J. Membrane Biol. 209, 3–20 (2006)
DOI: 10.1007/s00232-005-0838-4

The Journal of
Membrane
Biology

K^+ Channels in Apoptosis

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Received: 25 January 2006/Online publication: 17 April 2006

Abstract. A proper rate of programmed cell death or apoptosis is required to maintain normal tissue homeostasis. In disease states such as cancer and some forms of hypertension, apoptosis is blocked, resulting in hyperplasia. In neurodegenerative diseases, uncontrolled apoptosis leads to loss of brain tissue. The flow of ions in and out of the cell and its intracellular organelles is becoming increasingly linked to the generation of many of these diseased states. This review focuses on the transport of K^+ across the cell membrane and that of the mitochondria via integral K^+-permeable channels. We describe the different types of K^+ channels that have been identified, and investigate the roles they play in controlling the different phases of apoptosis: early cell shrinkage, cytochrome *c* release, caspase activation, and DNA fragmentation. Attention is also given to K^+ channels on the inner mitochondrial membrane, whose activity may underlie anti- or pro-apoptotic mechanisms in neurons and cardiomyocytes.

Key words: Apoptosis — K^+ channels — Plasma membrane — Mitochondria

Introduction

Programmed cell death, apoptosis, is vital to an organism to maintain normal tissue homeostasis, limit cell growth and rid the organism of unwanted cells. An imbalance in cell apoptosis and survival (or proliferation) is a characteristic feature in many disease states such as hypertension, cancer, and neurodegenerative disorders, and is also an important feature in immune responses, embryonic development and response to ischemia. In the last decade, deregulated transmembrane ion flow via ion channels has been implicated in the initiation and progression of apoptosis. These ion channels include those on the plasma membrane, as well as those on intracellular membranes, such as in the mitochondria. Potassium ion (K^+) channels are ubiquitously found in many cell types, including neurons, cardiomyocytes, skeletal muscle cells, erythrocytes, pancreatic β cells, smooth muscle cells, and lymphocytes. Activation of K^+ channels has generally been associated with the control of the resting membrane potential and the rate of repolarization after an action potential in excitable cells such as neuronal, cardiac, and smooth muscle cells. However, numerous studies have implicated K^+ channel (dys) function in the regulation of apoptosis. This review provides an overview of the role of intracellular K^+ and sarcolemmal (and mitochondrial) K^+ channels in cell volume regulation and apoptosis.

Functional Classification and Structure of K^+ Channels

Currently, there are five main functional classes of K^+ channels: voltage-gated K^+ (Kv) channels, Ca^{2+}-activated K^+ (K_{Ca}) channels, ATP-sensitive K^+ (K_{ATP}) channels, inwardly rectifying K^+ (K_{IR}) channels, and two-pore domain K^+ (K_{2P}) channels. Their molecular identity, electrophysiological and pharmacological properties, and structure and function are described below.

Voltage-gated K^+ (Kv) Channels

Characterization

Kv channels are characterized by their blockade by 4-aminopyridine (4-AP), dendrotoxin and tityustoxin as well as by their sensitivity to membrane potential (E_m).

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They open upon membrane depolarization, enabling K^+ efflux based on its electrochemical driving force ($[K^+]_{in} >> [K^+]_{out}$), causing membrane hyperpolarization. The properties of Kv currents are diverse due to association of the pore-forming α subunits with modulatory β subunits and alternative splicing of primary transcripts (Isom, De Jongh & Catterall, 1994; Chandy & Gutman, 1995; Martens, Kwak & Tamkun, 1999). There are twelve Kv subfamilies (Kv1-Kv12). Kv1-Kv4 were initially identified in mutant *D. melanogaster* (Papazian et al., 1987), and all have mammalian homologues. Although their sequences vary, these Kv channels can be distinguished based on current kinetics (Chandy & Gutman, 1995; Coetzee et al., 1999). For example, classical delayed rectifier Kv channels (e.g., Kv1.5 and Kv2.1) activate after a delay following membrane depolarization and inactivate slowly, whereas *A*-type channels (e.g., Kv1.4 and Kv4) activate and inactivate rapidly (Coetzee et al., 1999; Hille, 2001). Kv7 channels generate the "*M* current", a non-inactivating, voltage-dependent current with a role in regulating neuronal excitability and action potential firing frequency (Delmas & Brown, 2005; Gu et al., 2005).

Kv5 (Zhao et al., 1994), Kv6 (Post, Kirsch & Brown, 1996; Kramer et al., 1998; Zhu et al., 1999; Sano et al., 2002), Kv8 (Hugnot et al., 1996), and Kv9 (Patel, Lazdunski & Honoré, 1997, 1999b; Salinas et al., 1997; Kerschensteiner, Soto & Stocker, 2005) subunits cannot form functional channels, but associate with and alter the current properties and pharmacology of other Kv channels. Kv2.1 is an example of a functional Kv channel whose properties (oxygen sensitivity, inactivation kinetics, sensitivity to TEA, voltage-dependence) are altered by the electrically silent pore-forming α (or γ) subunits, such as Kv6.1, Kv8.1, and Kv9.3 (Hugnot et al., 1996; Post et al., 1996; Patel et al., 1997; Salinas et al., 1997; Kramer et al., 1998; Hulme et al., 1999; Zhu et al., 1999; Ottschytsch et al., 2002; Sano et al., 2002; Kerschensteiner et al., 2005). Kv10 and Kv11 are also quiescent regulatory subunits in some tissues (Ottschytsch et al., 2002; Vega-Saenz de Miera, 2004). However, in others, Kv10 (*eag*), Kv11 (*erg*), and Kv12 (*elk*) subunits underlie a family of functional voltage-dependent K^+ channels, such as the human *ether a go-go* channel (*HERG*) whose functional alteration is linked to cardiac arrhythmia (Shi et al., 1998; Ganetzky et al., 1999; Keller, Platoshyn & Yuan, 2005).

Structure

Native Kv channels exist as homo- or hetero-tetramers of pore-forming α subunits associated with regulatory β subunits (in a stoichiometry of $\alpha_4\beta_4$). The Kv channel α subunits have six transmembrane domains (S1–S6) and cytoplasmic N- and C- termini (Fig. 1*A*). The highly-conserved S5–S6 region constitutes the channel's conduction pore (P-region) and K^+ selectivity filter (Doyle et al., 1998; Choe, 2002), while the S4 domain contains a positively charged voltage sensor (Butler et al., 1989; Long, Campbell & Mackinnon, 2005b); transduction of the 'open conformation' message to the pore may involve the interaction of the S4–S5 linker with the S6 α helix (Chanda et al., 2005; Choe, 2002; Long et al., 2005b; Posson et al., 2005). An N-terminal heteromerization domain is involved in α-α (Hugnot et al., 1996; Post et al., 1996; Choe, 2002) and α-β interactions (Sewing, Roeper & Pongs, 1996; Yu, Xu & Li, 1996; Gulbis et al., 2000; Long, Campbell & Mackinnon, 2005a). In rapidly inactivating Kv channels, such as Kv1.4 and Kv4, a 20-amino acid region upstream of this domain forms a charged 'ball' that blocks the channel conduction pathway (Kurata, Wang & Fedida, 2004; Pourrier et al., 2004) or confers rapid inactivation upon slowly or non-inactivating Kv channels (Zagotta, Hoshi & Aldrich, 1990).

Association of regulatory Kv β subunits with functional Kv α subunits contributes to the diversity of Kv currents (Sewing et al., 1996). Cytoplasmic Kv β subunits *a*) confer rapid inactivation onto non-inactivating Kv channel α subunits (ball-and-chain model) (Rettig et al., 1994; Bähring et al., 2001), *b*) modify current biophysics (kinetics and amplitude) by acting as an open-channel blocker (Rasmusson et al., 1997), *c*) participate in α subunit assembly and transport to the plasma membrane (Martens et al., 1999), *d*) enhance α subunit interaction with kinases (Gong et al., 1999), and *e*) confer redox and O_2 sensitivity (Pérez-García, López-López & González, 1999; Coppock & Tamkun, 2001).

Ca^{2+}-Activated K^+ (K_{Ca}) Channels

Characterization

The first recordings of unitary K_{Ca} currents in rat myotubes characterized its K^+ selectivity, high-to-intermediate conductance (60–100 pS), and dependence on elevated cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) and E_m depolarization (Pallotta, Magleby & Barrett, 1981). K_{Ca} channels are subcategorized based on single-channel conductance and pharmacological properties.

Large-conductance (>200 pS) K_{Ca} channels (BK_{Ca} or maxi-K_{Ca}), are sensitive to inhibition by charybdotoxin and iberiotoxin (Nelson & Quayle, 1995). At physiological $[Ca^{2+}]_{cyt}$ ($\leq$ 100 nM), BK_{Ca} are only voltage dependent. However, at higher (i.e., in micromolar concentration) $[Ca^{2+}]_{cyt}$, BK_{Ca} channels activate at less depolarized potentials. In other words, increased $[Ca^{2+}]_{cyt}$ enhances voltage dependence of BK_{Ca} channels. The ability to open at less depolarized E_m makes BK_{Ca} channels ideal feedback

regulators of Ca^{2+} entry: Ca^{2+} entering through voltage-gated Ca^{2+} channels (VGCC) after depolarization activates BK_{Ca} channels, enabling E_m hyperpolarization (and repolrization), closure of VGCC, and diminished Ca^{2+} influx.

Intermediate conductance K_{Ca} (IK_{Ca}: 50–200 pS) are specifically inhibited by TRAM-34 (Köhler et al., 2003) and clotrimazole (Ouadid-Ahidouch et al., 2004). Small conductance K_{Ca} channels (SK_{Ca}, 2–15 pS) are sensitive to extracellular apamin (Stocker, 2004). Although they are sensitive to changes in $[Ca^{2+}]_{cyt}$ (300–700 nM) (Köhler et al., 1995; Neylon et al., 1999), prior binding of calmodulin is required for Ca^{2+} sensitivity of IK_{Ca} and SK_{Ca} channels (Keen et al., 1999; Neylon et al., 1999; Xia et al., 1998). Activation of IK_{Ca} and SK_{Ca} channels is weakly voltage-dependent and the currents show inward rectification at positive E_m due to voltage-dependent block by intracellular divalent cations (Köhler et al., 1995; Xia et al., 1998; Soh & Park, 2001).

Structure

The basic structure of the pore-forming α subunits of K_{Ca} channels is similar to that of Kv channels (Fig. 1*B* and *C*). BK_{Ca} channel α subunits have an additional N-terminus (SO) segment, which allows for interaction with membrane-spanning regulatory β subunits (Fig. 1*B*) (Meera et al., 1997). SK_{Ca} and IK_{Ca} channels have fewer positive residues in the voltage sensor (S4 transmembrane domain) than Kv and BK_{Ca} channels, possibly explaining their voltage independence (Stocker, 2004). BK_{Ca} and IK/SK_{Ca} channel subunits also assemble as tetramers, with β subunits in the case of BK_{Ca}. The long C-terminal region of the K_{Ca} channel α subunit (BK_{Ca}) is divided into hydrophobic domains (S7–S10) containing regulatory sites (Wang et al., 1999a). In the *Slo* gene (Butler et al., 1993; McCobb et al., 1995), which encodes for BK_{Ca} α subunits, a Ca^{2+} bowl in the S9–S10 domains underlies its Ca^{2+} sensitivity (Toro et al., 1998). This "Ca^{2+} bowl" is not present in IK_{Ca}/SK_{Ca} channels; the Ca^{2+} sensitivity of IK_{Ca}/SK_{Ca} channels is conferred by the interaction of calmodulin with sites on the C-terminus just distal to the S6 domain (Keen et al., 1999; Neylon et al., 1999) (Fig. 1*C*). The two-domain transmembrane-spanning regulatory BK_{Ca} β subunit is covalently linked to the S0 domain of BK_{Ca} α subunits (Knaus et al., 1994). It modulates the BK_{Ca} channels' voltage dependence, Ca^{2+} sensitivity, and kinetics (McCobb et al., 1995; Tseng-Crank et al., 1996; Brenner et al., 2000; Cox & Aldrich, 2000; Xia, Ding & Lingle, 2003; Orio & Latorre, 2005). The β subunit may also increase the affinity of BK_{Ca} channels for charybdotoxin (Hanner et al., 1997).

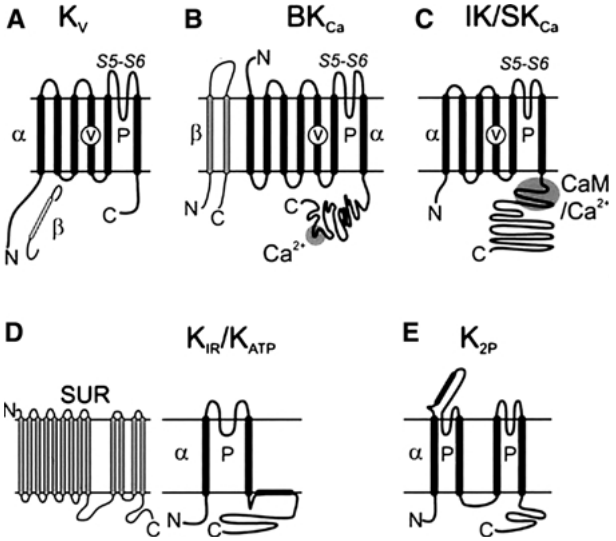

Fig. 1. Structure of K^+ channels. Schematic representation of Kv (*A*), BK_{Ca} (*B*), IK/SK_{Ca} (*C*), K_{IR}/K_{ATP} (*D*), and K_{2P} (*E*) channels. Pore-forming α subunits are highlighted in the gray boxes. Pores are identified as '*P*' and voltage sensor as '*V*'. C-terminal Ca^{2+} binding domains are depicted for BK_{Ca} and IK/SK_{Ca} channels.

Inward Rectifier K^+ (K_{IR}) Channels

Characterization

K_{IR} activity is modulated by two factors, E_m and extracellular K^+ concentration: E_m hyperpolarization (relative to the reversal potential or equilibrium potential for K^+) and moderately elevated (7–15 mM) external $[K^+]$ activate K_{IR} (Nelson & Quayle, 1995). Inward rectification at more positive potentials is due to time-dependent block by endogenous Mg^{2+} and polyamines (Ficker et al., 1994; Lopatin, Makhina & Nichols, 1994). K_{IR} are inhibited by Ba^{2+}, but not TEA, charybdotoxin, iberiotoxin, and glibenclamide (Quayle, Dart & Standen, 1996).

Structure

The functional pore-forming K_{IR} channel subunit has two membrane-spanning domains and a conserved pore-forming loop domain or P region (*Fig. 1D*) (Nishida & MacKinnon, 2002; Kuo et al., 2003) that is homologous to that of Kv and K_{Ca}. K_{IR} channels also assemble as tetramers (Kuo et al., 2003). An additional membrane-bound cytoplasmic α helix, the slide helix, can move laterally to displace the inner helix and is involved in K_{IR} channel gating (Kuo et al., 2003). The large C-terminal domain is also believed to contribute to pore structure formation, acting as an inner lining of β sheets, which regulate inward rectification by Mg^{2+} or polyamines (Yang, Jan & Jan, 1995; Nishida & MacKinnon, 2002; Kuo et al., 2003). In the closed state, the K_{IR} C-terminus

covers the cytoplasmic side of the pore and causes conformational changes that preclude K^+ permeation.

K_{IR} channels are subdivided into 7 subfamilies ($K_{IR}1$-$K_{IR}7$) based on sensitivity to endogenous signals (Doupnik, Davidson & Lester, 1995). $K_{IR}1$ (ROMK1), $K_{IR}4$, and $K_{IR}6$ consist of ATP-sensitive pore-forming subunits. $K_{IR}2$ (IRK) forms the classic, constitutively active, strong inward rectifiers found mostly in the heart, but also in vascular smooth muscle and endothelial cells, skeletal muscle and brain (Kubo et al., 1993a; Doupnik et al., 1995). $K_{IR}3$ (GIRK) is a strongly rectifying G protein-gated channel regulated by G protein βγ subunits (Kubo et al., 1993b); it has been implicated in regulating cardiac activity in response to vagal stimulation. $K_{IR}5$ does not generate currents when expressed alone, but it may coassemble with and modulate the properties of other K_{IR} subunits (Doupnik et al., 1995).

ATP-Sensitive K^+ (K_{ATP}) Channels

Characterization

K_{ATP} channels are inhibited by intracellular ATP and activated by nucleotide diphosphates (Beech et al., 1993; Clapp, 1995; Quayle, Nelson & Standen, 1997). K_{ATP} channels are also modulated by phospholipids (PIP_2) (Baukrowitz et al., 1998). Voltage-independent K_{ATP} channels can be subclassified as intermediate (5–50 pS) or large (~130 pS) unitary conductance channels. However, all are highly sensitive to inhibition by glibenclamide (Clapp, 1995), and are activated by K^+ channel openers such as pinacidil, lemakalim, and cromakalim (Clapp & Gurney, 1992; Bonev & Nelson, 1993; Clapp, 1995; Quayle et al., 1997). The variable conductance and pharmacological properties suggest that various species of K_{ATP} channels are expressed in different cell types where their roles vary dramatically (Noma, 1983; Ashcroft & Kakei, 1989; Clapp & Gurney, 1992; Bonev & Nelson, 1993; Conway, Nelson & Brayden, 1994; Liu et al., 1996; Ashcroft, 2005; Fitzpatrick et al., 2005).

Structure

The ion-conducting structure of K_{ATP} channels is the K_{IR} subunit (Ho et al., 1993). However, these subunits alone do not conduct ions; a functional K_{ATP} channel is formed from the octameric association of four $K_{IR}6$ subunits with four sulfonylurea receptor (SUR) subunits (Inagaki et al., 1995) (Fig. 1*D*). ATP sensitivity is conferred by association of K_{IR} with the ATP-binding SUR (Aguilar-Bryan et al., 1995; Inagaki et al., 1995). Raising intracellular ATP inhibits currents generated by weakly rectifying $K_{IR}6$ subunits. Activation of K_{ATP} by PIP_2 is believed to be due to decreased binding of ATP to the SUR (Baukrowitz et al., 1998), although PIP_2 can also bind directly to the K_{IR} subunit (Huang, Feng & Hilgemann, 1998). Modulation by PIP_2 is common to ATP-sensitive $K_{IR}1$, $K_{IR}2$, $K_{IR}3$, and $K_{IR}6$ subunits (Baukrowitz et al., 1998; Huang et al., 1998).

Two-pore K^+ (K_{2P}) Channels

Characterization

These voltage-insensitive and constitutively active K_{2P} channels represent a novel class of K^+ channels. They differ from all other K^+ channels in a few ways: *a*) the current shows no voltage dependence and does not inactivate, *b*) the current is active at all E_m, and *c*) the channels are not blocked by 4-AP or TEA, but rather by quinine, quinidine, and zinc (Lesage & Lazdunski, 2000). Activation of K_{2P} channels is associated with a strong membrane hyperpolarization; they may be important in setting, maintaining, and regulating the resting E_m (Lesage et al., 1996a; Gurney et al., 2003). There are six structural and functional groups of K_{2P} channels: *1*) tandem pore, weak inwardly rectifying (TWIK) K_{2P} channels; *2*) TWIK-related acid-sensitive (TASK) K_{2P} channels, *3*) TWIK-related (TREK) and arachidonic acid-stimulated (TRAAK) K_{2P} channels, *4*) TWIK-related alkaline pH-activated (TALK) K_{2P} channels; *5*) tandem pore halothane-inhibited (THIK) K_{2P} channels; and *6*) TWIK-related spinal cord (TRESK) K_{2P} channels (Patel & Lazdunski, 2004). TWIK, TALK, and TASK are sensitive to changes in pH (Lesage et al., 1996a; Duprat et al., 1997; Gurney et al., 2003). TASK, TREK, THIK, and TRAAK channels are regulated by inhalational general anesthetics (Patel et al., 1999a; Buckler, Williams & Honoré, 2000; Gurney et al., 2002). TASK and TALK channels are sensitive to changes in oxygen tension and the production of reactive oxygen species (Lewis et al., 2001; Johnson, O'Kelly & Fearon, 2004; Caley, Gruss & Franks, 2005; Duprat et al., 2005). TASK channels are also modulated by changes in cell volume (Niemeyer et al., 2001). In pulmonary artery smooth muscle cells, TASK-1 seems to be important in setting resting E_m (Gurney et al., 2002).

Structure

K_{2P} channels have a structure unlike any other K^+ channel, with four transmembrane domains and two ion-conducting pores (Fig. 1*E*). Where Kv channel α subunits are divided based largely on sequence homology, the latter is generally low ($< 45\%$ for human isoforms) among K_{2P} channels, with the notable exception of TWIK-1/TWIK-2 (58%) and TREK-1/TRAAK (54%) (Lesage & Lazdunski, 2000). Thus, sequence conservation may be associated more with functional properties (e.g., sensitivity to anesthetics),

although there are exceptions to this rule (e.g., TASK-1 and TASK-2 are acid-sensitive but have < 33% homology). Also, functional K_{2P} channels exist as homo- or hetero-dimers instead of tetramers. TASK-1 and TASK-3 form functional heterodimers in *Xenopus* oocytes (Czirják & Enyedi, 2002). TWIK-1 can self-associate to form a disulfide bridge homodimer (Lesage et al., 1996b). All K_{2P} channels (except TASK-1) contain a cysteine residue within an amphipathic α helix known as the "self-interacting domain" (found on the loop between the first transmembrane segments); this cysteine facilitates homodimerization (Lesage & Lazdunski, 2000) and may participate in binding of regulatory proteins.

Mitochondrial Ion Channels

The mitochondrion plays an intrinsic role in apoptosis. Altered mitochondrial membrane permeability (MMP) to ions and/or apoptotic factors is at the heart of apoptosis, whether it is mediated by the death receptor or mitochondrial pathways (Ferri & Kroemer, 2001; Remillard & Yuan, 2004). Under normal physiological conditions, the inner membrane is virtually impermeable, so the extrusion of H^+ by the electron transport chain creates a highly negative mitochondrial transmembrane potential ($\Delta\Psi_m$) ranging from −150 to −200 mV. Altered MMP may be due to disrupted ATP-ADP exchange by the inner membrane adenine nucleotide translocase (ANT) and the outer membrane voltage-dependent anion channels (VDAC). While dysfunctional ATP-ADP exchange renders the outer membrane permeable to soluble proteins, disrupted $\Delta\Psi_m$ renders the inner membrane permeable. In addition to VDAC and ANT, other channels and exchange proteins that populate the mitochondrial membranes contribute to the regulation of $\Delta\Psi_m$. Below, we briefly describe a few key mitochondrial channels (Fig. 2) that are implicated in the regulation of apoptosis. The specific role of mitochondrial K^+ channels in apoptosis will be discussed more extensively in the section Transmembrane K^+ Flow and Apoptosis.

VDAC is a weakly Cl^--selective channel. However, when co-assembled with the ANT in regions where the inner and outer membranes are adjacent, the VDAC-ANT complex is a nonselective channel, the "mitochondrial permeability transition pore (MPTP)", that opens to allow for the equilibration of ions within the matrix and intermembrane space, dissipating the proton gradient and causing mitochondrial membrane depolarization (Kroemer & Reed, 2000). Association of VDAC with Bax/Bak proteins (or cation channels) forms a large pore which allows cytochrome *c* (cyt *c*) to pass from the matrix to the cytosol upon mitochondrial membrane depolarization (Shimizu et al., 2000a). Activation of

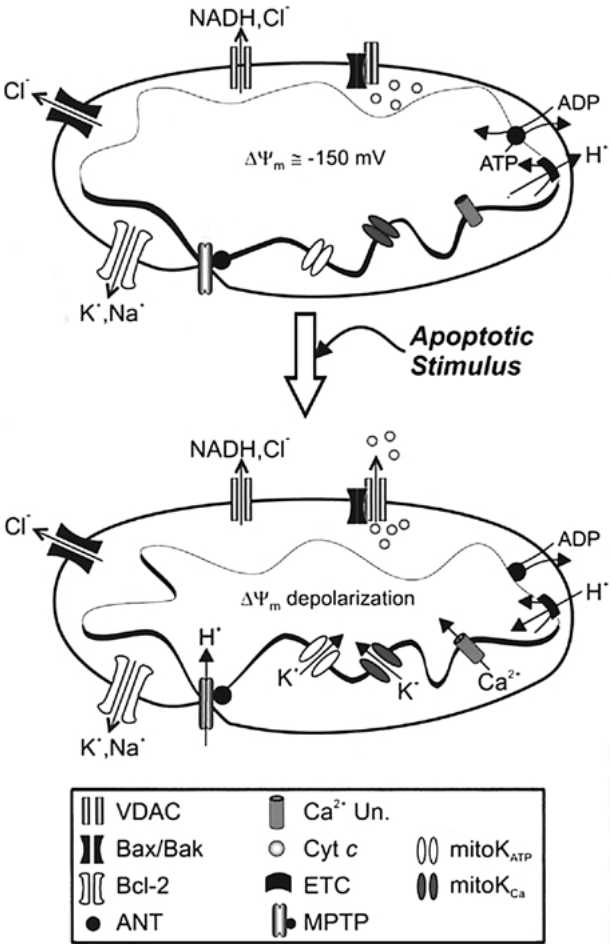

Fig. 2. Ion flux in the mitochondrion. Mitochondrial channels and simplified ion flux at rest (*top*) and during an apoptotic challenge (*bottom*). Channel properties are described in the text. Apoptotic mitochondrion shows increased K^+, Ca^{2+}, and H^+ influx, cyt *c* efflux via VDAC/Bax/Bak complexes, and matrix shrinkage. *ANT*, adenine nucleotide transporter; Ca^{2+} *Un.*, Ca^{2+} uniporter; *ETC*, electron transport chain; *MPTP*, mitochondrial permeability transition pore; *VDAC*, voltage-dependent anion channel.

outer membrane Bcl-2 and Bcl-x_L proteins (or channels) increases cell survival by promoting mitochondrial membrane hyperpolarization, which attenuates cyt *c* release, enhances H^+ efflux, helps maintain mitochondrial homeostasis, closes VDAC, and prevents outer membrane translocation and dimerization of Bax/Bak channels (Vander Heiden et al., 1997; Gross et al., 1998; Shimizu et al., 1998, 2000b).

K_{ATP} (mitoK_{ATP}) and K_{Ca} (mitoK_{Ca}) channels on the inner mitochondrial membrane (Inoue et al., 1991; Xu et al., 2002) are similar to their plasma membrane counterparts. Large-conductance (~300 pS) mitoK_{Ca} channels sensitive to increased $[Ca^{2+}]_{cyt}$ (Siemen et al., 1999; Xu et al., 2002) contribute to background K^+ conductance/influx and mitochondrial K^+ uptake, and play a role in protecting against ischemia. The mitoK_{Ca} currents are blocked by iberiotoxin and charybdotoxin, but not by the mi-

toK_{ATP} channel blocker, 5-hydroxydecanoate (Siemen et al., 1999; Xu et al., 2002). MitoK_{ATP} channels are inhibited by glibenclamide and elevated ATP. The activity of mitoK_{ATP} channels is stimulated by diazoxide and inhibited by 5-hydroxydecanoate, neither one of which significantly affects plasma membrane K_{ATP} channels, allowing for pharmacological distinction of the two channels (Inoue et al., 1991; Liu et al., 1998; Dębska et al., 2001). Structurally, mitoK_{ATP} are also composed of two subunits: a 55-kD mitoK_{IR} ($K_{IR}6$) and a 63-kD mitoSUR. The roles of mitoK_{ATP} channels in ischemic preconditioning, cardioprotection, and mitochondrial volume regulation through control of K^+ homeostasis will be discussed later.

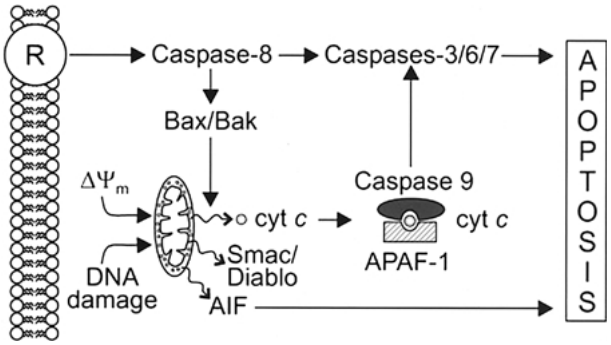

Fig. 3. Major proteins involved in extrinsic and intrinsic apoptosis. Extrinsic apoptosis involves binding to a death receptor '*R*' and subsequent caspase activation. Intrinsic apoptosis involves disruption of mitochondrial integrity, followed by cyt *c* release and formation of the apoptosome. Cross-talk between the two pathways is shown via the effect of Bax/Bak proteins on cyt *c* release. *AIF*, apoptosis-inducing factor.

Regulation of Cell Volume

Maintenance of cell volume depends largely on ionic movement into and out of the cell, which itself influences the passive transport of water across the plasma membrane through aquaporins to maintain normal hydrostatic pressure gradients. In smooth muscle cells, K^+ and Cl^- are the most concentrated intracellular cation and anion, respectively (Aickin & Brading, 1982). The large K^+ gradient (~28 times more concentrated inside the cell) is largely due to the activity of the Na^+/K^+ ATPase or Na^+ pump, which expels 3 Na^+ ions for every 2 K^+ ions it imports, creating a voltage potential across the cell membrane and an intracellular environment that is high in K^+ (~140 mM) and low in Na^+ (~10 mM). At rest, cell permeability to K^+ is greater than it is for other ions; therefore, K^+ permeability is an important regulator of cell volume. Increased K^+ efflux due to K^+ channel opening has been reported to be an initial step in cell shrinkage: K^+ efflux prompts Cl^- efflux from the cell, as both the electrical and concentration gradients favor its extrusion. The extrusion of K^+ and Cl^- cause an imbalance between intracellular and extracellular osmolarity. To restore osmotic balance to the cell, water exits through aquaporins (i.e., channels that allow water to pass), and the cell shrinks.

Experimentally, a hypotonic challenge causes an initial uptake of water and cell swelling, which subsequently triggers shrinkage, i.e., regulatory volume decrease (RVD), to return the cell to its original volume. RVD depends on efflux of both K^+ and Cl^-, followed by water extrusion to maintain osmolarity. Kv, K_{Ca}, K_{IR}, and K_{2P} channels known to be involved in the regulation of cell volume have been identified at the molecular level (reviewed in Lang et al., 1998). For example, an increased K^+ current was observed in human intestinal epithelium during hypotonicity-induced volume decrease; RVD was prevented by treating cells with clotrimazole, a non-selective blocker of K_{Ca}, Kv, and K_{IR} channels (Yuan et al., 1995; Wang, Morishima & Okada, 2003). In human bronchial epithelial cells, open probability of BK_{Ca} was enhanced in response to a hypotonic challenge (Fernández-Fernández et al., 2002); both the current and the RVD were Ca^{2+}-dependent and had a pharmacologic profile similar to that for BK_{Ca} (Fernández-Fernández et al., 2002). In addition to participating in RVD, K^+ channels have also been demonstrated to regulate apoptosis, as discussed further below.

Transmembrane K^+ Flow and Apoptosis

There are, at least, two pathways that can lead to caspase activation, proteolysis and DNA fragmentation, and apoptosis (Fig. 3). The extrinsic pathway, or the death receptor pathway, requires binding of ligands such as CD95, tumor necrosis factor α (TNF-α), or Fas-ligand to transmembrane death receptors, followed by the activation of membrane-proximal caspase 8 (and/or caspase 10). The intrinsic pathway, or the mitochondrial pathway, triggered by mitochondrial membrane depolarization (i.e., $\Delta\Psi_m$ disruption or dissipation) or DNA damage relies on disruption of the mitochondrial membrane and release of cyt *c* from the mitochondrial matrix. Both the extrinsic and intrinsic pathways converge with the activation of effector caspase 3, caspase 6, and caspase 7. However, the pathways are also not mutually exclusive of each other. For example, caspase 8 activation by death receptor activation can trigger the formation of Bax/Bak proteins, which translocate to the outer mitochondrial membrane and facilitate the extrusion of cyt *c* into the cytosolic space, further aggravating apoptosis. Apoptosis-inducing factor, another factor released from the mitochondria, can

also trigger apoptosis, albeit without the involvement of caspases.

The goal of apoptosis is to maintain normal tissue homeostasis by eliminating superfluous or damaged cells. Unregulated apoptosis and lack of apoptosis, the two ends of the apoptotic spectrum, have been implicated in a wide range of disease states, including cancer, heart disease, atherosclerosis, hypertension, and neurodegeneration (Yuan & Yankner, 2000; Dispersyn & Borgers, 2001; Mayr & Xu, 2001; Green & Evan, 2002). While the stresses or factors that trigger apoptosis are varied, apoptotic volume decrease (AVD) due to efflux of K^+, Cl^-, and H_2O is an early hallmark of apoptosis, occurring within 1–4 hours of challenge with apoptosis inducers (Darzynkiewicz et al., 1992; Wesselborg & Kabelitz, 1993; Bortner, Hughes & Cidlowski, 1997; Maeno et al., 2000; Chang et al., 2000; Platoshyn et al., 2002). This corresponds to the initial phase of AVD that takes place before the activation of the apoptosome (including cyt *c*, caspase 9, and APAF-1). Subsequent caspase activation during the late phase of AVD (by either pathway) leads to cleavage of the nuclear lamin and DNA fragmentation via the activation of deoxyribonucleases, followed by formation of apoptotic bodies, and eventual phagocytosis of unlysed cell remnants by resident macrophages. The time course of the effect of pro-apoptotic agents suggests that the initial phase of AVD occurs prior to caspase activation, cyt *c* release and translocation, and DNA/nuclear fragmentation. However, a rise in cytoplasmic cyt *c* and increased caspase activation also contributes to the late phase of AVD (Wolf et al., 1997; Hughes & Cidlowski, 1998; Schrantz et al., 1999; Vu, Bortner & Cidlowski, 2001; Platoshyn et al., 2002). Therefore, early and late phase AVD may result from different mechanisms. In both stages, membrane ion channels and transporters appear to be involved. Below, we describe the roles of plasmalemmal and mitochondrial K^+ channels in modulating the various stages of apoptosis.

Cytoplasmic K^+ Efflux Promotes Apoptosis

The use of K^+ ionophores (e.g., valinomycin) has proven useful in establishing the link between K^+ efflux and apoptosis. Beauvericin (Ojcius et al., 1991) and valinomycin (Allbritton et al., 1988; Inai et al., 1997; Yu et al., 1997; Dallaporta et al., 1998, 1999; Krick et al., 2001b), two ionophores specific for K^+, can induce apoptosis in neurons, thymocytes, ascites hepatoma cells, and pulmonary artery smooth muscle cells (PASMC) due to increased K^+ loss. In addition, transmembrane K^+ efflux can be enhanced by activation of large- and small/intermediate-conductance Ca^{2+}-activated K^+ channels in erythrocytes (Lang et al., 2003), human epithelial cells (Wang et al., 2003), and vascular smooth muscle cells (Krick et al., 2001b) as a result of increased $[Ca^{2+}]_{cyt}$. Exposure of erythrocytes to ionomycin, a Ca^{2+} ionophore that markedly raises $[Ca^{2+}]_{cyt}$ and activates Ca^{2+}-activated K^+ channels, has been shown to induce cell shrinkage, cell membrane blebbing, and apoptosis (Lang et al., 2003). In support of these findings, early studies clearly indicated that lymphocyte and neuron apoptosis was enhanced when K^+ efflux was increased or, conversely, apoptosis was attenuated by inhibiting K^+ efflux (Bortner et al., 1997; Yu et al., 1997).

That apoptotic K^+ efflux occurs via K^+ channels has been established using specific channel inhibitors. The activity of a Kv channel sensitive to 4-AP and/or TEA is enhanced by a number of pro-apoptotic agents, such as staurosporine (Yu et al., 1997; Ekhterae et al., 1999; Wang et al., 2000; Krick et al., 2001a), S-nitroso-acetyl-penicillamine (SNAP, an NO donor) (Krick et al., 2002), UV light (Wang et al., 1999b), sulfhydryl oxidizing agents (McLaughlin et al., 2001), and dexamethasone (Dallaporta et al., 1999). In pulmonary artery smooth muscle cells (PASMC) (Ekhterae et al., 2001; Krick et al., 2001a; Platoshyn et al., 2002), the maximum enhancement of whole-cell Kv currents, $I_{K(V)}$, by staurosporine occurs within 6 h (Fig. 4*A*), while apoptosis is maximal after ~24 h of treatment, suggesting that Kv channel activation in PASMC occurs rapidly following the challenge by apoptotic inducers or death triggers, and likely precedes caspase activation and DNA degradation in these cells as well. In another study, treatment of PASMC with dichloroacetate, a metabolic modulator that increases mitochondrial oxidative phosphorylation, also enhanced Kv function and expression (i.e., Kv1.5) in PASMC (McMurtry et al., 2004) and induced apoptosis.

In addition to Kv channels, other K^+ channels have also been implicated in apoptosis. In vascular smooth muscle cells, disruption of $\Delta\Psi_m$ by carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone (FCCP) causes an increase in $[Ca^{2+}]_{cyt}$, which enhances K^+ efflux via BK_{Ca} channels (Krick et al., 2001b). Activation of BK_{Ca} channels by SNAP, dihydroepiandrosterone (DHEA), and TNF-α also induces apoptosis (Nietsch et al., 2000; Krick et al., 2001a, 2002). Activation of K_{2P} channels, such as TREK (Trimarchi et al., 2002) and TALK (Duprat et al., 2005), is involved in hydrogen peroxide (H_2O_2)- and NO-mediated apoptosis, respectively, as is the activity of HERG K^+ channels (Wang et al., 2002). Activation of K_{ATP} channels by cromakalim induces neuronal apoptosis (Yu et al., 1997). Finally, Ba^{2+}- and quinine-sensitive channels, possibly K_{IR}, are also involved in staurosporine-, TNF-α-, and cyclohexamide-induced apoptosis (Maeno et al., 2000; Nietsch et al., 2000).

While the evidence is clear that pro-apoptotic agents activate K^+ channels, interaction of auxiliary regulatory proteins and kinases with K^+ channels

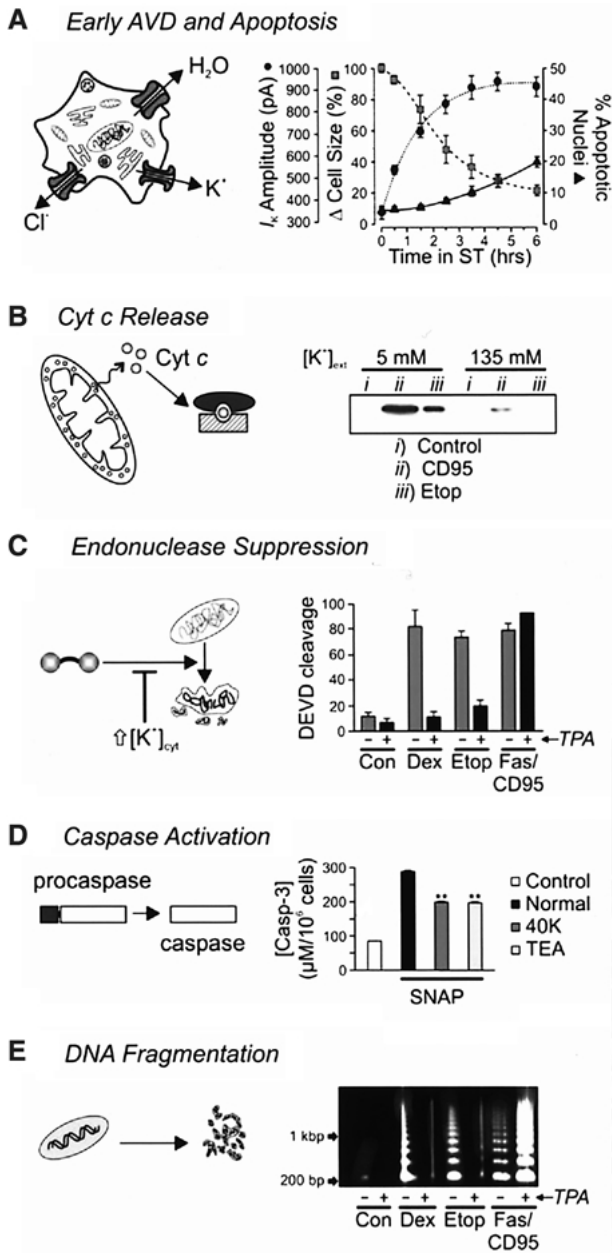

Fig. 4. Modulation of apoptotic steps by K^+ efflux. (*A*) Early AVD is accompanied by increased K^+ efflux via Kv channels on the plasma membrane. Both AVD and Kv channel activation occur within 1 h of the apoptotic trigger, staurosporine (ST) in this case, while apoptosis is not maximal even 6 h after the initial apoptotic stimulus. (*B*) Decreasing K^+ efflux by reducing the driving force for K^+ (i.e., by elevating extracellular $[K^+]$, $[K^+]_{out}$) attenuates cyt *c* release induced by CD95 and etoposide (*Etop*). (*C*) Maintaining high $[K^+]_{cyt}$ suppresses endonuclease activation, as during K^+ channel inhibition by TPA in the presence of dexamethasone (*Dex*), etoposide (*Etop*), but not Fas/CD95. Endonucleases are not activated with TPA or in the absence of apoptotic stimuli (*Con*). (*D*) Caspase activation induced by SNAP is increased by K^+ efflux and prevented by attenuating K^+ efflux by K^+ inhibition with TEA or 40 mM $[K^+]_{cyt}$. (*E*) DNA fragmentation (and loss of nuclear membrane integrity) is abolished by treatment with TPA in the presence of *Dex* and *Etop*, but not *Fas/CD95* or in control (*Con*) conditions. Reproduced with permission from Platoshyn et al., 2002; Am. J. Physiol. 283:C1298–C1305 (*A*), Thompson et al., 2001; Biochem. J. 357, 137–145© the Biochemical Society (*B*), Dallaporta et al., 1999. The American Association of Immunologists, Inc.,) (*C, E*), and Remillard & Yuan, 2004; Am. J. Physiol 286:L49–L67 (*D*).

under apoptotic conditions may occur. For example, KChAP (K^+ channel-associated protein/protein inhibitor of activated STAT) induces apoptosis in prostate cancer cells by increasing K^+ efflux and AVD (Wible et al., 2003); KChAP expression is enhanced by staurosporine. Protein kinase C may also be involved since its inhibition promotes cell shrinkage caused by Fas-ligand, reactive oxygen species, H_2O_2, and TNF-α (Gómez-Angelats, Bortner & Cidlowski, 2000; Li et al., 1999; Nietsch et al., 2000; Storey et al., 2003; Ryer et al., 2005). Tyrosine kinase inhibition also induces Fas-and ceramide-mediated apoptosis by enhancing Kv channel activity (Szabò et al., 1996; Yu et al., 1999), although this effect may also involve suppression of Na^+-K^+ ATPase activity (Bortner, Gómez-Angelats & Cidlowski, 2001; Mann et al., 2001; Wang & Yu, 2002).

Role of K^+ Channels in Apoptotic Volume Decrease (AVD) and Early Apoptosis

AVD is a prerequisite for apoptosis. This conclusion comes from studies where apoptosis was induced by staurosporine (mitochondrial pathway) or TNF-α/cyclohexamide (death receptor pathway). AVD occurred before caspase 3 activation, DNA laddering, and cellular structural alterations (Hughes et al., 1997; Maeno et al., 2000). Increased K^+ efflux is required for cell shrinkage, and is one of the earliest indicators of apoptosis. Cidlowski and associates (Bortner et al., 1997; Vu et al., 2001) showed that a decrease in cytosolic $[K^+]$ ($[K^+]_{cyt}$) correlated with the number of shrunken cells in lymphocytes treated with Fas ligand, dexamethasone, staurosporine, and anisomycin. Conversely, raising extracellular $[K^+]$ prevented K^+ efflux and attenuated AVD (Bortner et al., 1997; Yu et al., 1997; Gómez-Angelats et al., 2000; Krick et al., 2001a, 2001b).

By the late 1990's, many studies suggested that K^+ efflux occurred via sarcolemmal K^+ channels. The cornerstone study by Yu et al. clearly demonstrated that neuronal apoptosis was accompanied by an early increase in K^+ conductivity via TEA-sensitive Kv channels (Yu et al., 1997). Studies in lymphocytes (Bortner et al., 1997; Dallaporta et al., 1999; Gómez-Angelats et al., 2000; Maeno et al., 2000; Vu et al., 2001; Bock et al., 2002), neurons (Xiao et al., 2002), and vascular smooth muscle cells (Krick et al., 2001b, 2002; Platoshyn et al., 2002) showed similar findings. Maeno et al. (2000) also

demonstrated a role for a quinine- and Ba^{2+}- sensitive K^+ channel in lymphocytes. Finally, Dallaporta et al. indicated that treatment of lymphocytes with a TEA analogue inhibited early stage apoptosis brought on by γ irradiation, dexamethasone, or ceramide (Dallaporta et al., 1999).

Precisely which K^+ channels are involved in AVD and early apoptosis is still uncertain. TREK channels' activation has been implicated in H_2O_2-induced AVD and apoptosis in mouse embryos (Trimarchi et al., 2002). Specific Kv channel isoforms have also been identified as key mediators of apoptosis. Apoptosis induced by the cytostatic drug, actinomycin D, was shown to rely on the presence of Kv1.3 channels in human lymphocytes (Bock et al., 2002); Kv1.3-deficient lymphocytes did not exhibit mitochondrial membrane depolarization or cyt *c* release in response to actinomycin D.

Interestingly, however, in thymocytes from Kv1.3-knockout mice, which lacked any voltage-dependent K^+ current, there was a compensatory 50-fold increase in Cl^- current and no detectable defects in apoptosis (Koni et al., 2003). Nonetheless, Kv1.3 channels have also been implicated in Fas-induced early AVD and apoptosis in human T cells (Storey et al., 2003). Recently, we demonstrated that overexpression of 4-AP-sensitive Kv1.5 channels enhanced "basal" and staurosporine-induced caspase-3 activation (Fig. 5), AVD and apoptosis (in COS-7 and PASMC), whereas blockade of Kv1.5 decelerates AVD and inhibits apoptosis (Brevnova et al., 2004). These results suggest that Kv1.5 channel activation occurred in the early stages of apoptosis. Nonetheless, a residual component of K^+ currents not sensitive to 4-AP remained. In other experiments, we found that both Kv and BK_{Ca} channels were activated during NO- or FCCP-induced apoptosis in rat and human PASMC (Krick et al., 2001b, 2002). The involvement of BK_{Ca} is supported by studies in hepatocytes where intracellular Ca^{2+} chelation attenuated the early increase in K^+ promoted by TNF-α (Nietsch et al., 2000).

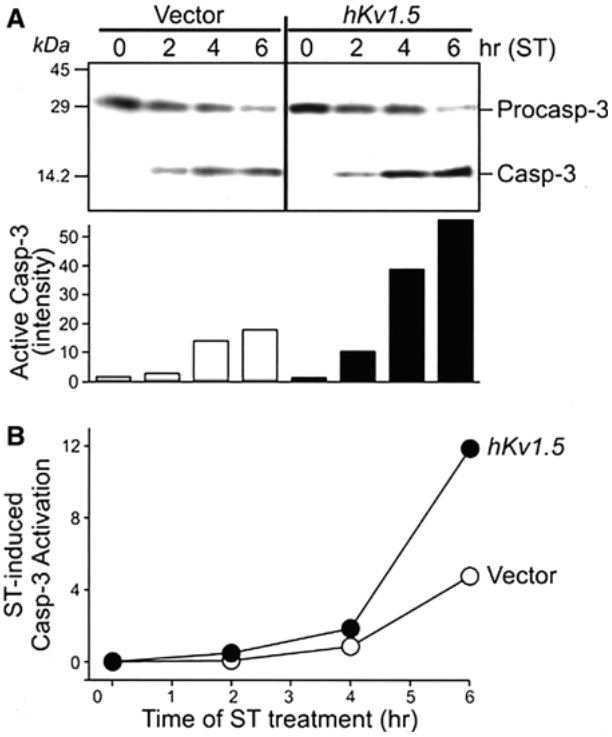

Fig. 5. Overexpression of Kv channels in PASMC enhances and accelerates staurosporine-induced caspase-3 activation. (*A*) Western blot analysis (*upper panel*) and the time course (*lower panel*) of procaspase-3 (*Procasp-3*) and active caspase-3 (*Casp-3*) protein levels in COS-7 cells transfected with pCMS-EGFP (*Vector*) and human Kv1.5 gene (*hKv1.5*) before (0 h) and after treatment with 1 μM staurosporine (ST) for 2, 4, and 6 h. The bar graph shows the protein level (determined by band intensity of active caspase-3. (*B*) Normalized increases of active caspase-3 (the intensity of the active caspase-3 band divided by the intensity of the procaspase-3 band) showing the relative change of active caspase- protein levels after treatment with ST for 2, 4, and 6 h in Vector- (*empty circles*) and *hKv1.5*/GFP- (*filled circles*) transfected cells. The two curves are significantly different ($P < 0.001$).

Mid- and Late-Phase Apoptosis Effects of K^+ Channels

After the initial triggering by the apoptotic stimulus (e.g., staurosporine) and subsequent early AVD and mitochondrial membrane depolarization, cyt *c* release and caspase activation lead to the ultimate degradation of the nuclear contents of the cell and disruption of cell membrane integrity. Changes in $[K^+]_{cyt}$ can modulate these steps in the apoptotic cascade as well.

Cytochrome c Release

The release of cyt *c* from the mitochondrial intermembrane space is pivotal to activation of caspase 9, formation of the apoptosome, activation of the effector caspases-3/-6/-7, and ultimately apoptosis. Increased extracellular $[K^+]$, by reducing the transmembrane chemical driving force for K^+ efflux, inhibits death receptor-mediated apoptosis; the inhibitory effect occurs before cyt *c* release and caspase-8 activation (Cain et al., 2001; Thompson et al., 2001). In Jurkat T cells and lymphocytes, increased extracellular $[K^+]$ prevents cyt *c* release by CD95 and etoposide (Thompson et al., 2001) (Fig. 4*B*). Quinine and Ba^{2+} inhibits cyt *c* release in lymphocytes (Maeno et al., 2000), and TEA and 4-AP inhibit cyt *c* release in enterocytes (Grishin et al., 2005), implicating K^+ channels in this process. Findings such as these support the contention that early K^+ efflux is a necessary trigger for apoptosis. However, evidence for a second phase (i.e., post-cyt *c* release) of K^+ efflux via plasmalemmal K^+ channels is also apparent. We have demonstrated that cytoplasmic application of recombinant cyt *c* can enhance 4-AP sensitive $I_{K(V)}$ in

PASMC pretreated with caspase 9 inhibitors (Platoshyn et al., 2002). The increased current in response to cyt *c* was seen ~5 minutes after cyt *c* application and occurred independently of caspase 9 activation, while nuclear condensation occurred ~15 h after cyt *c*-induced increase in K^+ currents (Krick et al., 2001a; Platoshyn et al., 2002), suggesting that cyt *c* release also activates K^+ channels and increased K^+ currents or efflux also occurs in the intermediate phase of apoptosis.

Endonuclease and Endogenous Caspase Suppression

The loss of intracellular K^+, in addition to causing AVD, also creates a permissible environment for apoptosis by relieving the inhibition of endogenous caspases and nucleases (Montague et al., 1999). Hughes and colleagues showed that intracellular levels of K^+ (~140 mM) were sufficient to inhibit the activity of endogenously active nucleases, as well as the nuclease-induced chromatin fragmentation (Hughes et al., 1997). High concentrations of intracellular K^+ also inhibited apoptosis in an in vitro thymocyte autodigestion model of cell death where nuclease enzyme activity was monitored (Hughes et al., 1997). In thapsigargin-, staurosporine-, and dexamethasone-induced thymocyte apoptosis, physiological concentration of cytoplasmic K^+ suppressed the activation of effector caspases, and loss of K^+ was associated with activation of effector caspases and a DNA nuclease (Bortner et al., 1997; Hughes et al., 1997). Endonuclease and caspase suppression was mimicked by K^+ channel inhibition with tetrapentylammonium (TPA, a TEA analogue) in dexamethasone- or etoposide-treated lymphocytes (Dallaporta et al., 1999) (Fig. 4*C*). Similar findings were shown using quinine and Ba^{2+} to attenuate K^+ channel activity in staurosporine-, TNF-α-, and cyclohexamide-treated cells (Maeno et al., 2000).

Caspase Activation

In cell lysates, elevated K^+ concentration inhibited the formation or oligomerization of the cyt *c*–caspase-9–APAF-1 apoptosome, and attenuated subsequent caspase activation. However, once the apoptosome assembled, it was insensitive to K^+ (Cain et al., 2001; Thompson et al., 2001). In PASMC, increasing extracellular $[K^+]$ significantly attenuated apoptosis induced by activated caspase-3 (Mandegar, Remillard & Yuan, 2002). Caspase-3 inhibition by the altered transmembrane K^+ gradient was mimicked by K^+ channel inhibition with TEA in PASMC (Remillard & Yuan, 2004) (Fig. 4*D*), and with Ba^{2+} in lymphocytes (Maeno et al., 2000). Inhibition of K^+ channels attenuates K^+ efflux and helps maintain the high concentration of K^+ in the cytosol, which suppresses cytoplasmic caspase and nuclease activity, and inhibits apoptosis.

DNA Fragmentation

In lymphocytes challenged with apoptosis inducers (e.g., thapsigargin, dexamethasone, staurosporine, Fas-ligand), DNA fragmentation was observed only in cells that demonstrated shrinking (or AVD) and low intracellular K^+ concentration, supporting an early role of K^+ loss or K^+ channel activation as an essential early step in apoptosis (Bortner et al., 1997; Hughes et al., 1997; Dallaporta et al., 1998; Montague et al., 1999). In contrast, lymphocyte DNA fragmentation induced by dexamethasone, etoposide, and Fas/CD95 was prevented by co-treatment with TPA to inhibit K^+ channels (Dallaporta et al., 1999) (Fig. 4*E*); TPA by itself did not affect DNA integrity in the absence of apoptotic stimuli.

Role of Mitochondrial K^+ Channels in $\Delta\Psi_m$, Cyt *c* Release, and Apoptosis

Mitochondrial membrane depolarization is believed to occur early in the onset of apoptosis and results in release of cyt *c* into the cytosol from the intermembrane space. Various apoptotic inducers, including staurosporine, TNF-α/cyclohexamide, NO, Fas-ligand, and UV irradiation cause cyt *c* release through depolarization of mitochondrial inner membrane or decrease in $\Delta\Psi_m$. In the mitochondrial pathway of apoptosis, cytosolic cyt *c* is necessary for the formation of the apoptosome, which then activates downstream effector caspases. Various studies have established a link between $\Delta\Psi_m$ and cytosolic K^+ levels. In apoptotic T cell hybridoma cells treated with dexamethasone or Fas, a significant loss of K^+ was seen only in cells that had completely dissipated $\Delta\Psi_m$ (or mitochondrial membrane depolarization). Moreover, K^+ loss could be inhibited with Bcl-2 (an antiapoptotic protein), bongkrekic acid, or cyclosporin A, presumably by preventing $\Delta\Psi_m$ dissipation (Dallaporta et al., 1998). Additionally, if isolated nuclei were exposed to supernatant of mitochondria that had already undergone permeability transition, chromatin fragmentation was only observed if K^+ was lowered to certain levels (Dallaporta et al., 1998), suggesting that lowering cytoplasmic K^+ concentration ($[K^+]_{cyt}$) is a prerequisite for apoptosis induced by mitochondrial membrane depolarization or $\Delta\Psi_m$ disruption.

In contrast, although K^+ efflux across the plasma membrane and mitochondrial membrane depolarization occur during apoptosis, they may not be causally linked. In human PASMC, staurosporine increased $I_{K(V)}$ ~35 minutes after application and induced mitochondrial membrane depolarization and

apoptosis 6 h later (Krick et al., 2001a). Reduction of the transmembrane driving force for K^+ by increasing extracellular [K^+] and blockade of plasmalemmal K^+ channels with 4-AP (and other K^+ channel blockers) significantly decreased whole-cell $I_{K(V)}$ and markedly inhibited AVD and apoptosis, although mitochondrial membrane depolarization still occurred (Krick et al., 2001a). Therefore, it seems likely that the presence of high concentration of cytoplasmic K^+ inhibits caspases and nucleases, even in the presence of cyt *c*. However, in a cell-free apoptosome reconstitution assay, the inhibition of apoptosome formation by K^+ can be relieved in a dose-dependent manner by increasing cyt *c*, suggesting that the massive leakage of cyt *c* during mitochondrial membrane depolarization and/or mitochondrial permeability transition can overcome K^+ inhibition of caspases (Cain et al., 2001). Another study showed that only the apoptotic cells undergoing AVD show mitochondrial membrane depolarization and DNA fragmentation (Bortner & Cidlowski, 1999). Although cell shrinkage, K^+ efflux across the plasma membrane, and mitochondrial membrane depolarization were shown to be linked during the apoptotic process, they appear to occur independently of DNA fragmentation and are therefore caspase-independent (Bortner & Cidlowski, 1999).

Role of mitoK_{ATP} and mitoK_{Ca} in Cell Survival

Based on the K^+ electrochemical gradient across the mitochondrial membrane, opening of K^+ channels on the inner mitochondrial membrane would lead to an influx of K^+ into the mitochondrial matrix, thereby causing mitochondrial membrane depolarization (or decrease in $\Delta\Psi_m$). As discussed above, apoptosis stimuli, which induce apoptosis via the mitochondrial pathway, cause mitochondrial membrane depolarization and subsequently release cyt *c* from the mitochondrial intermediate space to the cytosol. That is, mitochondrial membrane depolarization (or disruption of $\Delta\Psi_m$) triggers cyt *c* release. Based on these observations, opening of mitochondrial K^+ channels indirectly enhances cyt *c* release by causing mitochondrial membrane depolarization, and induces apoptosis by activating cytoplasmic caspase 9 (along with subsequent activation of the effector caspases). Furthermore, opening of mitochondrial K^+ channels has a widely divergent effect compared to its plasma membrane counterparts as it relates to apoptosis. However, whether mitochondrial membrane depolarization causes cyt *c* release is still controversial.

In initial studies in hippocampal homogenates, diazoxide, a K_{ATP} channel opener, caused mitochondrial membrane depolarization and cyt *c* release (Dębska et al., 2001), which would be expected to result in caspase activation and apoptosis. A later study showed that part of the diazoxide-mediated apoptotic effect may also be explained by the release of K^+ from the mitochondrion to the cytosol along its concentration gradient (Garlid, 1996), with subsequent extrusion of cytosolic K^+ via the sarcolemmal K^+ channels, AVD, and apoptosis (Liu et al., 2003). Furthermore, treatment of neurons with 5-hydroxydecanoate, a mitochondrial K_{ATP} channel blocker, promoted cell survival by inhibiting cellular K^+ efflux, decreasing oxygen radical production, stabilizing $\Delta\Psi_m$, and attenuating cyt *c* release (Liu et al., 2003).

In contrast to the above-mentioned studies, treatment of neurons with diazoxide protected the cells against amyloid β peptide toxicity (Goodman & Mattson, 1996) and cerebral ischemia (Liu et al., 2002), suggesting that activation of mitoK_{ATP} channels might also be neuroprotective. Indeed, in hippocampal neurons, diazoxide prevented cyt *c* release and inhibited staurosporine- and cyanide-induced apoptosis, although it caused mitochondrial depolarization (Liu et al., 2002). The antiapoptotic effects of diazoxide were blocked by 5-hydroxydecanoate, indicating that the effect was due to specific activation of mitoK_{ATP} channels. Diazoxide also increased antiapoptotic Bcl-2 protein levels in the mitochondria and inhibited Bax translocation to the mitochondrial membrane (Liu et al., 2002), thereby promoting neuronal survival. In all, preconditioning of neurons with diazoxide protects them against apoptosis following later apoptotic stimulation.

Similar to neurons, increased mitoK_{ATP} channel activity also protected against ischemic injury in cardiomyocytes (Liu et al., 1998, 1999; Sasaki et al., 2000; Akao et al., 2001; Murata et al., 2001). In ischemic cardiomyocytes, activation of mitoK_{ATP} channels with, for example, diazoxide, protected the cells from death by suppressing mitochondrial Ca^{2+} overload during stimulated ischemia (Holmuhamedov et al., 1998; Murata et al., 2001; Zhu et al., 2003; Rousou et al., 2004). NO-induced cardioprotection has also been attributed to mitoK_{ATP} activation (Sasaki et al., 2000). Diazoxide was also found to preserve mitochondrial integrity and prevent H_2O_2-induced apoptosis in neonatal rat ventricular myocytes (Akao et al., 2001). MitoK_{ATP}-mediated cardioprotection may also involve production of reactive oxygen species during preconditioning (Forbes, Steenbergen & Murphy, 2001, Liu et al., 1999; Sasaki et al., 2000; Xi, Cheranov & Jaggar, 2005; Yao et al., 1999; Zhang et al., 2002).

In direct contrast to the latter studies, others have suggested that the protective effect of diazoxide may not be due to its effect on mitoK_{ATP} channels, but on mitochondrial volume. Kowaltowski et al. (2001) showed that diazoxide causes $\Delta\Psi_m$ depolarization and Ca^{2+} uptake at concentrations higher than that needed to activate mitoK_{ATP} channels, and that it does not stimulate respiration at doses that

affect $\Delta\Psi_m$ and Ca^{2+} uptake. Rather, their data suggest that increased mitochondrial K^+ influx during mitoK_{ATP} activation causes the mitochondron to rapidly take in osmotically-obligated water, thereby increasing matrix volume by 15–20% (Kowaltowski et al., 2001); matrix swelling by diazoxide is attenuated by 5-hydroxydecanoate. Matrix contraction or shrinkage, and the ensuing expansion of the intermembrane space, can lead to increased VDAC function (Garlid & Paucek, 2001) and diffusion of cyt *c* from the mitochondria. Therefore, matrix swelling during an apoptotic challenge preserves the 'normal' architecture of the intermembrane space, therefore preserving efficient energy transfer between the mitochondria and cytosol and enhancing cell survival. Increased cardio- and neuro-protection due to matrix volume protection has since been substantiated by others (Rousou et al., 2004; Brustovetsky, Shalbuyeva & Brustovetsky, 2005; Korge, Honda & Weiss, 2005).

Charybdotoxin-sensitive mitoK_{Ca} channels have been identified in neuron (Siemen et al., 1999) and cardiomyocyte (Xu et al., 2002) inner mitochondrial membranes. As for mitoK_{ATP} channels, activation of mitoK_{Ca} channels should theoretically cause complete and irreversible uncoupling of mitochondrial integrity, leading to apoptosis. However, two studies have suggested that activation of mitoK_{Ca} channels also promotes cardiomyocyte survival. Both (Xu et al., 2002; Cao et al., 2005) showed that preischemic exposure to NS-1619, a mitoK_{Ca} opener, significantly (~50%) decreased infarct size, similar to that caused by mitoK_{ATP} channel activation previously (*see* above). Similarly, pretreatment with paxilline, a mitoK_{Ca} inhibitor, prevented cardiomyocyte survival induced by ischemic preconditioning (Cao et al., 2005). In addition, paxilline also impaired left ventricular function while NS-1619 improved it (Cao et al., 2005). Therefore, like mitoK_{ATP} channels, mitoK_{Ca} channels are also important in protecting against cardiac ischemia/reperfusion injury.

Antiapoptotic Proteins and Plasma Membrane K^+ Channel Modulation

Bcl-2 and ARC (apoptosis repressor with caspase domain) are antiapoptotic proteins that act, at least in part, through the modulation of sarcolemmal K^+ channels. In cardiomyocytes, ARC blocked the staurosporine-mediated increase in $I_{K(V)}$ and apoptosis (Ekhterae et al., 2003). Bcl-2, an antiapoptic protein found in both the endoplasmic reticulum and mitochondrial membranes, prevents apoptosis primarily by blocking the release of cyt *c* into the cytosol (Kluck et al., 1997; Yang et al., 1997). In thymocytes, inhibition of mitochondrial $\Delta\Psi_m$ loss by Bcl-2 prevented K^+ leakage and subsequent apoptosis (Dallaporta et al., 1998). In staurosporine-treated rat PASMC, overexpression of Bcl-2 abolished the enhancement in $I_{K(V)}$ and significantly attenuated apoptosis (Ekhterae et al., 2001). Decreased $I_{K(V)}$ by Bcl-2 was due not only to altered channel activation kinetics and current amplitude, but also to downregulation of Kv1.1, Kv1.5, and Kv2.1 mRNA expression (Ekhterae et al., 2001). Recently, McMurtry et al. also demonstrated that survivin, an apoptosis inhibitor which targets procaspase-3 activation, prevented Kv channel activation and attenuated apoptosis in PASMC (McMurtry et al., 2005). These findings in PASMC with Bcl-2 and survivin are important findings for the treatment of pulmonary arterial hypertension, a disease whose etiological bases include (but are not limited to) deregulated PASMC proliferation and altered K^+ channel expression and/or function.

Summary

Traditionally, transmembrane K^+ flow has been associated with the regulation of cell excitability and, consequently, of muscle tension. From the discussions presented in this review, it is apparent that the roles of K^+ ions and K^+ channels have dramatically expanded to include the regulation of cell volume and of cell survival/apoptosis, the latter at many levels of the signaling pathways, whether the K^+ channels in question are located on the plasma membrane or the inner mitochondrial membrane. While apoptosis itself bears negative connotations, we also provide compelling evidence to suggest that the targeting of K^+ channels is of great import in controlling unregulated apoptosis, and may play a vital role in the treatment of diseases such as pulmonary hypertension, cardiac ischemic injury, and stroke.

Research and data presented here were supported in part by NIH/NHLBI grants (HL 064945, HL 054043, HL 66012, HL 69758, and HL66941).

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