSION**

Primary pulmonary hypertension (PPH) is a fatal disease of unidentified etiological cause in which increased pulmonary arterial pressure and vascular resistance lead to right heart failure and death. Pulmonary vasoconstriction, pulmonary vascular wall remodeling, and in situ thrombosis are the main causes for the elevated pulmonary vascular resistance in patients with PPH (6, 49, 139). Pulmonary vascular remodeling is characterized by arterial wall thickening as a result of increased fibroblast, PASMC, and endothelial cell proliferation in the tunica adventitia, media, and intima, respectively (151). The pulmonary vascular medial hypertrophy in pulmonary hypertension is mainly due to increased PASMC growth and/or decreased PASMC apoptosis (40, 168). Therefore, the precise control of the balance between PASMC proliferation and apoptosis plays a critical role in maintaining 1) the normal structural and functional integrity of the pulmonary vasculature and 2) the low pulmonary arterial pressure in normal subjects.

In normal human PASMC, our preliminary data showed that apoptosis inducers, such as ST and cyt-c, increased K⁺ channel activity (Figs. 4–6), whereas antiapoptotic proteins (e.g., Bcl-2) decreased K⁺ channel activity (Fig. 9). Inhibition of K⁺ currents by pharmacological blockade of Kv channels or by reducing the transmembrane K⁺ gradient (e.g., raising extracellular K⁺ concentration) attenuated ST-induced apoptosis. In PASMC from PPH patients, expression of functional Kv channels...
nels and the resulting macroscopic currents were markedly reduced compared with PASMC from normal subjects and patients with secondary pulmonary hypertension (SPH) (190–192). Accordingly, ST-induced apoptosis was also significantly inhibited in PPH-PASMC compared with SPH-PASMC (196). Therefore, inhibition of apoptosis in PASMC as a result of gene downregulation and/or dysfunction of KV channels (8, 190, 192, 193) plays a critical role in the development of pulmonary vascular medial hypertrophy and the increased pulmonary vascular resistance and arterial pressure in PPH patients.

Vasoactive agonists, growth factors, and cytokines regulate PASMC proliferation and apoptosis and pulmonary vascular remodeling (Fig. 14) (96, 111, 114, 127). Bone morphogenetic proteins (BMP) are part of the greater transforming growth factor-β family of polypeptides that regulate a wide spectrum of cellular functions, such as proliferation, differentiation, migration, and apoptosis (107). Mutations of the BMP receptor type II gene (BMP-RII) have been identified in patients with familial and sporadic PPH (34, 92, 102, 117). The protein expression level of BMP-RII is significantly decreased in lung tissues from PPH patients with or without mutations in the BMP-RII gene compared with normal subjects and patients with SPH (9). These observations suggest that dysfunction and/or downregulation of BMP-RII and/or its downstream signaling may play an important role in the development of pulmonary vascular medial hypertrophy.

In PASMC from normotensive patients, low doses (10–100 nM) of BMPs (e.g., BMP-2, -4, and -7) inhibit cell proliferation (determined by [3H]thymidine incorporation), whereas high doses (100–200 nM) of BMPs (e.g., BMP-2 and -7) induce cell apoptosis (113, 196). The BMP-mediated antiproliferative and proapoptotic effects on PASMC are significantly inhibited in PPH from PPH patients compared with PASMC from normal subjects and patients with SPH (196). These results provide compelling evidence that BMPs and their receptors and downstream signal transduction are involved or required for preventing normal PASMC from overgrowth (i.e., hypertrophy and hyperplasia), which is important in maintaining the thin pulmonary vascular wall and low pulmonary vascular resistance under normal conditions. Mutation and/or downregulation of BMP ligands and receptors as well as defects in the downstream signaling pathway would therefore enhance pulmonary vascular remodeling and increase pulmonary vascular resistance and arterial pressure in patients with PPH (9, 32, 113, 140, 196).

The precise mechanisms by which BMPs induce apoptosis in normal human PASMC are still unknown. Our preliminary study indicates that treatment of normal PASMC with BMP-2 decreases the mRNA and protein expression of Bcl-2, an antiapoptotic protein that attenuates apoptosis by inhibiting cyt-c release (81, 183), blocking K⁺ channels (43), downregulating K⁺ channel gene expression (43), maintaining Ca²⁺ in the sarcoplasmic/endoplasmic reticulum (65, 91), and regulating proton flux in mitochondria (145). Geraci et al. (54) show that the mRNA expression of Bcl-2 was upregulated in lung tissues from sporadic and familial PPH patients. The upregulated Bcl-2 gene transcription may be related to the mutations in BMP-RII gene and/or dysfunction of BMP signaling. These results imply that modulation of Bcl-2 gene expression is a critical mechanism in directing human PASMC to undergo proliferation or apoptosis.

**INDUCTION OF APOPTOSIS AS A THERAPEUTIC APPROACH FOR PATIENTS WITH PULMONARY HYPERTENSION**

Apoptosis is a highly regulated process in which cells that are no longer needed during development and vascular cells that undergo “misguided” hypertrophy and hyperplasia are removed. Activation of apoptosis is implicated in the regression of pulmonary vascular medial hypertrophy, whereas inhibition of apoptosis may lead to the progression of pulmonary vascular wall thickening (Fig. 14B).

In addition to the protein-protein interactions that regulate apoptosis, protein-matrix interactions are also involved in the arrest of induced apoptosis.
cell proliferation and migration associated with vascular wall remodeling. Therefore, another avenue of treatment for pulmonary hypertension is the modulation of extracellular matrix glycoproteins, whose expression and deposition is 1) increased in clinical and experimental progressive pulmonary hypertension and 2) linked to increased cell migration, proliferation, and apoptosis (132, 133). Tenascin-C, one such matrix glycoprotein, is induced by matrix metalloproteinases and amplifies the response of smooth muscle to growth factors. Treatment of pulmonary hypertensive (due to hypoxia or monocrotaline) rats with serine elastase and matrix metalloproteinase inhibitors suppresses tenascin-C induction and collagen and elastin deposition, resulting in complete reversal of pulmonary hypertension (26–28, 138). Antisense tenascin-C treatment (with osteopontin blockade) also induces apoptosis in hypertrophied pulmonary arteries. Therefore, disrupted matrix glycoprotein attachment appears to be a powerful trigger for apoptosis, making it a logical target in the treatment of pulmonary intimal hypertrophy.

Similar to pulmonary vascular thickening (or medial hypertrophy) in pulmonary hypertension, cancers arise not only due to unrestrained cell proliferation, but also due to insufficient apoptotic turnover (60). In recent years, the modulation of apoptosis has gained much interest as a potential therapeutic target for diseases, such as cancer, where increased cell proliferation or decreased cell apoptosis is a primary diagnostic tool. As discussed earlier, many enzymes, ligands, receptors, signal transduction proteins, and transcription factors are involved in the apoptotic cascade; very few conventional drugs are available that specifically target these factors. Combined with the fact that drug therapies effective in the brain may not work in the heart and lung, attention has been focused on gene antisense therapies aimed at intermediary apoptotic proteins, particularly Bcl-2. G-3139 is an antisense oligonucleotide that targets the first six codons of human bcl-2; its binding to mRNA precludes translation into Bcl-2 protein (119). As of late 2000, G-3139 was involved in phase III clinical trials for the treatment of malignant melanoma, non-Hodgkin’s lymphomas, and leukemia, in which Bcl-2 levels are elevated. Attempts are also underway to combine Bcl-2 antisense therapy with common chemotherapeutic therapies to combat lung, prostate, breast, and colorectal cancers (119).

As with cancers, antisense Bcl-2 strategies may also prove effective in the treatment of pulmonary hypertension. In normal PASMC, overexpression of Bcl-2 inhibits apoptosis by downregulating K+ channel gene expression and by decreasing K+ efflux via sarcolemmal Kv channels (43). In familial and sporadic PPH patients, Bcl-2 levels in lung tissues are significantly higher than in normal subjects and patients with SPH (54). In PPH patients, the mRNA expression and activity of Kv channels in PASMC are also decreased compared with normal subjects and patients with SPH (190, 191, 194). Therefore, inhibition of bcl-2 gene expression using antisense and overexpression of K+ channels using adenoviral vectors can both be potential therapeutic approaches for patients with PPH. Indeed, Pozeg and colleagues (130) recently reported that in vivo gene transfer of Kv1.5, a delayed-rectifier Kv channel that is downregulated in PPH patients (190, 194) and in animals with hypoxia-mediated pulmonary hypertension (7, 128, 150, 171), significantly reduced pulmonary hypertension and right heart hypertrophy in rats.

Another avenue that bears more scrutiny is the use of NO as a proapoptotic stimulus. We have shown in the past that NO (SNAP) and DHEA induce apoptosis of human PASMC by promoting K+ efflux through activated KCa and Kv channels (88) and that DHEA also has an additive effect on SNAP-induced apoptosis. Recently, one group has shown that DHEA ingestion by hypoxic rats dose dependently inhibits the hypoxia-induced increase in pulmonary artery pressure, total pulmonary resistance, and right ventricular hypertrophy, while having minimal effects on systemic pressure or vascular resistance (122). Therefore, it is possible that treatment with K+ channel openers may reverse the physiological effects of pulmonary hypertension via parallel (but not necessarily related) processes that target both pulmonary vasoconstriction and the increased medial hypertrophy. Figure 15 presents a schematic summary of how modulation of K+ channel expression and function by genetic and environmental factors influences both pulmonary vascular remodeling and hypertrophy in the development of pulmonary hypertension.

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Fig. 15. Schematic diagram showing the potential roles played by ion channel activity and intracellular Ca2+ in modulating cell proliferation and apoptosis in regulating pulmonary vascular tone and remodeling in patients with pulmonary arterial hypertension. CREB, cAMP response element-binding protein; Ca2+/CaM, calcium-calmodulin complex; PVR, pulmonary vascular resistance; VDCC, voltage-dependent Ca2+ channels.
CONCLUSIONS

Ion permeation through transmembrane channels regulates a variety of processes, including, but not limited to, excitation-contraction coupling, cell volume regulation, protein trafficking, DNA replication and fragmentation, nerve transmission, cell metabolism, cell proliferation, and cell death. Apoptosis is a process that plays a critical role in embryonic development and tissue homeostasis; a balance between proliferation and apoptosis controls cell number or density. In humans, dysfunction of target cells by cytotoxic lymphocytes: molecular and cellular aspects. Annu Rev Immunol 12: 735–773, 1994.


Dallaporta B, Marchetti P, de Pablo MA, Maisse C, Duc H-T, Dallaporta B, Hirsch T, Susin SA, Larochette N, Bernardi P. Bone morphogenetic proteins, in particular, play an especially important role in the maintenance of so-called ‘normal’ physiology. Regulated apoptosis has been linked to the pathogenesis of cancer, atherosclerosis, and pulmonary vascular disease. 

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ACTIVATION OF K⁺ CHANNELS


Invited Review

ACTIVATION OF K⁺ CHANNELS


